Ajay Kumar Tiwari *Editor*

Advances in Seed Production and Management

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Dedicated to my mentor Dr. Govind Pratap Rao, Principal Scientist, Division of Plant Pathology, Indian Agriculture Research Institute, Pusa Campus, New Delhi, and Chief Editor—Sugar Tech, Phytopathogenic Mollicutes, *and* Medicinal Plants.

Sir,

You are a wonderful teacher, boss, leader, and friend and are everything one could look for in a good mentor. It would be impossible to count all the ways that you have helped me in my career. I cannot thank you enough for your mentorship over the years. You have been such an integral part of my career. I always hope to inspire others as you have inspired me.

It is all because of you.

Introduction

Seed quality is basic to good and healthy crops. There is still an intense need to enhance the supply and quality of seeds. Plant geneticists and breeders now have many techniques and tools for crop improvement. The accomplishments of the past half-century are many, but will be extended as newer and more improved methods are developed. Scientific and technical competence in crop improvement, in the areas of production, processing, and utilization, has been improving in general and in the vegetable and flower seed industry in particular. Finding and developing better seeds for agricultural crops that can resist drought, heat and cold, the threat of the disease, and attack of insect pests is important to ensure adequate food for all populations. The seeds in use today have enabled farmers to produce a healthy variety of hardy food and fiber crops unknown even a few years ago. The continued efforts of plant breeders and geneticists to accomplish miracles in the development of more useful crop plants could rescue people worldwide from the fear of hunger. This book comprises updated information related to the advancement in seed production in legumes, sugar crops, vegetables, seed biology, seed dormancy, role of quarantine, diseases, insect pest management, effect of climate, organic packages, seed treatment, weed management, etc.

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About the Editor

Ajay Kumar Tiwari, PhD is a Scientific Officer at the UP Council of Sugarcane Research, Shahjahanpur, UP, India. He completed his PhD on cucurbit viruses at the CCS University, Meerut, UP, India, in 2011. He is a member of the British Society of Plant Pathology, Indian Phytopathological Society, Sugarcane Technologists Association of India, International Society of Sugarcane Technologists, Society of Sugarcane Research and Promotion, Prof H. S. Srivastava Foundation, and Society of Plant Research. He has published 75 research articles and 12 review articles in respected national and international journals. He has also published six book chapters and has also authored seven books published by Springer, Taylor & Francis, and Nova. He has submitted more than 200 plant pathogen nucleotide sequences to Genbank.

Dr. Tiwari is a regular reviewer and member of the editorial boards of several international journals and is managing editor of *Sugar Tech* (IF 1.08) and chief editor of the *Agrica* journal. He received the CIPAM Young Researcher Award in 2011 and the DST-SERB Young Scientist Award and was nominated for the Narasimhan Award by the Indian Phytopathological Society. He was selected for the Young Scientist Award by the Chief Minister of the State Government of UP for his outstanding contributions in the area of plant pathology and was the recipient of many international travel awards conferred by DST, DBT, and CSIR in India; PATHOLUX in Luxembourg; and IOM in Brazil. He has attended conferences and workshops in China, Italy, Germany, Vietnam, and Thailand and has delivered invited talks on phytoplasma diseases of sugarcane in

Vietnam and Thailand. He also received a postdoctoral fellowship from Oman and DST India.

He is currently involved in research on the molecular characterization and management of agricultural plant pathogens and the production of healthy sugarcane seed materials and their distribution in UP through cane societies.

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Role of Quarantine in Management of Transboundary Seed-Borne Diseases

1

V. Celia Chalam, Ruchi Sharma, Vaishali Dutt Sharma, and A. K. Maurya

Abstract

The global movement of seeds has the potential of introducing new pathogens which may cause potential risk to the agriculture of the importing country. The National Plant Protection Organizations assume responsibility for preventing entry of new pathogens and for eradicating those that have entered and are still confined. The exclusion can be achieved by a combination of technical and regulatory approaches that can ensure biosecurity for a country/region. In India, the Directorate of Plant Protection, Quarantine and Storage under the Ministry of Agriculture and Farmers' Welfare is responsible for enforcing quarantine regulations and for inspection and disinfection/disinfestation of agricultural commodities meant for commercial purpose. The imported germplasm material including transgenics is quarantined at the ICAR-National Bureau of Plant Genetic Resources, New Delhi. The strategies for biosecurity for plant viruses include stringent quarantine measures for the imported material, domestic quarantine and use of certified disease-free seed and other planting material within the country. Adopting a workable strategy, several pathogens of quarantine significance have been intercepted, and the risk of introduction of these pathogens into India was thus eliminated. Adopting the appropriate technique and the right strategy for pathogen detection and disinfection would go a long way in ensuring safe exchange of germplasm and trade and the biosecurity of Indian agriculture from transboundary introduction of plant pathogens.

Keywords

Biosecurity · Quarantine · Plant pathogens · Trade · Germplasm · India

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1.1 Introduction

Plant diseases substantially reduce crop production every year, resulting in serious economic losses throughout the world. Infected or contaminated seed is a primary source of infection for a large number of destructive diseases of important food, fodder and fibre crops and is an excellent carrier for the dissemination of pathogens to long geographical distances. Trade and exchange of germplasm at international level play a key role in the long-distance dissemination of a destructive pathogen or its virulent pathotype/race/strain along with agri-horticultural produce. Due to liberalization under WTO, the recent years have seen a significant growth in trade and exchange of agri-horticultural crops. The global movement of seed material has the potential of introducing new pathogens which may pose potential risk to the agriculture of the importing country. The present-day definition of a pest is any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products. A quarantine pest is the pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed, and being officially controlled (https://www.ippc.int/largefiles/adopted.../en/ISPM_05_2007_En_2007-07-26.pdf).

The devastating effects resulting from pathogens introduced along with international movement of seed and other planting material are well documented. The Irish famine of 1845, which forced the people to migrate en masse from Europe, was the result of almost total failure of potato crop due to attack of late blight pathogen (*Phytophthora infestans*) introduced from Central America. Coffee rust (*Hemileia vastatrix*) appeared in Sri Lanka in 1875 and reduced the coffee production by >90% in 1889. The disease entered India in 1876 from Sri Lanka, and within a decade, the coffee industry of South India was badly affected. Bulk import of seeds and other planting material without proper phytosanitary measures, indiscriminate exchange of germplasm and the distribution of seed and other planting material by international agencies have increased the possibility of dissemination of pathogens in areas previously considered pathogen-free (Khetarpal et al. 2006). Further, the threat may become severe, if more virulent strains or races of the pathogen are introduced into previously disease-free areas. Even a low seed transmission rate of a pathogen, especially viruses, may lead to an epiphytotic proportion of the disease in field, if other conditions of field spread and climate are favourable. The worldwide distribution of many economically important viruses such as *bean common mosaic virus*, *soybean mosaic virus*, *pea seed-borne mosaic virus*, *wheat streak mosaic virus* and *peanut mottle virus* is attributed to the unrestricted exchange of seed lots.

Like in other countries, a number of exotic plant pathogens got introduced into India along with imported planting material causing serious crop losses from time to time. These included potato late blight (*Phytophthora infestans* in 1883), coffee rust (*Hemileia vastatrix* in 1879), *banana bunchy top virus* (BBTV) in 1940 and flag smut of wheat (*Urocystis tritici*) in 1906. These introductions highlighted the fact that increased pace of international travel and trade had exposed countries to the danger of infiltration of exotic pathogens harmful to the agriculture.

The most fundamental approach to the management of a disease is to ensure that it is not present through exclusion (quarantine) or eradication. National Plant Protection Organizations assume responsibility for protecting their countries from the unwanted entry of new pests and for coordinating programmes to eradicate those that have recently arrived and are still sufficiently confined for their elimination to be realistic. The strategies for plant health management include certified disease-free seed and other planting material, chemical control, biological control, cultural control and use of resistant varieties. In the context of quality control, bulk samples of seed lots need to be tested by drawing workable samples as per norms. The detection of pathogens is then carried out by the approved or available techniques. Over the years, a great variety of methods have been developed that permit the detection and identification of pathogens. The successful detection and control of pathogens in seed and other planting material depend upon the availability of rapid, reliable, robust, specific and sensitive methods for detection and identification of pathogens.

Detection and diagnosis of pathogens are crucial for seed trade and for exchange of germplasm. Early, sensitive and accurate diagnosis is indispensable for certification of seed and other planting material under exchange. The selection of a diagnostic method for evaluating plant health depends on the host to be tested and the type of pathogens that may be carried in the seed. The technique should be reliable for quarantine requirements; reproducible within statistical limits; economical with regard to time, labour and equipment; and rapid to provide results of large samples in the shortest time.

The bulk samples of seed lots need to be tested by drawing workable samples as per norms. The detection of pathogens is then carried out by the approved or available techniques. Over the years, a great variety of methods have been developed that permit the detection and identification of pathogens.

1.2 Diagnostics for Detection of Pathogens

The successful detection and control of pathogens in seed and other planting material depend upon the availability of rapid, reliable, robust, specific and sensitive methods for detection and identification of pathogens. The various techniques, conventional and modern, that are employed for detection of pathogens are enumerated below in brief.

1.2.1 Conventional Methods

The conventional techniques which are adapted are briefly described below:

1.2.1.1 Examination of Dry Seed

Examination of dry seed under a low-power stereomicroscope with magnification up to 50–60 times may reveal symptoms such as discolouration, malformation, fruiting

bodies of fungi, hyphae, eggs of insects on seed surface, insect feeding holes and even bacterial spores or growth on the surface of the seed.

1.2.1.2 Examination of Seed Washing

This technique is employed for detecting various fungi, adhered to the surface of seed and also the spore load in a short time. Weighed seeds are shaken in a known volume of water for a fixed time on a mechanical shaker. The washing is examined under a compound microscope. By the use of phase-contrast microscope, unstained bacteria can also be observed. Failure to detect infection internal to the seed and inability to distinguish between spores of saprophytic fungi from spores of pathogenic fungi are the limitations of this technique.

1.2.1.3 Examination of Soaked Seed

This technique is practiced for detecting paddy bunt fungus, *Neovossia horrida*. Rice seeds are soaked in 0.2% sodium hydroxide for 24 h at 25 °C. The infected seeds appear brown, dull or shiny black. The infection is confirmed by rupturing the seed in a drop of water. A stream of smut spores is released from the shiny black discolouration.

1.2.1.4 Examination of Whole Embryo

This technique is used for detecting obligate pathogens, i.e. *Ustilago segetum* var. *hordei* and *U. segetum* var. *tritici* (loose smut of barley and wheat). Seeds are soaked overnight in 10% sodium hydroxide, containing trypan blue stain at 25 °C, and washed with warm water through sieves of decreasing size, and embryos are finally cleared in lactophenol. The infected embryos under stereomicroscope reveal bluish stained mycelium which may be present in scutellum, plumule bud or the whole embryo.

1.2.1.5 Incubation Tests

Incubation tests are used successfully against surface-borne as well as internal infections. After plating of seed, the incubation period gives an opportunity to the dormant mycelium of fungal spores to grow along with the host. Most commonly used incubation tests are blotter method and agar plate method, in which seeds are placed on moist blotters and agar media, respectively.

1.2.1.6 Phage Sensitivity Test

Most of the bacteria are sensitive to bacteriophages, and as a result, they are lysed. Formation of lytic zones around the phage spot confirms the identity/presence of the bacterium.

1.2.1.7 Staining of Inclusion Bodies

Inclusion bodies are aggregate of virus particles or virus-induced proteins or special structure characteristic of virus infection either in the cytoplasm or in the nucleus. The virus infection is detected by cutting freehand sections with a razor blade and

staining with Azure A and O-G combination. The tissue is observed under light microscope, and inclusion bodies are located and characterized.

1.2.1.8 Electron Microscopy

The transmission electron microscope (TEM) can be used directly to detect the presence of virus in the plant tissue. It reveals the shape and size of the virus particle. The shape and size of the virus particle give an idea of the group to which it may belong. This helps in limiting the number of antisera to be used in serological tests such as enzyme-linked immunosorbent assay (ELISA) for further identification of the virus, as only antisera of viruses of a particular shape can thus be used for identification (Chalam and Khetarpal 2008). However, the TEM remains a very expensive equipment and is often not available. Moreover, electron microscopy is not suited for routine virus indexing, whereas the highly sensitive immunosorbent electron microscopy (ISEM), developed by Derrick (1973), is occasionally used to detect viruses in seeds or to verify results of other detection methods.

1.2.1.9 Growing-On Test

Certain seed-borne diseases need longer periods for their expression than provided in the normal incubation tests. The pathogens are identified based on symptoms followed by tests of infectivity/electron microscopy/ELISA in case of viruses.

1.2.1.10 Infectivity Test

Healthy young seedlings or mature plants are exposed to infected material to produce the symptoms. This approach is quite old; however, the technique has been used successfully to detect fungi, bacteria and viruses. In case of viruses, their presence is assayed by inoculating leaf extracts of seedlings, which may or may not be showing symptoms, on indicator hosts. The indicator hosts may reveal the symptoms by producing local lesions or systemic infection. Long span of time required for development of symptoms on them, requirement of large greenhouse space and the prodigious labour and time for working with large samples are the limitations of this test.

1.2.2 Serological Tests/Immunoassays

This technique is based on the principle that a substance having high molecular weight (>10,000 Da) when introduced into an animal causes the formation of specific proteins (the immunoglobulins) in the blood, which are commonly called antibodies. The causative substance is called antigen, and the blood serum containing antibodies is called antiserum. The antigen-antibody reaction can be examined in vitro as well as in situ. Serodiagnostic tests are very sensitive and reliable to detect the presence of virus and bacteria.

Earlier serological tests based on immunoprecipitation, immunodiffusion, and latex agglutination were very popular. Nowadays, enzyme-linked immunosorbent assay (ELISA) and Dot-immunobinding assay (DIBA) are the most widely used

methods of serological detection of plant viruses and also bacteria as they are much more sensitive than diffusion and agglutination methods, use less antibody and can be employed for simultaneous handling of a large number of samples in routine testing. Thirty-three seed-transmitted viruses, which are either not known to occur in India or known to possess virulent strains or not known to occur in India on particular host(s), have been intercepted in germplasm including transgenics imported from many countries (Chalam et al. 2005a, b, 2008, 2009a, b, 2012a, b, c, 2014a, b, c; Chalam and Khetarpal 2008):

1.2.2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

The adoption of ELISA test has created new interest in serological diagnosis of plant viruses. Two types of ELISA commonly used are (1) double-antibody sandwich ELISA (DAS-ELISA) and (2) direct antigen coating ELISA (DAC-ELISA). The advantages of ELISA are it is reasonably sensitive, less susceptible to 'false positives' and low cost per sample; can handle large number of samples; and can be subjected to automation and detection kits are available commercially. Forty-one seed-transmitted viruses which are either not known to occur in India or known to possess virulent strains or not known to occur in India on particular host(s) have been intercepted in germplasm including transgenics imported from many countries (Chalam and Khetarpal 2008; Chalam et al. 2009a, b, 2014a, b, c; Chalam 2016).

1.2.2.2 Dot-Immunobinding Assay (DIBA)

It is a variant of ELISA test used for detection of viruses wherein instead of using microtitre plates as solid support, nitrocellulose membranes are used. A few microlitres of extract of infected samples are blotted on this membrane which is then submerged in primary antibody (crude antiserum). The precipitated antibody is then detected with enzyme-labelled second antibody.

DIBA has an edge over conventional ELISA as it does not require any special equipment and it requires only a crude specific antiserum to each of the viruses/bacteria and a single enzyme conjugate. Above all, the blotted membranes can be mailed to long distances for further processing in a centralized laboratory.

1.2.2.3 Tissue Blotting Immunoassay/Tissue Print Immunoassay/Tissue Print Immunoblotting

Tissue blot immunoassay is a detection method similar to DIBA, except that it does not involve tissue extraction. Instead of dotted extracts, freshly cut tissue surfaces are printed directly onto nitrocellulose membranes. The antigens trapped in the tissue blots are then reacted with antibodies, conjugate and substrate in the same way as in DIBA. The method has gained popularity for many purposes, not only due to its simplicity, eliminating the need for an extraction step, but also due to its high sensitivity, comparable with or in some cases even higher than ELISA and DIBA.

1.2.2.4 Lateral Flow Strip Method

Lateral flow strip method is a variation of ELISA used for detecting viruses/bacteria, and the antibodies are immobilized onto a test strip in specific zones. The test is

provided in kit form and does not require any major equipment. Lateral flow strips are suitable for field or on-site use, with minimal training required. Sample preparation simply involves crushing the sample and mixing it with the extraction solution provided in the kit. These tests generally provide qualitative or semi-quantitative results using antibodies and colour reagents incorporated into a flow strip.

1.2.3 Molecular Methods

1.2.3.1 Polymerase Chain Reaction

This involves rapid and highly specific in vitro amplification of selected DNA sequences, for which specific primers are synthesized. With its relative simplicity and high sensitivity (detecting picogram quantities of viral nucleic acids in infected tissues), PCR method has high potential in detection of viruses/viroids/bacteria/fungi. However, the prerequisite of having known sequences to select and synthesize suitable primers limits its application to well-characterized viruses/viroids/fungi/bacteria.

Variants of PCR

Reverse Transcription-PCR (RT-PCR)

Most of the plant viruses consist of RNA, which require the introduction of a preliminary reverse transcription (RT) step before the PCR amplification process (RT-PCR), thus allowing the amplification of RNA sequences in a cDNA form. Many viruses have been detected using RT-PCR (Siljo et al. 2014; Chalam and Khetarpal 2008; Chalam et al. 2004, 2012a, b).

Immunocapture-PCR (IC-PCR)

This is a variant of PCR which utilizes antibodies to trap viral particles without prior viral RNA extraction, which would presumably facilitate its use in routine testing. Moreover, because antibodies are involved in the first step, it may be assumed that this method could also selectively detect viruses. Thus, this method could be very useful and practical in virus indexing programme (Phan et al. 1998).

The advantages of PCR are it is highly sensitive (can detect picogram quantities of target nucleic acid); the process is automated, very rapid; it takes 2 h or less for the test; it can be used for detecting RNA or DNA; and it is very useful where ELISA is not effective (viroids, geminiviruses).

Real-Time PCR/Real-Time RT-PCR

Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (i.e. in real time) as opposed to the endpoint detection. The real-time progress of the reaction can be viewed. Real-time PCR quantitation eliminates post-PCR processing of PCR products. This helps to increase throughput and reduce the chances of carry-over contamination. No post-PCR processing (no electrophoretical separation of amplified DNA) is required.

The advantages of real-time PCR/real-time RT-PCR are it is not influenced by non-specific amplification, amplification can be monitored in real time, no post-PCR processing of products is required (high throughput, low contamination risk), there is ultra-rapid cycling (30 min to 2 h), it requires 1000-fold less RNA than conventional assays and it is most specific, sensitive and reproducible. The technique has been successfully exploited for detecting viruses (Chalam et al. 2004, 2012a), fungi and bacteria.

1.2.3.2 Nucleic Acid Hybridization Assays

Detection of viruses/bacteria by nucleic acid hybridization is based on the specific pairing between the target nucleic acid sequence (denatured DNA or RNA) and a complementary nucleic acid probe to form double-stranded nucleic acids. Thus, either RNA or DNA sequences may be used as probes. It has a potential of detecting extremely low level of inoculum or latent infections in plant/planting materials. Basically, the method involves the immobilization of a spot or dot of sap extract from the plant under test on a solid matrix and the detection of viral/bacterial nucleic acid sequences in that spot by use of a hybridization probe.

1.2.3.3 Double-Stranded RNA (dsRNA) Analysis

For poorly characterized or unknown viruses, detection methods either are not available or are not sensitive. In such cases, double-stranded RNA analysis is a rapid tool that can supplement the information obtained from bioassays. Analysis of dsRNA is based on the isolation of disease-specific dsRNAs from virus-infected tissues and their electrophoretic separation on a gel, which is then stained and viewed. However, the presence of non-viral dsRNAs in healthy plants and the apparent absence of dsRNA profile in some viruses may result in false negatives or false positives. Negative dsRNA tests should be confirmed by other methods before plant material is indexed as virus-free.

1.2.3.4 Microarrays: High-Throughput Technology

In the context of phytodiagnostics, the simplest analogy that could be drawn is essentially a dot blot in reverse, where the probe rather than the sample is bound to the solid phase. The logical extension of this approach is to immobilize a number of different spatially separated probes to the solid phase such that the samples can be tested for multiple targets. DNA capture probes (or spots) for each of the genes/pathogens to be detected are immobilized onto a solid support in a spatially separated and individually addressable fashion. Nucleic acid from the sample to be tested is extracted and labelled, and this labelled nucleic acid (known as the target) is then hybridized to the array. The array is scanned such that the hybridization events can be identified, and the presence of the gene/pathogen or insect pest is resolved by the predefined position of the DNA capture probe in the array.

Microarrays look promising for high-throughput analysis, i.e. screening of multiple viruses/bacteria/fungi simultaneously, provided the relevant sequence information is available. Microarray analysis in theory can combine detection, identification and quantification of a large number of fungi/bacteria/viruses/nematodes/insect pests

in one single assay. Hundreds of tests could be run simultaneously and in a cost-effective manner (Boonham et al. 2007).

1.2.3.5 Loop-Mediated Isothermal Amplification

Loop-mediated isothermal amplification (LAMP) is a novel technique that requires only one enzyme having strand displacement activity for amplification under isothermal conditions. LAMP has a higher specificity than PCR because its four primers recognize six distinct regions on the targeted genome. LAMP has been successfully used for detection of viruses (Arif et al. 2012; Bhat et al. 2013; Siljo and Bhat 2014).

1.2.3.6 Helicase-Dependent Amplification

Helicase-dependant amplification (HDA) requires no thermocycler for enhanced isothermal DNA amplification and has been successfully used for detection of *bean pod mottle virus* (Chalam et al. 2012a).

1.2.3.7 Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) technologies sequence the total nucleic acid content in disease samples for the identification of pathogens by bioinformatics tools. Unlike existing methods, the metagenomics approach does not require prior knowledge of the pathogens and can potentially identify both the known and new pathogens including viruses and viroids in a disease sample (Boonham et al. 2014; Hadidi et al. 2016).

The various techniques, conventional and modern, that are employed for seed health testing of different pathogens are summarized in Table 1.1.

1.3 Exclusion of Pathogens Through Quarantine

1.3.1 International Scenario: Imports and Exports

The recent trade-related developments in international activities and the thrust of the WTO agreements imply that countries need to update their quarantine or plant health services to facilitate pest-free import/export.

The establishment of the WTO in 1995 has provided unlimited opportunities for international trade of agricultural products. History has witnessed the devastating effects resulting from diseases and insect pests introduced along with the international movement of planting material, agricultural produce and products. It is only recently, however, that legal standards have come up in the form of sanitary and phytosanitary (SPS) measures for regulating the international trade. The WTO Agreement on the Application of SPS Measures concerns the application of food safety and animal and plant health regulations. It recognizes government's rights to take SPS measures but stipulates that they must be based on science; should be applied to the extent necessary to protect human, animal or plant life or health; and

Table 1.1 Summary of various techniques for detecting seed-borne pathogens of quarantine significance

Techniques	Fungi	Bacteria	Viruses	Viroids
Dry seed examination	+	+	+	+
Seed washing test	+	+	–	–
Soaked seed test	+	–	–	–
Whole embryo test	+	–	–	–
Incubation tests	+	+	–	–
Phage sensitivity test	–	+	–	–
Staining of inclusion bodies	–	–	+	–
Electron microscopy	–	–	+	+
Growing-on test	+	+	+	+
Infectivity test	+	+	+	+
Enzyme-linked immunosorbent assay (ELISA)	–	+	+	–
Dot-immunobinding assay (DIBA)	–	–	+	–
Tissue blotting immunoassay	–	–	+	–
Immunosorbent electron microscopy (ISEM)	–	–	+	–
Lateral flow strips	–	+	+	–
Polymerase chain reaction (PCR)	+	+	+	+
Reverse transcription-PCR (RT-PCR)	–	–	+	–
Immunocapture-RT-PCR (IC-RT-PCR)	–	–	+	–
Real-time PCR	+	+	+	+
Real-time RT-PCR	–	–	+	–
Microarrays	+	+	+	+
Loop-mediated isothermal amplification (LAMP)	+	+	+	+
Helicase-dependent amplification (HDA)	+	+	+	+
Next-generation sequencing (NGS)	+	+	+	+

should not unjustifiably discriminate between members where identical or similar conditions prevail (http://www.wto.org).

The SPS Agreement aims to overcome health-related impediments of plants and animals to market access by encouraging the 'establishment, recognition and application of common SPS measures by different Members'. The primary incentive for the use of common international norms is that these provide the necessary health protection based on scientific evidence and improve trade flow at the same time.

SPS measures are defined as any measure applied within the territory of the member state to protect animal or plant life or health from risks arising from the entry, establishment or spread of pests, diseases and disease-carrying/causing organisms; to protect human or animal life or health from risks arising from additives, contaminants, toxins or disease-causing organisms in food, beverages or foodstuffs; to protect human life or health from risks arising from diseases carried by animals, plants or their products or from the entry and establishment/spread of pests; or to prevent or limit other damage from the entry, establishment or spread of pests.

The SPS Agreement explicitly refers to three standard-setting international organizations commonly called as the 'three sisters' whose activities are considered to be particularly relevant to its objectives: International Plant Protection Convention (IPPC) of Food and Agriculture Organization (FAO) of the United Nations, World Organization for Animal Health (OIE) and Codex Alimentarius Commission of Joint FAO/WHO. The IPPC develops the International Standards for Phytosanitary Measures (ISPMs) which provides guidelines on pest prevention, detection and eradication. To date, 43 ISPMs (https://www.ippc.int/en/core-activities/standards-setting/ispms/) have been developed (Appendix).

Prior to the establishment of WTO, governments on a voluntary basis could adopt international standards, guidelines, recommendations and other advisory texts. Although these norms shall remain voluntary, a new status has been conferred upon them by the SPS Agreement. A WTO member adopting such norms is presumed to be in full compliance with the SPS Agreement.

1.3.2 National Scenario: Imports

Plant quarantine is defined as all activities designed to prevent the introduction and/or spread of quarantine pests or to ensure their official control. Quarantine pest is a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2019).

As early as in 1914, the Government of India passed a comprehensive act, known as Destructive Insects and Pests (DIP) Act, to regulate or prohibit the import of any article into India likely to carry any pest that may be destructive to any crop, or from one state to another. The DIP Act has since undergone several amendments. In October 1988, New Policy on Seed Development was announced, liberalizing the import of seeds and other planting material. In view of this, Plants, Fruits and Seeds (Regulation of Import into India) Order (PFS Order) first promulgated in 1984 was revised in 1989. The PFS Order was further revised in the light of World Trade Organization (WTO) agreements, and the Plant Quarantine (Regulation of Import into India) Order 2003 (hereafter referred to as PQ Order), came into force on January 1, 2004 to comply with the Sanitary and Phytosanitary Agreement (Khetarpal et al. 2006). A number of amendments of the PQ Order were notified, revising definitions and clarifying specific queries raised by quarantine authorities of various countries, with revised lists of crops under Schedules VI and VII and quarantine weed species under Schedule VIII. The revised list under Schedules VI and VII now includes 699 and 519 crops/commodities, respectively, and Schedule VIII now includes 57 quarantine weed species. Schedule IV includes 15 crops and countries from where import is prohibited along with the name of pest(s). The PQ Order ensures the incorporation of 'Additional/Special Declarations' for import commodities free from quarantine pests, on the basis of pest risk analysis (PRA) following international norms, particularly for seed/planting material (http://www.

agricoop.nic.in/gazette.htm). The Agriculture Biosecurity Bill was discussed in Lok Sabha in March 2013.

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture and Farmers' Welfare is responsible for enforcing quarantine regulations and for quarantine inspection and disinfestation of agricultural commodities. The quarantine processing of bulk consignments of grain/pulses, etc. for consumption and seed/planting material for sowing is undertaken by the 70 Plant Quarantine Stations located in different parts of the country, and many pests were intercepted in imported consignments (Sushil 2016; http://ppqs.gov.in/divisions/plant-quarantine/strengthening-modernisation-plant-quarantine-facilities-india). Import of bulk material for sowing/planting purposes is authorized only through seven Regional Plant Quarantine Stations. There are 42 designated inspection authorities who inspect the consignment being grown in isolation in different parts of the country. Besides, DPPQS has developed 22 standards on various phytosanitary issues such as on PRA, pest-free areas for fruit flies and stone weevils, certification of facilities for treatment of wood packaging material and methyl bromide fumigation. Also, two standard operating procedures have been notified on export inspection and phytosanitary certification of plants/plant products and other regulated articles and **post-entry quarantine inspection** (www.plantquarantineindia.org/standards.htm).

The following are the seed-borne pathogens (fungi, bacteria, viruses and viroids) of quarantine significance for India which are not reported from India/included in the PQ Order 2003 as regulated pests (Chalam et al. 2005b, 2012d, 2013a; Dev et al. 2005; Gupta et al. 2013; http://plantquarantineindia.nic.in/pqispub/html/PQO_amendments.htm):

1.3.2.1 Fungi

1. *Acremonium strictum* (acremonium wilt)
2. *Ascochyta abelmoschi* (leaf spot)
3. *Ascochyta fabae* (leaf and pod spot)
4. *Aspergillus wentii*
5. *Beltrania* sp.
6. *Blumeriella jaapii* (cherry leaf spot)
7. *Botryosphaeria dothidea*
8. *Ceratobasidium cereale* (sharp eyespot of cereals)
9. *Cercospora abelmoschi*
10. *Cercospora apii*
11. *Cercospora elaeidis* (freckle)
12. *Cladosporium caryigenum*
13. *Cladosporium geniculata*
14. *Claviceps gigantea* (ergot)
15. *Claviceps purpurea* (ergot)
16. *Colletotrichum antirrhini*
17. *Colletotrichum coffeanum* var. *virulens* (coffee berry disease)
18. *Colletotrichum gossypii* var. *cephalosporioides* (witches' broom of cotton)

19. *Colletotrichum hibisci* (anthracnose)
20. *Colletotrichum higginsianum*
21. *Colletotrichum linicola* (anthracnose)
22. *Colletotrichum violaetricoloris* (anthracnose)
23. *Cristulariella moricola* (zonate leaf spot)
24. *Diaporthe phaseolorum* var. *caulivora* (stem canker)
25. *Dibotryon morbosum* (black knot)
26. *Didymella chrysanthemi* (ray blight)
27. *Drechslera maydis* race T (southern corn blight)
28. *Elsinoë phaseoli* (scab)
29. *Embellisia allii*
30. *Eutypa armeniacae* (gummosis)
31. *Fusarium culmorum* (culm rot: cereals)
32. *Fusarium oxysporum* f. sp. *apii*
33. *Fusarium oxysporum* f. sp. *callistephi* (wilt)
34. *Fusarium oxysporum* f. sp. *cucumerinum* (fusarium wilt)
35. *Fusarium oxysporum* f. sp. *elaeidis* (vascular wilt)
36. *Fusarium oxysporum* f. sp. *lagenariae* (bottle gourd wilt)
37. *Fusarium oxysporum* f. sp. *matthiolae* (wilt)
38. *Fusarium oxysporum* f. sp. *passiflorae* (base rot disease of passionfruit)
39. *Fusarium oxysporum* f. sp. *phaseoli* (wilt of bean)
40. *Fusarium culmorum* (culm rot: cereals)
41. *Gaeumannomyces graminis* var. *graminis* (crown sheath rot)
42. *Gloeotinia granigena* (blind seed disease: grasses)
43. *Graphium* sp.
44. *Grovensinia pyramidalis* (zonate leaf spot of Indian jujube)
45. *Heterobasidion annosum*
46. *Heteropatella antirrhini*
47. *Kabatiella caulivora* (northern anthracnose)
48. *Kabatiella zeae* (anthracnose)
49. *Leptosphaeria maculans* (black leg)
50. *Marssonina panottoniana* (anthracnose)
51. *Marasmiellus cocophilus*
52. *Monilinia fructicola* American strain (brown rot)
53. *Monilinia laxa* (blossom blight and fruit rot)
54. *Moniliophthora perniciosa* (witches' broom disease of cacao)
55. *Moniliophthora roreri*
56. *Monographella nivalis* (foot rot of cereals)
57. *Mycena citricolor*, syn. *Omphalia flavida* (American leaf spot of coffee)
58. *Mycocentrospora acerina* (anthracnose of caraway, halo blight)
59. *Mycosphaerella zeae-maydis*
60. *Nectria radicicola* (black root)
61. *Nodulisporium* sp.
62. *Periconia circinata* (milo disease)
63. *Peronospora dianthi* (downy mildew)

64. *Peronospora dianthicola* (downy mildew)
65. *Peronospora farinosa* (downy mildew)
66. *Peronospora hyoscyami* f. sp. *tabacina* (angular tobacco leaf spot)
67. *Peronospora manshurica* (downy mildew)
68. *Peronospora tabacina* (blue mould of tobacco)
69. *Pezicula alba*
70. *Phaeoacremonium aleophilum*
71. *Phaeosphaeria avenaria* f. sp. *avenaria* (leaf spot of oats)
72. *Phakopsora meibomiae* (soybean rust)
73. *Phoma andigena*
74. *Phoma matthiolicola* (leaf spot)
75. *Phomopsis longicolla* (*Phomopsis* seed decay, pod and stem blight)
76. *Phomopsis sclerotioides* (black spot)
77. *Phyllosticta antirrhini*
78. *Phymatotrichopsis omnivora*
79. *Physopella zeae* (tropical rust)
80. *Phytomonas staheli* (fatal wilt or hart rot)
81. *Phytophthora cryptogea* (tomato foot rot)
82. *Phytophthora katsurae* (chestnut downy mildew)
83. *Phytophthora megakarya* (black pod of cocoa)
84. *Phytophthora megasperma* var. *sojae* (root and stem rot)
85. *Phytophthora phaseoli* (downy mildew of lima bean)
86. *Phytophthora sojae* (*Phytophthora* root and stem rot)
87. *Plasmopara halstedii* (downy mildew)
88. *Pleiochaeta setosa* (lupin leaf spot)
89. *Pleospora herbarum* (leaf blight of onion)
90. *Puccinia antirrhini*
91. *Pyrenochaeta lycopersici* (brown rot of tomato)
92. *Pyrenopeziza medicaginis* (yellow leaf blotch)
93. *Pyrenophora dictyoides* (net blotch of fescues, *Festuca* spp.)
94. *Pythium spinosum* (root rot)
95. *Pythium tracheiphilum* (bottom rot of lettuce)
96. *Ramularia lacteal* (white spot)
97. *Rhizopus* sp.
98. *Sclerotinia borealis* (snow blight of grass)
99. *Sclerotinia homoeocarpa* (dollar spot: grasses)
100. *Sclerotinia minor* (*Sclerotinia* disease of lettuce, *Sclerotinia* rot)
101. *Sclerotinia trifoliorum* (*Sclerotinia* wilt)
102. *Septoria callistephi* (leaf spot)
103. *Septoria cucurbitarum* (*Septoria* leaf spot)
104. *Septoria tageticola* (leaf spot)
105. *Sorosporium spaonariae* (smut)
106. *Sphaceloma violae* (scab)
107. *Sphaceloma arachidis* (scab of groundnut)
108. *Stemphylium callistephi* (leaf spot)

109. *Tilletia controversa* (dwarf bunt of wheat)
110. *Urocystis cepulae*
111. *Urocystis violae* (smut)
112. *Uromyces dianthi* (rust)
113. *Venturia carpophila* (scab)
114. *Venturia cerasi (scab)*
115. *Verticillium albo-atrum*

1.3.2.2 Bacteria

1. *Acidovorax avenae* subsp. *avenae* (bacterial blight)
2. *Acidovorax avenae* subsp. *citrulli* (bacterial fruit blotch of watermelon)
3. *Burkholderia andropogonis* (bacterial blight, bacterial leaf stripe of sorghum and corn)
4. *Burkholderia glumae*
5. *Burkholderia plantarii*
6. *Burkholderia solanacearum* African strains (bacterial wilt of groundnut)
7. *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker)
8. *Clavibacter michiganensis* subsp. *nebraskensis* (Nebraska wilt)
9. *Clavibacter michiganensis* subsp. *sepedonicus*
10. *Corynebacterium michiganense* pv. *insidiosum* (bacterial wilt)
11. *Curtobacterium flaccumfaciens* pv. *betae* (silvering disease)
12. *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Bacterial wilt)
13. *Erwinia rhapontici* (*Pectobacterium rhapontici*) (rhubarb crown rot)
14. *Pantoea agglomerans*
15. *Pantoea stewartii* (bacterial leaf blight of maize)
16. *Pantoea stewartii* subsp. *stewartii* (Stewart's wilt)
17. *Phyllosticta impatiens*
18. *Pseudomonas ananas*
19. *Pseudomonas atrofaciens* (spike rot of wheat)
20. *Pseudomonas cepacia*
21. *Pseudomonas cichorii* (bacterial blight, leaf spot of coffee)
22. *Pseudomonas fuscovaginae* (bacterial rot of rice sheaths, sheath brown rot of rice)
23. *Pseudomonas glumae* (seedling rot of rice)
24. *Pseudomonas marginalis* pv. *marginalis*
25. *Pseudomonas passiflora*
26. *Pseudomonas putida*
27. *Pseudomonas savastanoi* pv. *phaseolicola* (halo blight of beans)
28. *Pseudomonas syringae* pv. *aptata* (bacterial blight)
29. *Pseudomonas syringae* pv. *atrofaciens* (basal: wheat glume rot)
30. *Pseudomonas syringae* pv. *atropurpurea*
31. *Pseudomonas syringae* pv. *coronafaciens* (chocolate spot of maize, halo blight)
32. *Pseudomonas syringae* pv. *delphinii* (leaf spot)
33. *Pseudomonas syringae* pv. *lachrymans* (angular leaf spot)
34. *Pseudomonas syringae* pv. *maculicola* (bacterial leaf spot, cabbage leaf spot)

35. *Pseudomonas syringae* pv. *persicae* syn. *P. morsprunorum* (bacterial dieback of peach)
36. *Pseudomonas syringae* pv. *primulae* (leaf spot)
37. *Pseudomonas syringae* pv. *punctulens* (bacterial pustule)
38. *Pseudomonas syringae* pv. *striafacians*
39. *Pseudomonas syringae* pv. *tabaci* (wildfire)
40. *Pseudomonas syringae* pv. *tagetis* (bacterial: *Tagetes* spp. leaf spot)
41. *Pseudomonas syringae* pv. *tomato* (bacterial leaf spot)
42. *Pseudomonas syringae* pv. *striafacians*
43. *Pseudomonas syringe* pv. *atrofaciens* (glume rot)
44. *Pseudomonas syringe* pv. *garcae* (halo blight of coffee)
45. *Pseudomonas viridiflava* (bacterial leaf blight of tomato)
46. *Rhizobium rhizogenes* (gall)
47. *Xanthomonas arboricola* pv. *corylina* (hazelnut blight)
48. *Xanthomonas axonopodis* pv. *vitians* (leaf spot)
49. *Xanthomonas campestris* pv. *campestris* (black rot)
50. *Xanthomonas campestris* pv. *incanae*
51. *Xanthomonas campestris* pv. *raphani* (*Raphanus* leaf spot)
52. *Xanthomonas campestris* pv. *malvacearum* African strain (bacterial blight of cotton)
53. *Xanthomonas campestris* pv. *pelargonii* (bacterial spot)
54. *Xanthomonas campestris* pv. *raphani* (leaf spot)
55. *Xanthomonas hortorum* pv. *carotae* (bacterial blight of carrot)
56. *Xanthomonas melonis* (soft rot)
57. *Xanthomonas translucens* pv. *translucens* (bacterial leaf streak)
58. *Xanthomonas vasicola* pv. *holcicola* (bacterial leaf streak)
59. *Xanthomonas vesicatoria* (bacterial scab)
60. *Xylella fastidiosa* (Pierce's disease of grapevines)

1.3.2.3 Viruses

1. *Alfalfa mosaic virus* (AMV)
2. *Arabis mosaic virus* (ArMV)
3. *Artichoke yellow ringspot virus* (AYRSV)
4. *Asparagus virus 1* (AV-1)
5. *Asparagus virus 2* (AV-2)
6. *Barley stripe mosaic virus* (BSMV)
7. *Bean mild mosaic virus* (BMMV)
8. *Bean pod mottle virus* (BPMV)
9. *Bean yellow mosaic virus* (BYMV)
10. *Blueberry leaf mottle virus* (BLMoV)
11. *Broad bean mottle virus* (BBMV)
12. *Broad bean stain virus* (BBSV)
13. *Broad bean true mosaic virus* (BBTMV)
14. *Broad bean wilt virus* (BBWV)

15. *Cacao swollen shoot virus* (CSSV)
16. *Carnation cryptic virus 1* (CCV-1)
17. *Cherry leaf roll virus* (CLRV)
18. *Cherry rasp leaf virus* (CRLV)
19. *Citrus leaf blotch virus* (CLBV)
20. *Clover yellow mosaic virus* (ClYMV)
21. *Cocoa necrosis virus* (CoNV)
22. *Coffee ringspot virus* (CoRSV)
23. *Cowpea mottle virus* (CPMoV)
24. *Cowpea severe mosaic virus* (CPSMV)
25. *Elm mottle virus* (EMoV)
26. *Grapevine Algerian latent virus* (GALV)
27. *Grapevine chrome mosaic virus* (GCMV)
28. *Grapevine fan leaf virus* (GFLV)
29. Grapevine line pattern virus (GLPV)
30. *Grapevine rupestris stem pitting-associated virus* (GRSPaV)
31. *High plains virus* (HPV)
32. *Lettuce mosaic virus* (LMV)
33. *Lucerne Australian latent virus* (LALV)
34. *Maize chlorotic mottle virus* (MCMV)
35. *Melon necrotic spot virus* (MNSV)
36. *Mulberry ringspot virus* (MRSV)
37. Odontoglossum ringspot virus
38. Papaya meleira virus (PMeV)
39. *Pea early-browning virus* (PEBV)
40. *Pea enation mosaic virus* (PEMV)
41. *Peach rosette mosaic virus* (PRMV)
42. *Peanut stripe virus* (PStV)
43. *Peanut stunt virus* (PSV)
44. *Pepino mosaic virus* (PepMV)
45. *Pepper mild mottle virus* (PMMoV)
46. *Prune dwarf virus* (PDV)
47. *Raspberry bushy dwarf virus* (RBDV)
48. *Raspberry ringspot virus* (RpRSV)
49. *Red clover mosaic virus* (RCIMV)
50. *Red clover vein mosaic virus* (RCVMV)
51. *Satsuma dwarf virus* (SDV)
52. *Sowbane mosaic virus* (SoMV)
53. *Squash mosaic virus* (SqMV)
54. *Strawberry latent ringspot virus* (SLRSV)
55. Sunflower crinkle virus (SuCV)
56. *Sunn-hemp mosaic virus* (SHMV)
57. *Tobacco mosaic virus* (TMV)
58. *Tobacco necrosis virus* (TNV)
59. *Tobacco rattle virus* (TRV)

60. *Tobacco ringspot virus* (TRSV)
61. *Tobacco streak virus* (TSV)
62. *Tomato aspermy virus* (TAV)
63. *Tomato black ring virus* (TBRV)
64. *Tomato bushy stunt virus* (TBSV)
65. *Tomato mosaic virus* (ToMV)
66. *Tomato ringspot virus* (ToRSV)
67. *Turnip yellow mosaic virus* (TYMV)
68. *Vicia cryptic virus* (VCV)
69. *Wheat streak mosaic virus* (WSMV)
70. *Zucchini yellow mosaic virus* (ZYMV)

1.3.2.4 Viroids

1. *Australian grapevine viroid* (AGVd)
2. *Avocado sunblotch viroid* (ASBVd)
3. *Chrysanthemum stunt viroid* (CSVd)
4. *Coconut cadang-cadang viroid* (CCCVd)
5. *Coconut tinangaja viroid* (CTiVd)
6. *Grapevine yellow speckle viroid* (GYSVd)
7. *Potato spindle tuber viroid* (PSTVd)

The ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), the nodal institution for exchange of plant genetic resources (PGR), has been empowered under the PQ Order to handle quarantine processing of germplasm including transgenic planting material imported for research purposes into the country by both public and private sectors. ICAR-NBPGR has developed well-equipped laboratories and post-entry quarantine greenhouse complex. Keeping in view the biosafety requirements, National Containment Facility of level-4 (CL-4) has been established at NBPGR to ensure that no viable biological material/pollen/pathogen enters or leaves the facility during quarantine processing of transgenics. Till date, >16,000 samples of transgenic crops comprising *Arabidopsis thaliana*, *Brassica* spp., chickpea, corn, cotton, potato, rice, soybean, tobacco, tomato and wheat with different traits imported into India for research purposes were processed for quarantine clearance, wherein they are tested for associated exotic pests, if any, and also for ensuring the absence of terminator gene technology (embryogenesis deactivator gene) which are mandatory legislative requirements. At ICAR-NBPGR, some of the important pathogens intercepted include fungi *Fusarium nivale*, *Peronospora manshurica* and *Uromyces betae* and bacterium *Xanthomonas campestris* pv. *campestris* (Bhalla et al. 2018b). In the last three decades by adopting a workable strategy such as PEQ growing in PEQ greenhouses/containment facility and inspection, PEQ inspection at indenter's site, electron microscopy, enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR), 45 viruses of great economic and quarantine importance have been intercepted in exotic germplasm including transgenics. The interceptions include 17 seed-transmitted viruses not yet reported from India, viz. *barley stripe mosaic*

virus (BSMV), *bean mild mosaic virus* (BMMV), *bean pod mottle virus* (BPMV), *broad bean mottle virus* (BBMV), *broad bean stain virus* (BBSV), *broad bean true mosaic virus* (BBTMV), *cherry leaf roll virus* (CLRV), *cowpea mottle virus* (CPMoV), *cowpea severe mosaic virus* (CPSMV), high plains virus (HPV), *maize chlorotic mottle virus* (MCMV), *pea enation mosaic virus* (PEMV), *peanut stunt virus* (PSV), *pepino mosaic virus* (PepMV), *raspberry ringspot virus* (RpRSV), *tomato ringspot virus* (ToRSV) and *wheat streak mosaic virus* (WSMV). Besides, 21 viruses not known to occur on particular host(s) in India have been intercepted, and these are also of quarantine significance for India. Twenty viruses have been intercepted in germplasm imported from CGIAR centres (Chalam 2016, 2014; Chalam et al. 2005a, 2007, 2008, 2009b, 2012a, b, d, 2013b, c, d, 2014a, b, c, 2015a, b, 2016, 2017, 2018; Chalam and Khetarpal 2008; Chalam and Maurya 2018; Khetarpal et al. 1992, 1994, 2001; Kumar et al. 1991; Parakh et al. 1994, 2005, 2006, 2008; Prasada Rao et al. 1990, 2004, 2012; Singh et al. 2003). Even though some of the intercepted viruses are not known to occur in India, their potential vectors exist and so also the congenial conditions for them to multiply, disseminate and spread the destructive exotic viruses/strains and even native strains more efficiently. The risk of introduction of 45 viruses or their strains into India was thus eliminated. All the plants infected by the viruses were uprooted and incinerated.

The infected samples were salvaged by using suitable techniques, and the disease-free germplasm was only used for further distribution and conservation. If not intercepted, some of the above quarantine pests could have been introduced into our agricultural fields and caused havoc to our productions. Thus, apart from eliminating the introduction of exotic pathogens from our crop improvement programmes, the harvest obtained from disease-free plants ensured conservation of pest-free exotic germplasm in the National Genebank.

1.3.3 National Scenario: Exports

The Directorate of Plant Protection, Quarantine and Storage (DPPQS) under the Ministry of Agriculture is responsible for enforcing quarantine regulations and for quarantine inspection and disinfestation of agri-horticultural commodities. All the material meant for export should be accompanied by phytosanitary certificate giving the details of the material and treatment in the model certificate prescribed under the IPPC of FAO. The Ministry of Agriculture, Government of India, has notified 161 officers to grant phytosanitary certificate for export of plants and plant materials.

The ICAR-NBPGR, the nodal institution for exchange of plant genetic resources (PGR), is vested with the authority to issue phytosanitary certificate for seed material and plant propagules of germplasm meant for export for research purposes after getting approval from DARE. NBPGR has developed well-equipped laboratories and greenhouse complex. ICAR-NBPGR undertakes detailed examination of germplasm meant for export for presence of various pests using general and pest-specific detection techniques and issues phytosanitary certificate giving the details of the material and treatment in the model certificate prescribed under the IPPC.

1.3.4 National Domestic Quarantine

Domestic quarantine or internal quarantine is aimed to prevent the spread of introduced exotic species or an indigenous key pest to clean (pest-free) areas within the country, and this has its provisions in the DIP Act, 1914, and is enforced by the notification issued by the central and state governments. More than 30 pest species seem to have been introduced into India, while notifications have been issued against the spread of nine introduced pests only, namely, fluted scale, San Jose scale, codling moth, coffee berry borer, potato wart disease, potato cyst nematode, *Apple mosaic virus*, BBTV and *banana mosaic virus* (Khetarpal et al. 2006). According to notifications issued under the DIP Act, an introduced pest, for example, BBTV, has been declared a pest in states of Assam, Kerala, Orissa, Tamil Nadu (TN), West Bengal (WB), and banana, which come out of these states, have to be accompanied by a health certificate from the state pathologist or other competent authorities that the plants are free from it. However, due to absence of domestic quarantine, BBTV has spread to most banana-growing areas in the country. The limitations and constraints of domestic quarantine include lack of basic information on the occurrence and distribution of major key pests in the country (in other words, pest distribution maps are lacking for most of the key pests); absence of concerted action and enforcement of internal quarantine regulations by the state governments; lack of interstate border quarantine checkposts at rail and road lines greatly added to the free movement of planting material across the states; lack of close cooperation and effective coordination between state governments and centre for timely notification of introduced pests, organizing pest detection surveys for delineating the affected areas and immediate launching of eradication campaigns in affected areas; lack of public awareness; lack of rapid diagnostic tools/kits for quick detection/identification of exotic pests at the field level; and lack of rigorous seed/stock certification or nursery inspection programmes to make available the pest-free seed/planting material for farmers (Bhalla et al. 2014).

There is a dire need to revisit the **existing domestic quarantine** scenario for strengthening interstate quarantine checkposts and eventually for monitoring movement of viruses of significance. Also, review and update the list of viruses to be regulated under domestic quarantine. For example, BBTV and banana mosaic virus (*cucumber mosaic virus*) need to be deleted as regulated pests under domestic quarantine as they are widely spread across the country.

The following viruses are known to occur only in certain parts of the country:

- *Indian citrus ringspot virus*: Known to occur in Haryana, Maharashtra (MH), Punjab and Rajasthan
- *Citrus mosaic virus*: Known to occur in Andhra Pradesh (AP), Karnataka and parts of TN
- *Tomato spotted wilt virus*: Reported from TN on Chrysanthemum
- *Banana bract mosaic virus*: Known to occur in AP, Karnataka, Kerala and TN
- *Arabis mosaic virus*: Known to occur in AP, Karnataka and parts of TN

- *Red clover vein mosaic virus*: Known to occur on rose in Palampur, Himachal Pradesh (HP)

Thus, there is a need to consider the above viruses and others for inclusion as regulated pests for domestic quarantine to prevent their spread to other parts of the country, and there is also a need to effectively implement domestic quarantine. India must develop organized services of plant quarantine at state level parallel to Australia and the USA.

1.3.5 The Agricultural Biosecurity Bill 2013

In order to meet the challenges of globalization and free trade, the Agricultural Biosecurity Bill 2013, was introduced in the Parliament of India on March 11, 2013. The main provisions of the Bill are to set up an autonomous authority encompassing the four sectors of agricultural biosecurity, viz. plant health, animal health, living aquatic resources (fisheries, etc.) and agriculturally important microorganisms. It provides for modernizing the legal framework to regulate safe movement of plants and animals within the country and in international trade and harmonize the legal requirements of the various sectors of agricultural biosecurity. The proposed legislation is expected to ensure agricultural biosecurity of the country for common benefit and for safeguarding the agricultural economy. The bill repeals DIP Act, 1914, and the Livestock Importation Act, 1898, and will give direct powers to the quarantine officers to deport or destroy or confiscate the consignment or lodge complaints under the Indian Penal Code.

The bill establishes the Agricultural Biosecurity Authority of India (Authority) having functions such as (1) regulating the import and export of plants, animals and related products; (2) preventing the introduction of quarantine pests from outside India; and (3) implementing post-entry quarantine measures. The administrative and technical control of existing Plant Quarantine Stations, Central Integrated Pest Management Centres and other laboratories under the DPPQS shall be transferred to and vested in the Authority (http://www.indiaenvironmentportal.org.in/files/file/Agricultural%20Biosecurity%20Bill.pdf).

1.4 Challenges in Diagnosis of Pathogens in Quarantine

The issues related to quarantine methodology were analyzed/reviewed by Khetarpal (2004). The challenge prior to import is preparedness for pest risk analysis (PRA). PRA is now mandatory for import of new commodities into India. The import permit will not be issued for the commodities not covered under Schedules V, VI and VII under the PQ Order. Hence, for import of new commodities in bulk for sowing/planting, the importer should apply to the Plant Protection Adviser to the Government of India for conducting PRA. In case of germplasm, import permit shall be

issued by the director, NBPGR, after conducting PRA based on international standards (http://agricoop.nic.in/Gazette/Psss2007.pdf).

The PRA process requires detailed information on pest scenario in both countries importing and exporting the commodity. Database on all pests, including information on host range, geographical distribution and strains, should be made available for its use as a ready reckoner by the scientists, extension workers and quarantine personnel. NBPGR has compiled pests of quarantine significance for cereals (Dev et al. 2005), grain legumes (Chalam et al. 2012c), oilseeds (Gupta et al. 2013) and tropical and sub-tropical fruit crops (Bhalla et al. 2018a). The Crop Protection Compendium of CAB International, the United Kingdom, is a useful asset to scan for global pest data (CAB International 2007).

As we face challenges to crops from intentional or unintentional introductions of pests, speed and accuracy of detection become paramount. Intense efforts are under way to improve detection techniques. The size of consignment received is very critical in quarantine from processing point of view. Bulk seed samples of seed lots need to be tested by drawing workable samples as per norms. The prescribed sampling procedures need to be followed strictly, and there is a need to develop/adapt protocols for batch testing, instead of individual seed analysis (Maury et al. 1985). On the other hand, germplasm samples are usually received as a few seeds/sample, and thus, it is often not possible to do sampling because of few seeds and also because of the fact that a part of the seed is also to be kept as voucher sample in the National Genebank in India apart from the pest-free part that has to be released. Hence, extreme precaution is needed to ensure that the result obtained in the test does not denote a false positive or a false negative sample. Removal of exotic viruses from germplasm by growing in PEQ greenhouses inevitably causes a delay in the release of seeds as it takes one crop season to release the harvest only from the indexed virus-free plants. Samples received after the stipulated sowing time would require the indenter to wait for another season. Non-destructive testing of the seeds could shorten this time, and therefore, more attention needs to be given to non-destructive techniques wherever possible (Khetarpal 2004; Chalam and Khetarpal 2008).

1.5 Perspectives

Detection and diagnosis of pathogens are crucial for application of mitigation strategies, seed trade and exchange of germplasm.

Need to develop the **Web-based information portal** for sharing diagnostics including database on diagnosticians.

The biotechnological interventions for detection of pathogens in quarantine assume great significance, but other areas need to be properly addressed. There is a need to have antisera for all the plant viruses in the quarantine laboratories to facilitate the interception of exotic viruses and their strains. Information access and exchange on diagnostics of plant pathogens and quarantine are crucial for effective working. Establishment of a **National Diagnostic Network for Plant Pests** including antisera bank, database of primers, seeds of indicator hosts, national DNA bank

of plant pests, lateral flow strips/dip sticks which can detect multiple pests, multiplex PCR/RT-PCR, real-time PCR/RT-PCR, loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HAD), microarray technology, DNA barcoding and, ultimately, a **national biosecurity chip** for diagnosis of all current threats to crop plants would be the backbone for strengthening the programme on plant quarantine. The *National Diagnostic Network for Plant Pests*, if established, can be a storehouse of information on biology of plant pests, diagnostic procedures and policies, international standards and related issues. Also, **Regional Working Groups of Experts for Detection and Identification of Plant Pests** thus needs to be formed to explore future cooperation in terms of sharing of expertise and facilities, for example, in South Asia where the borders are contiguous. This would help in avoiding the introduction of plant pests not known in the region and also the movement of plant pests within the region.

Adopting the reliable conventional, serological and molecular techniques with an appropriate strategy for the detection of pathogens would go a long way in ensuring the management through quarantine, disease-free trade and exchange of germplasm. Besides preventing the introduction of exotic pathogens, the role of the diagnostics especially advanced biotechnological interventions in certification of the planting material of the agri-horticultural crops against indigenous pathogens needs a great impetus in boosting our production and trade.

1.6. Appendix

1. ISPM 1: Principles of plant quarantine as related to international trade
2. ISPM 2: Guidelines for pest risk analysis
3. ISPM 3: Code of conduct for the import and release of exotic biological control agents
4. ISPM 4: Requirements for the establishment of pest-free areas
5. ISPM 5: Glossary of phytosanitary terms
6. ISPM 6: Guidelines for surveillance
7. ISPM 7: Export certification system
8. ISPM 8: Determination of pest status in an area
9. ISPM 9: Guidelines for pest eradication programmes
10. ISPM 10: Requirements for the establishment of pest-free places of production and pest-free production site
11. ISPM 11: Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms
12. ISPM 12: Guidelines for phytosanitary certificates
13. ISPM 13: Guidelines for the notification of non-compliance and emergency action
14. ISPM 14: The use of integrated measure in a systems approach for pest risk management

15. ISPM 15: Guidelines for regulating wood packaging material in international trade
16. ISPM 16: Regulated non-quarantine pests: concept and application
17. ISPM 17: Pest reporting
18. ISPM 18: Guidelines for the use of irradiation as a phytosanitary measure
19. ISPM 19: Guidelines on list of regulated pests
20. ISPM 20: Guidelines for phytosanitary import regulatory system
21. ISPM 21: Pest risk analysis for regulated non-quarantine pests
22. ISPM 22: Requirements for the establishment of areas of low pest prevalence
23. ISPM 23: Guidelines for inspection
24. ISPM 24: Guidelines for the determination and recognition of equivalence of phytosanitary measures
25. ISPM 25: Consignments in transit
26. ISPM 26: Establishment of pest-free areas for fruit flies (Tephritidae)
27. ISPM 27: Diagnostic protocols for regulated pests
28. ISPM 28: Phytosanitary treatments for regulated pests
29. ISPM 29: Recognition of pest-free areas and areas of low pest prevalence
30. ISPM 30: Establishment of areas of low pest prevalence for fruit flies (Tephritidae)
31. ISPM 31: Methodologies for sampling of consignments
32. ISPM-32: Categorization of commodities according to their pest risk
33. ISPM 33: Pest-free potato (*Solanum* spp.) micropropagative material and minitubers for international trade
34. ISPM 34: Design and operation of post-entry quarantine stations for plants
35. ISPM 35: Systems approach for pest risk management of fruit flies (Tephritidae)
36. ISPM 36: Integrated measures for plants for planting
37. ISPM 37: Determination of host status of fruit to fruit flies (Tephritidae)
38. ISPM 38. International movement of seeds
39. ISPM 39: International movement of wood
40. ISPM 40: International movement of growing media in association with plants for planting
41. ISPM 41: International movement of used vehicles, machinery and equipment
42. ISPM 42: Requirements for the use of temperature treatments as phytosanitary measures
43. ISPM 43: Requirements for the use of fumigation as a phytosanitary measure

References

Agricultural Biosecurity Bill (2013) Ministry of Agriculture and Farmers Welfare, Government of India. http://www.indiaenvironmentportal.org.in/files/file/Agricultural%20Biosecurity%20Bill.pdf

Arif M, Daniels J, Chalam VC, Fletcher J, Ochoa Corona FM (2012) Detection of *High plains virus* with loop-mediated isothermal amplification and qPCR. pp 7 In: 2012 American Phytopathological Society Meeting Abstracts of Presentations, August 4-8, 2012, Rhode Island, USA. Phytopathology 102(Suppl 4, Issue 7):S4.6. 168 p

Bhalla S, Chalam VC, Tyagi V, Lal A, Agarwal PC, Bisht IS (2014) Teaching manual on germplasm exchange and plant quarantine. National Bureau of Plant Genetic Resources, New Delhi. 340 p + viii

Bhalla S, Chalam VC, Khan Z, Singh B, Dubey SC (eds) (2018a) Potential quarantine pests for India in tropical and sub-tropical fruit crops. ICAR-National Bureau of Plant Genetic Resources, New Delhi. x+510 p. ISBN 978-81-937111-0-1

Bhalla S, Chalam VC, Singh B, Gupta K, Dubey SC (2018b) Biosecuring plant genetic resources in India: role of plant quarantine. ICAR-National Bureau of Plant Genetic Resources, New Delhi. 216 p + vi. ISBN 978-81-937111-1-8

Bhat AI, Siljo A, Deeshma KP (2013) Rapid detection of *Piper yellow mottle virus* and *Cucumber mosaic virus* infecting black pepper (*Piper nigrum*) by loop-mediated isothermal amplification (LAMP). J Virol Methods 193:190–196

Boonham N, Tomlinson J, Mumford R (2007) Microarrays for rapid identification of plant viruses. Annu Rev Phytopathol 45:307–328

Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J, Mumford R (2014) Methods in virus diagnostics: from ELISA to next generation sequencing. Virus Res 186:20–31

CAB International (2007) Crop protection compendium. CAB International, Wallingford

Chalam VC (2014) Biotechnological interventions for biosecuring Indian agriculture from transboundary plant viruses. In: Mishra SK (ed) Current trends and future challenges in biotechnology and biomedicine for human welfare and sustainable development. Part II. Department of Botany & Biotechnology, Govt. New Science College, Rewa, pp 16–24. 163 p

Chalam VC (2016) Challenges for plant virus diagnosis in certification and transboundary movement of planting material. In: Chalam VC, Akhtar J, Dubey SC (eds) Souvenir and Abstracts of Zonal Annual Meeting of Delhi Zone, Indian Phytopathological Society (IPS) & National Symposium on "Biosecurity in Food Value Chain", February 20, 2016, Delhi Zone. IPS and ICAR-National Bureau of Plant Genetic Resources, New Delhi, pp 5–17. 76 p

Chalam VC, Khetarpal RK (2008) A critical appraisal of challenges in exclusion of plant viruses during transboundary movement of seeds. Indian J Virol 19:139–149

Chalam VC, Maurya AK (2018) Role of quarantine in ensuring biosecurity against transboundary plant viruses. Agric Res J 55(4):612–626

Chalam VC, Pappu HR, Druffel KL, Khetarpal RK (2004) Detection of potyviruses in legume seeds by RT-PCR and real-time RT-PCR. In: Proceedings of National Symposium on Molecular Diagnostics for the Management of Viral Diseases, October 14-16, 2004. Indian Agricultural Research Institute and Indian Virological Society, New Delhi

Chalam VC, Khetarpal RK, Parakh DB, Maurya AK, Jain A, Singh S (2005a) Interception of seed-transmitted viruses in French bean germplasm imported during 2002-03. Indian J Plant Prot 33:134–138

Chalam VC, Parakh DB, Singh S, Khetarpal RK (2005b) Viruses of quarantine significance in cereals. In: Dev U, Khetarpal RK, Agarwal PC, Lal A, Kapur ML, Gupta K, Parakh DB (eds) Potential quarantine pests for India: cereals. National Bureau of Plant Genetic Resources, New Delhi, pp 82–92. 144 p

Chalam VC, Khetarpal RK, Prasada Rao RDVJ, Parakh DB, Maurya AK (2007) Transboundary movement of plant viruses: a case study of India. Indian J Virol 19(1):97–98

Chalam VC, Parakh DB, Khetarpal RK, Maurya AK, Jain A, Singh S (2008) Interception of seed-transmitted viruses in cowpea and mungbean germplasm imported during 2003. Indian J Virol 19:12–16

Chalam VC, Parakh DB, Khetarpal RK, Maurya AK, Pal D (2009a) Interception of seed-transmitted viruses in broad bean (*Vicia faba* L.) germplasm imported into India during 1996-2006. Indian J Virol 20:83–87

Chalam VC, Bhalla S, Gupta K, Singh B, Rajan, Goswami S, Chhabra R, Maurya AK, Randhawa GJ, Kapur ML, Khetarpal RK (2009b) Methodology for processing transgenic germplasm in quarantine. In: Tyagi RK, Mal B, Sharma SK, Khetarpal RK, Brahmi P, Singh N, Tyagi V, Archak S, Gupta K, Agarwal A (eds) National Symposium on Recent Developments in the

Management of Plant Genetic Resources, December 17–18, 2009. Souvenir and Abstracts. Indian Society of Plant Genetic Resources, New Delhi, pp 320–321. 370 p

Chalam VC, Arif M, Caasi DR, Fletcher J, Ochoa-Corona FM (2012a) Discrimination among CLRV, GFLV and ToRSV using multiplex RT-PCR. Phytopathology 102(Suppl 4):21

Chalam VC, Arif M, Fletcher J, Ochoa-Corona FM (2012b) Detection of *Bean pod mottle virus* using RT-PCR, RT-qPCR and isothermal amplification. Phytopathology 102(Suppl 7):S4.20

Chalam VC, Bhalla S, Singh B, Rajan (2012c) Potential quarantine pests for India in grain legumes. National Bureau of Plant Genetic Resources, New Delhi. 324 p + xii

Chalam VC, Parakh DB, Kaur H, Kumar A, Maurya AK, Khetarpal RK (2012d) Viruses of quarantine significance in grain legumes. In: Chalam VC, Bhalla S, Singh B, Rajan (eds) Potential quarantine pests for India in grain legumes. National Bureau of Plant Genetic Resources, New Delhi, pp 174–211. 324 p + xii

Chalam VC, Parakh DB, Kumar A, Maurya AK (2013a) Viruses, viroids and phytoplasma of quarantine significance in edible oilseeds. In: Gupta K, Singh B, Akhtar J, Singh MC (eds) Potential quarantine pests for India in edible oilseeds. National Bureau of Plant Genetic Resources, New Delhi, pp 175–212. 295 p + vii

Chalam VC, Parakh DB, Maurya AK, Khetarpal RK, Agarwal PC (2013b) Quarantine certification of seed and other planting material for viruses. In: Proceedings of Asia Pacific Congress of Virology, VIROCON 2013, December 17–20, 2013. Amity University, Noida, pp 67–69. 221 p

Chalam VC, Parakh DB, Sharma R, Maurya AK (2013c) *Maize chlorotic mottle virus*: a quarantine pest for India. Technical bulletin. National Bureau of Plant Genetic Resources, New Delhi. 4 p

Chalam VC, Parakh DB, Sharma R, Maurya AK (2013d) *Wheat streak mosaic virus*: a quarantine pest for India. Technical bulletin. National Bureau of Plant Genetic Resources, New Delhi. 4 p

Chalam VC, Parakh DB, Maurya AK (2014a) Biosecurity umbrella for Indian agriculture against exotic plant viruses: a case study of quarantine of exotic germplasm. In: Proceedings of XXIII National Conference (VIROCON 2014) on Recent trends in Virology Research in the Omics Era, December 18–20 2014. Tamil Nadu Agricultural University, Coimbatore

Chalam VC, Parakh DB, Maurya AK, Singh S, Khetarpal RK (2014b) Biosecuring India from seed-transmitted viruses: the case of quarantine monitoring of legume germplasm imported during 2001-2010. Indian J Plant Prot 42(3):270–279

Chalam VC, Parakh DB, Sharma R, Maurya AK (2014c) High plains virus: a quarantine pest for India. Technical bulletin. National Bureau of Plant Genetic Resources, New Delhi. 4 p

Chalam VC, Parakh DB, Maurya AK (2015a) Biosecuring Indian agriculture from transboundary plant viruses: biotechnological interventions and phytosanitary issues. In: Proceedings of National Symposium on Challenges and Management Approaches for Crop Diseases of National Importance - Status and Prospects, February 12–14, 2015. TNAU, Madurai

Chalam VC, Parakh DB, Sharma R, Maurya AK (2015b) *Barley stripe mosaic virus*: a quarantine pest for India. Technical bulletin. National Bureau of Plant Genetic Resources, New Delhi. 4 p

Chalam VC, Khetarpal RK, Agarwal PC (2016) Addressing plant health management using diagnostics and certification issues. In: Katti G, Kodaru A, Somasekhar N, Laha GS, Sarath Babu B, Varaprasad KS (eds) Plant health management for food security issues and approaches. Astral International Pvt. Ltd., New Delhi, pp 39–59. 230 p

Chalam VC, Parakh DB, Maurya AK (2017) Role of viral diagnostics in quarantine for plant genetic resources and preparedness. Indian J Plant Genet Resour 30:271–285

Chalam VC, Parakh DB, Maurya AK, Priyadarshini C, Tripathi A, Sharma R (2018) Viruses, viroids, phytoplasma and spiroplasma of quarantine significance in tropical and sub-tropical fruit crops. In: Bhalla S, Chalam VC, Khan Z, Kandan A, Dubey SC (eds) Potential quarantine pests for India in tropical and sub-tropical fruit crops. ICAR-National Bureau of Plant Genetic Resources, New Delhi, pp 277–373. 510 p

Chalam VC, Dubey SC, Murali Krishna C, Bhalla S, Singh K (eds) (2018) Transboundary movement of living modified organisms: strengthening capacities of enforcement agencies. ICAR-National Bureau of Plant Genetic Resources and Ministry of Environment, Forest and Climate Change, New Delhi. vi + 159 p. ISBN 978-81-937111-2-5

Dev U, Khetarpal RK, Agarwal PC, Lal A, Kapur ML, Gupta K, Parakh DB (eds) (2005) Pests of quarantine significance in cereals. National Bureau of Plant Genetic Resources, New Delhi. 142 p

Derrick KS (1973) Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56:652–653

FAO (2019) Glossary of phytosanitary terms. International standard for phytosanitary measures no. 5. Published by FAO on behalf of the Secretariat of the International Plant Protection Convention (IPPC), Rome. 35 p. Licence: CC BY-NC-SA 3.0 IGO

Gupta K, Singh B, Akhtar J, Singh MC (eds) (2013) Potential quarantine pests for India in edible oilseeds. National Bureau of Plant Genetic Resources, New Delhi. 295 p + vii

Hadidi A, Flores R, Candresse T, Barba M (2016) Next-generation sequencing and genome editing in plant virology. Front Microbiol 7:1–12. https://doi.org/10.3389/fmicb.2016.01325

Khetarpal RK (2004) A critical appraisal of seed health certification and transboundary movement of seeds under WTO regime. Indian Phytopathol 57:408–421

Khetarpal RK, Parakh DB, Singh S, Nath R (1992) ELISA detection of soybean mosaic virus in testas, embryos and seedlings from mottled and unmottled seeds of imported soybean germplasm. Indian J Virol 8(2):106–110

Khetarpal RK, Parakh DB, Singh S, Nath R, Jain RK, Varma A (1994) Bean common mosaic virus detected by DAC-Indirect ELISA in exotic *Phaseolus vulgaris* L. Indian J Virol 10:13–16

Khetarpal RK, Singh S, Parakh DB, Maurya AK, Chalam VC (2001) Viruses intercepted in exotic germplasm during 1991-2000 in quarantine. Indian J Plant Genet Resour 14:127–129

Khetarpal RK, Lal A, Varaprasad KS, Agarwal PC, Bhalla S, Chalam VC, Gupta K (2006) Quarantine for safe exchange of plant genetic resources. In: Singh AK, Srinivasan K, Saxena S, Dhillon BS (eds) Hundred years of plant genetic resources management in India. National Bureau of Plant Genetic Resources, New Delhi, pp 83–108

Kumar CA, Singh S, Parakh DB (1991) Detection of pea seed borne mosaic virus (PSbMV) in pea germplasm during quarantine processing. Indian Phytopathol 44:366–369

Maury Y, Duby C, Bossenec JM, Boudazin G (1985) Group analysis using ELISA: determination of the level of transmission of soybean mosaic virus in soybean seed. Agronomie 5:405–415

Parakh DB, Khetarpal RK, Singh S, Nath R (1994) Post entry quarantine detection of soybean mosaic virus by ELISA in soybean germplasm. Indian J Virol 10:17–21

Parakh DB, Chalam VC, Khetarpal RK, Maurya AK, Jain A, Singh S (2005) Interception of seed-transmitted viruses in soybean germplasm imported during 2003 and 2004. Indian J Plant Prot 33:119–124

Parakh DB, Chalam VC, Maurya AK, Khetarpal RK, Singh S, Kaur A (2006) Interception of *Pea seed-borne mosaic virus* in pea germplasm imported during 2003. Indian J Virol 17:35–38

Parakh DB, Khetarpal RK, Chalam VC (2008) Risk of seed-transmitted viruses associated with exchange of soybean germplasm and the South Asian scenario. Indian J Virol 19(1):47–49

Phan TTH, Khetarpal RK, Le TAH, Maury Y (1998) Comparison of immunocapture-PCR and ELISA in quality control of pea seed for pea seed borne mosaic potyvirus. In: Hutchins JD, Reeves JE (eds) Seed health testing: progress towards the 21st century. CAB International, Wallingford, pp 193–199

Plant quarantine (Regulation of Import into India) Order 2003 and amendments. Department of Agriculture and Cooperation, Ministry of Agriculture, India, http://www.agricoop.nic.in/gazette.htm

Prasada Rao RDVJ, Chakrabarty SK, Reddy AS (1990) Interception of Peanut stripe virus in imported groundnut seed from Myanmar. FAO Plant Prot Bull 38:48–49

Prasada Rao RDVJ, Reddy AS, Chakrabarty SK, Anitha K (2004) Interception of seed transmitted viruses in peanut germplasm imported into India during 1986 to 2003. Indian J Plant Prot 32:102–104

Prasada Rao RDVJ, Anitha K, Chakrabarty SK, Girish AG, Sarath Babu B, Rameash K, Abraham B, Varaprasad KS (2012) Quarantine pathogen interceptions on crop germplasm in India during 1986-2010 and their possible economic impact. Indian J Agric Sci 82(5):436–441

Siljo A, Bhat AI (2014) Reverse transcription loop-mediated isothermal amplification assay for rapid and sensitive detection of *Banana bract mosaic virus* in cardamom (*Elettaria cardamomum*). Eur J Plant Pathol 138:209–214

Siljo A, Bhat AI, Biju CN (2014) Detection of *Cardamom mosaic virus* and *Banana bract mosaic virus* in cardamom using SYBR Green based reverse transcription-quantitative PCR. Virus Dis 25:137–141

Singh B, Rajan, Bhalla S, Chalam VC, Pandey BM, Singh SK, Kumar N, Khetarpal RK (2003) Quarantine processing of imported transgenic planting material. Indian J Agric Sci 73 (2):97–100

Sushil SN (2016) Agricultural biosecurity system in India. In: Chalam VC, Akhtar J, Dubey SC (eds) Souvenir and abstracts of zonal annual meeting of Delhi zone, Indian Phytopathological Society (IPS) & National Symposium on "biosecurity in food value chain", February 20, 2016, Delhi zone. IPS and ICAR-National Bureau of Plant Genetic Resources, New Delhi, pp 26–35. 101 p

Application of Advanced Seed Production Techniques in Sugarcane Crop Improvement

2

Sangeeta Srivastava and Pavan Kumar

Abstract

Sugarcane is one of the socioeconomic crops of the world. The production of sugarcane in India is spread across the country and is divided into three productivity groups, viz., high, medium, and low, according to their productivity. The main cause of decreased production of sugarcane is reported to be the unhealthy seed as it affects seed cane multiplication rate. Various methods such as the spaced transplanting (STP) method, polybag technique, bud chip technique, cane node technique, and three-tier seed program have been developed. Besides tissue culture technique using meristem, artificial seed technology is also used to develop high seed multiplication as well as disease-free seed cane with high cane productivity. The Department of Biotechnology (DBT), Government of India, has established a National Certification System for Tissue Culture-raised Plants (NCS-TCP), where Accredited Test Laboratories (ATLs) play a crucial role in testing and certifying tissue culture-raised plants for virus indexing and genetic fidelity. A massive breeder's seed production program of improved sugarcane varieties has been launched under Mega Seed Project of Indian Council of Agriculture Research (ICAR), Government of India, to produce good quality planting material to be supplied to sugar mills every year.

Keywords

Spaced transplanting (STP) · Polybag technique · Bud chip technique · Three-tier seed program · Meristem culture

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2.1 Introduction

Sugarcane, a socioeconomic C-4 crop, belongs to Poaceae family and stores carbohydrate in the form of sucrose. Globally, it is cultivated at latitude between 36.7°N and 31.0°S in tropical and subtropical zones extending from equator. The sugarcane crop is grown in more than 100 countries of the world not only for sugar but also more recently for energy. It produces approximately 80% of the world's sugar to fulfill the demand of the growing population of the world, which is increasing day by day; that is, in the coming decades, more food and energy will be required along with high yield (FAOS 2017). In India, there are two agroclimatic zones for production of sugarcane, tropical and subtropical, of which the area of tropical zone is around 45% and yields 55%, while subtropical region accounts for 55% area and yields 45% of the total sugarcane produced.

2.1.1 Tropical Sugarcane Region

The tropical sugarcane region consists of peninsular zone and coastal zone, which includes the states of Maharashtra, Andhra Pradesh, Tamil Nadu, Karnataka, Gujarat, Madhya Pradesh, Goa, Pondicherry, and Kerala. In the coastal areas of Andhra Pradesh and Tamil Nadu, there is extensive sugarcane cultivation with high sugarcane productivity. The climatic conditions in tropical region are more or less ideal for its growth; hence, it contributes about 55% to the total cane production in the country. Abiotic stresses due to floods, water logging, and diseases such as red rot are the foremost problems. Moisture stress during the early part of the cane growth (from March to June) is an important problem. Smut and red rot affect sugarcane production in the plateau region and coastal areas, respectively. Besides, early shoot borer is a problem in the late planted crops.

2.1.2 Subtropical Sugarcane Region

The subtropical states of Uttar Pradesh (UP), Bihar, Haryana, Punjab Rajasthan, and West Bengal occupying around 55% of total cane area in the country come under this region. The area is characterized by extremes of climatic conditions. Sugarcane suffers due to floods and water logging during monsoon months in Eastern UP, Bihar, and West Bengal. The weather is very hot and dry, but July to October is rainy season, accounting for most of the rainfall from southwest monsoons. The months of November to March are cool with December and January being very cold, touching subzero levels at many places. Pests and diseases, particularly top borer, pyrilla, and red rot are serious problems. The cane yields are lower in the subtropics due to all the above factors.

2.1.3 Low Sugarcane Productivity: A Concern

The production of sugarcane in India is spread across the country. The major sugarcane-growing states, viz., Uttar Pradesh, Bihar, Assam, Haryana, Gujarat, Maharashtra, Karnataka, Andhra Pradesh, and Tamil Nadu, are classified into three groups according to their productivity. They are the high sugar-producing states (Maharashtra and Uttar Pradesh), medium sugar-producing states (Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, and Haryana), and low sugar-producing states (Bihar and Assam). It is well known that sugarcane crop productivity is greatly affected by biotic as well as abiotic stresses. More than 100 pathogens are known to cause various diseases in sugarcane (Rott et al. 2000), including fungi, bacteria, virus, phytoplasma, insect, and pest. Besides, the quality of seed and its multiplication ratio are also a determining factor for qualitative and quantitative yield of sugarcane throughout the world. Sugarcane is generally propagated via asexual or vegetative mode. It reproduces vegetatively through the three or two bud stem cuttings called cane setts. The application of genetically pure and disease-free seed of cane alone is usually able to improve the stalk yield by 10–15% in the field. Cane crop has low seed multiplication ratio (1:10), and as a result, it takes several years to develop plenty of seed stock of a newly released cane variety for cultivation over a large land area. Therefore, commercial production of sugarcane with high productivity in field basically depends on disease- and pest-free seed or planting material with rapid seed multiplication ratio. Biotechnology and advance field practicing method play an important role in enhancement of production of sugarcane as well as in countering the problems of seed production.

2.2 Sugarcane Seed Certification and Standards in India

2.2.1 The Seed Act

The Government of India passed the Seed Act in parliament in the year 1966; the draft was accepted in December 1966 for the first time by the Indian Standard Institution (ISI) and was published as ISI: 3866-1996, under the title "Specification for Sugarcane Seed Material." Minimum standard of seed certification in Section 8, under the Seed Act, 1966 has two initiatives: common seed certification and standards for all crops and specific seed certification and standards applicable to specific crop or group of crops. Subsequent orders or amendments like the Seed (Control) Order in 1983 and Seed Bill in 2004 are implemented to regulate the seed quality and sale for commercial purpose including export and import. While a number of sugarcane varieties have been notified through the Seed Act, thus far, there was no certification of sugarcane seed by any certification agency, perhaps because of its bulkiness and non-storability of the seed, which makes it complex to pack, seal, certify, and transport. To view this, a task force was constituted under the chairmanship of Dr. Kishan Singh (the former director of Indian Institute of Sugarcane Research (IISR), Lucknow) in 1978 to design the standards for seed cane. After

a series of dialogues and arguments, a draft was prepared and finalized. It was also published by the Indian Institute of Sugarcane Research (IISR) as "Specification for Sugarcane Seed Materials" in 1990. A review committee was framed by the Indian Council of Agriculture Research (ICAR) in 1999 for the field and seed standards of sugarcane planting material. In October 2001, the Technical Committee of Central Seed Certification Board gave the approval of this draft, and subsequently, it was notified by the Central Seed Certification Board (Karuppaiyan and Ram 2012; Shukla et al. 2017).

2.2.2 Seed Certification for Sugarcane

The objective of seed certification is to maintain the availability of quality seed to the public or farmer through a certification process. Under this process, high-quality seeds and planting material of notified type and varieties are grown and distributed with ensuring genetic identity and purity. Certification of seed cane is performed by a certification agency notified under section 8, as mentioned in the Seed Act, 1966.

2.2.3 Standards for Seed Cane

The approved seed cane standards together with seed certification standards recommended for sugarcane are as follows: the age of seed cane crop (for harvest regarding for seed purpose) shall be 6–8 months in tropical zone and 8–10 months in subtropical zone; physical purity of seed cane should be 95%; it should be undamaged as well as reasonably clean with one sound bud present on each node of seed cane (the number of nodes lacking bearing of sound bud shall not exceed 5.0% of the total number of buds present per seed cane); buds should be green, swollen, well protected, without any spot, and viability of buds to be not less than 80%; the number of buds, which have swollen up or projected ahead 1 cm from the ring surface, shall not exceed 5.0% (by number) of the total number of buds; seed canes should not hold aerial roots or nodal roots (relaxation up to 05% may be given in water logged area); minimum permissible limit for red rot, smut, wilt, and grassy shoot disease (GSD) is 0%, but for mosaic, mild strains are permitted.

2.2.4 Classes of Seed Certification in Sugarcane

Four classes of seed have been defined, which are certified by an official certification agency: nucleus seed, breeder seed, foundation seed, and certified seed.

- **Nucleus Seed:** Nucleus seed is known as the basic seed for production, which are source of breeder seed. These seeds have high percentage of genetic purity

(100%) and developed by research center or breeder who develops it. These seeds are exempted from certification by an agency.

- **Breeder Seed:** Breeder seed is produced from the nucleus seed by a breeder or in university or research center. Breeder seed in case of sugarcane referred as propagating materials (setts) is produced from nucleus seed, and high genetic purity (100%) is maintained in research center. Breeder seed is also exempted from certification.
- **Foundation Seed:** Foundation seed is produced from breeder seed under supervision of breeder or original sponsor, and this is a third-stage seed production in sugarcane. These seeds are produced in Government farm, sugar factory, and progressive farmer field. Foundation seed is certified by competent authority or concerned breeder or expert team for certified seed cane production.
- **Certified Seed:** Seeds produced from foundation seed are defined as certified seed. The foundation seed thus produced are handed over to the state departments/sugar factories for organizing the production of certified seed in the field of farmer. Regular inspection ensures genetic purity and health of seed crop. The state certification agencies will perform the regular inspection of certified seed plot for commercial purpose.

2.3 Sugarcane Seed Production

As a result of sugarcane breeding, true sugarcane seed "Fluff" is produced, which needs fast multiplication for field testing. Once the canes are multiplied from few clones developed from true sugarcane seeds, they are tested for the following aspects:

1. Adoptability under different agroclimatic conditions
2. Desirable economic traits
3. Yield potential and sucrose content
4. Disease and insect-pest tolerance resistance
5. Ratooning ability

The identified clones should have good germination ability and should be free from insect-pest and diseases. The clones having all the required characters are multiplied for different categories of seed, viz., breeder, foundation, registered, and certified. The production of quality seed canes is done in the following steps:

1. Selection of healthy and best quality seed canes
2. Seed treatment to check sett-borne diseases
3. Planting of setts in field at desired interval
4. Field inspection to rogue off-type and appearance of disease symptoms if any

2.4 Healthy Seed Cane Production Techniques

In sugarcane cultivation, seed cane or planting material is the major input price, amounting to Rs. 25,000–30,000 per hectare. There are various seed cane production techniques that are classified as field practicing methods along with tissue culture techniques for better growth, higher yields, and high crop quality. The spaced transplanting (STP) method, polybag technique, bud chip technique, and three-tier seed program are methods of healthy seed cane development. Other technique is via application of biotechnology that is known as in vitro propagation and is used to produce quality seed with rapid seed multiplication ratio as well as disease-free seed. Currently, tissue culture techniques have been widely adopted in countries such as Australia and the Philippines and even also in India and in Asia-Pacific region for commercial disease-free planting material and high-quality seed.

2.4.1 Spaced Transplanting (STP) Technique

The spaced transplanting (STP) technique, a cost-effective technology, was developed by ICAR-IISR (Indian institute of Sugarcane Research), Lucknow (http://www.iisr.nic.in/research/technologies.htm). In this technique, the synchronization of tillering and rapid seed multiplication of sugarcane have been achieved *via* raising settling in small area in nursery prior to actual transplant in field. Raising settling nursery and transplantation of settlings are two key basic steps of the STP technique. In the STP technique, first of all, disease-free single setts are prepared to raise nursery by dipping in 0.2% carbendazim for 5 min and then planted vertically in the nursery bed of 3 ft width and length as desired. Chlorpyrifos (1 mL for 1 L of water) treatment is applied on nursery beds. The setts are covered with loose soil and irrigated immediately. Regular weeding and pouring of water is crucially important for good germination and weed-free nursery. Six- to 7-week-old settlings are used to transplant vertically in fertilized field with irrigated furrows. Transplantation should be done preferably in the evening. In this technique, seed multiplication ratio increases from 1:10 to 1:40 of sugarcane, and it is widely adopted to improve cane yield in neighboring countries of India. This technology is also important in case of late planting condition in subtropical India (Shukla et al. 2017).

2.4.2 Polybag Technique

Use of settlings raised in polythene bags for a luxurious of sugarcane is known as polybag technique of sugarcane cultivation. The technique apparently resembles spaced transplanting (STP) technique; however, there are many differences between polybag technique and STP as settlings are raised in polythene bags instead of nursery beds, only one irrigation is required instead of two to three in STP, and posttransplanting mortality and weed problem are very low in polybag technique.

In this technique, plantation of single bud cane settling has been developed in perforated polythene bags (12 × 8 cm) containing a mixture of soil, sand, and organic manure (FYM/press mud) in ratio of 1:2:5 for rapid seed multiplication. Single bud cane prepared by manual cutting is treated with 0.1% carbendazim for 5 min and then planted vertically. The developed settlings are transplanted into fertilized field to give good yield of sugarcane. This method has several advantages such as the following: there is no need for nursery bed for raising settling, this method requires only 1.5–2.0 tons seed cane per hectare as compared to 6–7 tons seed cane per hectare in conventional sett planting, germination is 90–95%, and seed multiplication ratio is 1:40 over conventional method (1:10).

2.4.3 Short Crop Method

This is in practice in Java, where a well-fertilized and well-watered short-duration crop known as seed nursery is raised and harvested (first cut) after 6 months, followed by another cut after 6 months. In this method, the seed multiplication rate is 10–25 times as compared to conventional methods. This system is also followed in Anakapalle (Andhra Pradesh) area of India, where the seed cane is planted during February–March and harvested during August–September (about 6 month age) and replanted again in August to be harvested in February. This equals to 25–40 times of the area planted in spring in a period of 12–14 months. The entire stalk can be used as seed.

2.4.4 Bud Chip Method

The bud chip technology has higher seed multiplication ratio (1:60) as compared to conventional method (1:10) and therefore widely suitable for multiplication of newly released varieties. Small portion of cane node with single bud is called bud chip. These single buds along with nodal region are prepared from bud chipping machine. These buds are used to raise the settlings in cavity trays or perforated polythene bags. Before planting, bud chips are treated with chlorpyrifos and carbendazim for 5 min and incubated overnight in moist gunny bag. A mixture of equal amount of soil, sand, and FYM is filled in polythene bags, and then, the bud chips are planted in vertical position facing the bud up and are covered with soil mixture. Six- to 7-week-old settlings are ready to use for transplanting vertically in fertilized field. This technology has several advantages over conventional method such as less expensive, labor saving, higher bud germination rate (90%) and cane yield, easy transportation and handling of planted settlings, and 1 ton of seed cane is required for planting of 1 hectare area. There is also a limitation of this technology such as poor survival of bud chips under field condition due to lower food reserves (1.2–1.8 g sugar/bud) as compared to the three bud setts (6–8 g sugar/bud) (Jain et al. 2014; Mall et al. 2018). Samant (2017) reported improved cane yield using bud chip method over conventional method.

2.4.5 Cane Node Technology

In this technology, a cane node, having a bud along with root band after priming in organic slurry, is kept under decomposed farmyard manure having 60% moisture for 4–5 days. During this period, the buds get sprouted. It has 25% higher sugarcane production over the conventional method, space transplanting technique (STP), and bud chip. It has reduced cost of cultivation and plant material requirement, as the seed requirement is less than 1 tons per hectare seed. The multiplication ratio is to the tune of 1:40–1:60 over the traditional multiplication ratio of 1:10. The technology has the potential to break the yield barriers by raising the cane yield significantly.

2.4.6 Rayungan Method

Conventionally, sugarcane propagation through bud setts gets a long time. **Rayungan**, an Indonesian term, means a developed cane shoot with single sprouted bud (http://agropedia.iitk.ac.in/content/sugarcane-planting-methods). In this technology, a portion of field is selected for Rayungan production and is left at harvesting time. The seed stalks are decapitated (topped off) about 4–6 weeks prior to the planting time, and the lateral shoots develop into tailed Rayungan, which are cut off and planted out in the trenches made ready. At least 2–3 nodes remain underground (Sugiyarta and Winarsih 2009). Hence, by eliminating the upper Rayungans, the lower buds are made to sprout, and they are also likewise used.

2.4.7 Single Bud Sett Planting

Single bud sett planting method is also known as regulated planting (RPT) method. This method is a faster way of multiplication and spread of new variety for which planting material is insufficient. In this method, the stalk portions having nodes containing single bud in the center are planted directly in the main field, placed end to end with buds facing laterally, after dipping in 0.1% carbendazim solution. Recommended dose of fertilizers is applied (25% higher N than the recommended dose should be applied 1 month before harvest of seed crop). In this method, the germination is quicker and higher, and there is a saving of seed cane of about 40–50%. The crop stand is uniform with higher stalk yield.

2.5 Three-Tier Seed Program

Three-tier seed program has been developed for providing disease-free healthy seed to growers. This technique is based on moist hot air (MHA) treatment using the moist hot air (MHA) equipment, which was developed in the ICAR-IISR, Lucknow in 1977. MHA equipment has been installed in many sugarcane factories in India. This three-tier system consists of three stages: breeder seed nursery, foundation seed

nursery, and certified seed nursery under the proper monitoring and cultural practices that facilitate the production of quality seed. Hence, each tier is completed in 1 year. The planting material developed from certified nursery seed is distributed to growers (Sawant et al. 2014; Mall et al. 2018).

2.5.1 Breeder's Nursery Seed

During the first year, genetically pure healthy seed cane is selected from plant crop free from biotic and abiotic stress and treated with recommended fungicide prior to planting. This seed cane is treated either with hot water at 50 °C for 2.0–2.5 h or subjected to moist hot air treatment (MHAT) at 54 °C for 4.0 h. These treatments generally lower the germination rate. The purity of crop is regularly checked every month from germination till harvesting stage. Any diseased plant, if found, is immediately removed. The seed cane thus obtained is called Breeder's nursery seed.

2.5.2 Foundation Nursery Seed

In the second year of three-tier program, breeder's seed is multiplied to obtain plant treatment nursery called foundation seed. All the recommended measures are applied except heat treatment. The crop is inspected thrice: first after 45–60 days, second after 120–130 days of planting, and finally at 15 days prior to harvest.

2.5.3 Certified Nursery Seed

Certified seeds are progeny of foundation seed, distributed to growers for planting in field for commercial production. Except heat therapy, all the recommended measures are applied during the multiplication, along with regular inspection of the crop. The crop is inspected thrice for any diseased plants.

The three-tier seed program is useful in controlling seed (sett)-borne infections such as red rot, grassy shoot disease (GSD), and smut disease as well as to control the pests such as mealy bug and scale insect. However, moist hot air treatment is not much effective against sugarcane mosaic virus disease. For this purpose, meristem culture technique is a well-known technique to develop seed cane free from viruses and other pathogens (Usman 2015).

2.6 Mega Seed Project

The farmers generally use the same age-old planting material year after year, which has resulted in stagnant sugarcane productivity at national level. In addition, the use of poor quality sugarcane seed material and monoculture reduce productivity. A

massive breeder's seed production program of improved sugarcane varieties has been launched under Mega Seed Project of ICAR, Government of India, to produce good quality planting material to be supplied to sugar mills every year. Sugar mills produce this planting material as foundation seeds on their farm, and such foundation seeds are provided by sugar mills for the production of certified seeds on selected farmer's fields. This seed multiplication chain has increased sugarcane productivity by 15–20%.

2.7 Sustainable Sugarcane Initiative (SSI)

Sustainable Sugarcane Initiative (SSI) fittingly suits the requirement of innovative techniques that can be adopted by farmers to address the age-old issue of enhancing productivity to ensure higher income to farmers. It is based on the principles of "more with less" in agriculture, encompassing the improvement in the productivity of water, land, labor, and the cane while reducing the overall pressure on water resources on one hand and the cost of cultivation on the other, thus addressing the problems of sugar sector to a large extent, hence useful to increase sugarcane production. Extrapolating the principles and practices of System of Rice Intensification (SRI), Sustainable Sugarcane Initiative (SSI) has been introduced in the sugarcane region among farmers to practice bud (seed) treatment (with lime and cow urine), seedling bed preparation on plastic cavity trays, single bud/young seedling (25–35 days) transplantation, wider spacing (5 ft $\times$ 2 ft), organic manure application, mulching of dried sugarcane leaves in the interrow spaces, and intercropping with other crops, such as onion, garlic, and lady fingers, as a solution to the problems of unpredictable climatic changes, inappropriate cultivation practices, improper plant protection measures, imbalanced nutrient management, and other practices like monocropping and for more effective utilization of land, in order to enhance the health and fertility of the soil, conserve surface moisture, control weeds, reduce the water loss, and produce more number of canes with less seeds and more economic benefits to the farmers.

2.8 Biotechnological Interventions for Quality Seed Cane

Biotechnology offers excellent opportunities for sugarcane crop improvement. Commercial sugarcane, mainly the interspecific hybrids of *S. officinarum* and *S. Spontaneum*, is greatly benefited from biotechnological improvements due to its complex polyploid-enabled genome, narrow genetic base, poor fertility, and susceptibility to various diseases and pests. More importantly, there is an ongoing requirement to provide durable disease and pest resistance in combination with superior agronomic performance in the commercially exploited clones. This has led to considerable research in different areas of biotechnology pertinent to sugarcane breeding and disease control. According to Sengar et al. (2009), biotechnological approaches for improvement of sugarcane crop have been applied in the fields of cell

and tissue culture for rapid propagation of sugarcane, molecular diagnostics to detect sugarcane pathogens in seed cane material, virus indexing, and molecular testing of plants for clonal fidelity. In vitro propagation of sugarcane has been achieved through the totipotent part of cane such as shoot tip, apical meristem, axillary shoot, bud, and leaf.

2.8.1 In Vitro Propagation or Micropropagation Technique

In sugarcane, in vitro propagation or plant tissue culture techniques have allowed the rapid multiplication of new varieties in shorter period over conventional method through micropropagation; rejuvenation of old deteriorated varieties; disease-free planting material or seed cane with high multiplication, growth, and yield; conservation of germplasm material; and facilitation of the exchange of in vitro plant material (Hendre et al. 1983; Sandhu et al. 2009; Sawant et al. 2014; Lal and Krishna 1994; Parmessur et al. 2002; Adilakshmi et al. 2014). In vitro propagation or tissue culture can increase the propagation rate by 20–35 times (Snyman et al. 2006; Belete 2017). Seed cane production using apical meristem or shoot tip culture is contributing to decrease the spread of bacterial, fungal, and viral diseases (Jalaja et al. 2008; Lal and Krishna 1994; Cha-Um et al. 2006).

The micropropagation using meristem culture has the following steps:

1. **Initial stage (mother plant selection):** First of all, donor variety or mother plant is selected from mother plant nursery and conditioned to initiate the in vitro culture. Mother plant should be genetically pure.
2. **First stage (in vitro establishment):** The apical meristem chosen as explants and their sterilization is carried out to initiate in vitro culture under aseptic condition.
3. **Second stage (multiplication):** In this stage, mass propagation is performed on appropriate media. Many new shoots develop from the tissue. It is recommended that up to eight subcultures are sufficient, and making more than eight subcultures is responsible to decrease the length and number of shoot.
4. **Third stage (elongation and root formation):** In this stage, shoots form their root system and simultaneously increase in size as well as changes and adaptation to the hardening or acclimatization state.
5. **Fourth stage (acclimatization or hardening):** This process is performed under greenhouse condition. In this stage, the plant taken out from vessel and transferred under controlled greenhouse condition.

The production of seed cane through micropropagation technique is widely adopted for quality seed for enhancing sugarcane yield. This technique has several advantages over other conventional method such as the following:

- Genetic purity
- Low cost and less man power
- Rapid seed multiplication

- Disease- and pest-free seed planting material
- Higher germination rate of bud of cane sett
- Quick multiplication (single shoot apex can give number of plants)

2.8.2 Synthetic or Artificial Seed for Sugarcane Production

Synthetic or artificial seed production is a new technology in the production of seed for sugarcane (Nieves et al. 2003). Vegetative parts such bud chip (Silva et al. 2018) or axillary bud or shoot bud, somatic embryo (Nieves et al. 2003), or any other micropropagules are used for artificial seed production. Artificial seed is defined as encapsulation of totipotent part with synthetic covering. Polymers, gelatin, sodium polyacrylate, sodium alginate, and calcium chloride are used for synthetic covering (Álvarez-Sánchez et al. 2018).

2.8.3 Sugarcane Tissue Culture-Raised Plant (TCP)

Over the years, the seed cane production using tissue culture for sugarcane is widely in tradition. On June 9, 2006, a session was organized at the Vasantdada Sugar Institute, Pune (India). During this, two technical sessions were held, viz., "Developing standards for tissue culture-raised planting material" and "Methodology for determining seed cane standards." On the recommendation of the sessions, a committee meeting was called at ICAR-IISR, Lucknow, on October 9, 2006 for the finalization of issues related to seed certification standards of Tissue Culture-Raised Planting (TCP) material of sugarcane. These (TCP) standards along with general seed cane certification are applicable on sugarcane plants, which are multiplied using tissue culture technique under laboratory and greenhouse.

2.8.4 Accredited Test Laboratory

Accredited Test Laboratory are the test laboratory financially aided by the Department of Biotechnology for virus indexing and genetic fidelity/uniformity testing for certification of batches of Tissue Culture-Raised Plants (https://dbtncstcp.nic.in/ATLs). At present, two Accredited Test Laboratories are operational, one at ICAR-IISR, Lucknow, and the other at Vasantdada Sugar Institute, Pune (India). These have been established under National Certification System for Tissue Culture-Raised Plant (NCS-TCP) and put into practice by the Department of Biotechnology (DBT), India, since 2006 as per gazette of India notification under the Seed Act, 1966 (Holkar et al. 2016).

2.9 Conclusion

The production of seed cane is very challenging for obtaining the larger stock of seed for the development new varieties with high yield and better productivity. At the same time, healthy seed cane of good quality with genetic purity is another important issue. Various techniques have been developed for seed cane development with standard certification technique. However, there is a need to implement these practices in field for all farmers.

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References

Adilakshmi D, Jayachandra K, Bebi P (2014) *In vitro* meristem tip culture of sugarcane varieties – 96A3 and Co6907. Int J Adv Life Sci 7:148–154

Álvarez-Sánchez GF, la Cruz A-d, Heidi L, Salgado-García S, Córdova-Sánchez S, Ortiz-Laurel H, Castelán-Estrada M, García-de la Cruz R, Castañeda-Ceja R (2018) Development of artificial sugarcane seed CP-54 from three cultivars (cv MEX 69-290; cv MEX 68-P-23; cv. CP 72-2086) using polymers in Tabasco, Mexico. Acta Agronóm 67(1):94–100

Belete G (2017) Review on *in vitro* propagation of sugarcane to advance the value of tissue culture. Agri Res Tech Open Access J 5(4):555670

Cha-Um S, Nguyen T-H, Kirdmanee C (2006) Disease-free production of sugarcane varieties (*Saccharum officinarum* L.) using *in vitro* meristem culture. Biotechnology 5:443–448

FAOSTAT (2017) Food and Agriculture Organization of the United Nations. Rome. http://www.fao.org/faostat/en/#data/QC

Hendre RR, Iyyar RS, Katwal M, Khuspe SS, Mascarenhas AF (1983) Rapid multiplication of sugarcane through tissue culture. Sugarcane 1:5–7

Holkar SK, Atul Kumar M, Singh N, Khan MS, Singh RK, Kumar S (2016) Testing and certification of tissue culture-raised quality planting material at accredited test laboratory, ICAR-IISR, LUCKNOW. In: Centennial Conference: Challenges, Opportunities and Innovative Approaches in Sugarcane: Agriculture, Bio-energy and Climate Change. UPCSR, Shahjahanpur

Jain R, Chandra A, Shrivastava AK, Solomon S (2014) Bud chip technology for rapid seed multiplication and improving cane yield. Indian Farming 63(10):2–3

Jalaja NC, Neelamathi D, Sreenivasan TV (2008) Micropropagation for quality seed production in sugarcane in Asia and the Pacific. Food and Agriculture Organization of the United Nations; Asia–Pacific Consortium on Agricultural Biotechnology; Asia-Pacific Association of Agricultural Research Institutions, Rome; New Delhi; Bangkok. i–x + 46 p

Karuppaiyan R, Ram B (2012) Sugarcane seed production. Training manual. SBI centenary publication 5. Sugarcane Breeding Institute, Regional Centre, Haryana

Lal N, Krishna R (1994) Sugarcane and its problems: tissue culture for pure and disease free seed production in sugarcane. Indian Sugar 44:847–848

Mall AK, Misra V, Singh D, Kumar M, Pathak AD (2018) Methods and procedures for high yielding seed cane production. Int J Agric Sci 10(4):6660–6664

Nieves N, Zambrano Y, Tapia R, Cid M, Pina D, Castillo R (2003) Field performance of artificial seed derived sugarcane plants. Plant Cell Tissue Organ Cult 75:279–282

Parmessur Y, Aljanabi SM, Saumtally S, Dookun-Saumtally A (2002) Sugarcane yellow leaf virus and sugarcane yellows phytoplasma: elimination by tissue culture. Plant Pathol 51:561–566

Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (2000) A guide to sugarcane diseases. La Librairie du Cirad, Montpellier. 339 p

Samant TK (2017) Bud chip method: a potential technology for sugarcane (*Saccharum officinarum*) cultivation. J Med Plant Stud 5(3):355–357

Sandhu SK, Gosal SS, Thind KS, Uppal SK, Sharma B, Meeta M, Singh K, Cheema GS (2009) Sugar Tech 11:34

Sawant RA, Tawar PN, Meti NT, Ranjekar PK (2014) Role of sugarcane micropropagation for production of quality seed. Int J Rec Biotech 2:34–41

Sengar AS, Thind S, Kumar B, Pallavi M, Gosal SS (2009) *In vitro* selection at cellular level for red rot resistance in sugarcane (*Saccharum* sp.). Plant Growth Regul 58(2):201–209

Shukla SK, Sharma L, Awasthi SK, Pathak AD (2017) Sugarcane in India: package of practices for different agro-climatic zones. ICAR-All India Coordinated Research Project on Sugarcane, Lucknow, pp 1–64

Silva JC, Neto AR, Severiano ED, Silva FG, Dornelles DP (2018) Sugarcane bud chip encapsulation for *ex vitro* synthetic seed formation. J Agric Sci 10:104

Snyman S, Meyer G, Richards J, Ramgareeb S, Huckett B et al (2006) Refining the application of direct embryogenesis in sugarcane: effect of the developmental phase of leaf disc explants and the timing of DNA transfer on transformation efficiency. Plant Cell Rep 25(10):1016–1023

Sugiyarta E, Winarsih S (2009) Rapid propagation of sugarcane planting materials by using stratified Rayungan method. Sugar Tech 11:22–27

Usman IS (2015) Biotechnology interventions for production of good quality seed canes. Int J Sci Res Innov Technol 2:96–104

Agroecological Management of Stem Borers for Healthy Seed Production in Sugarcane

3

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Abstract

Sugarcane and interspecific hybrids are the main source of raw sugar in the globe. This plant is cultivated on more than 20 million hectares in more than 100 countries such as in South America, the United States, Australia, South Africa, Southeast and Southwest Asia, and overseas territories of Europe. Sugarcane has also been considered as eco-friendly source of energy in the form of bioethanol as energy cane. Sugarcane is vulnerable to biotic and abiotic stressors which increase the cost of production. Moth stem borers are the main entomological problems in all sugarcane-producing countries except Australia. The larval stage of stem borers feeds directly on the vegetative tissues that store sucrose and therefore directly reduces yield, but larval feeding also provides sites for the introduction of disease organisms. Both forms of damage can drastically affect yield and quality. Tunneling into stalks leads to reduced growth, weakening the stalks and resulting to stalk breakage. When severely damaged, stalks may rot, apical dominance can be lost resulting in the formation of side shoots, and late

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tillering may occur. Efficient management of moth stem borers through agroecological pest management programs is multi-tactic and requires that several ecologically sound control methods be used. We proposed the wide range of management strategies of stem borers based on agroecological practices.

Keywords

Stem borers · Sugarcane · Larval feeding · Agroecological

3.1 Introduction

Sugarcane (*Saccharum* spp. hybrid) is the world's leading sugar-producing crop, accounting for more than 80% of the world sugar supply; besides, it is also important for ethanol production in many countries, mainly in Brazil and the United States. There has been an increasing global interest in expanding and improving sugarcane production considering its potential over other tropical grasses as a converter of solar energy into biomass, consolidating the concept of "energy cane."

Sugarcane is produced in more than 100 countries in tropical and subtropical regions in both hemispheres. Although Brazil is the biggest sugarcane-producing country in the world, India's production is expected to rise to a record of 35.9 million tons for the 2018/2019 season due to its higher area and yields. Thus, Brazil's production would be eclipsed for the first time in over 15 years. Brazil's production is estimated to be down to 30.1 million tons due to lower sugarcane yields and more sugarcane being diverted toward ethanol production (USDA 2018).

Insect pests constitute one of the most important biotic stresses that affect sugarcane production. Major lepidopteran insect pests of sugarcane are stem borer, root borer, sugarcane top borer, pink borer, and Mexican rice borer (Table 3.1). They may reduce the yield up to 80% (Kalra and Sidhu 1955). Among them, the sugarcane stem borer (*Diatraea saccharalis* Fabricius, Lepidoptera: Crambidae) is the most important pest of this crop in the Americas (Bleszynski 1969). This pest produces severe agricultural and industrial problems annually causing more than 10% loss in sugarcane yield worldwide (Junior et al. 2010; Ricaud et al. 2012). In Brazil, the losses caused by insect generate an annual cost of nearly U$ 500 million, including spending on control measures (da Silveira 2017).

Neonate larval stage of stem borers may feed through the leaf tissue or tunnel through the midrib. In young plants, their attack may compromise the meristematic tissue producing the symptom known as "dead heart" and the death of the inner whorls of leaves (Barrera et al. 2017). After the first or second molt, larvae burrow into the stalk-forming tunnels which are gateways for pathogens (fungi and bacteria), producing red rot (*Colletotrichum falcatum*). The damage incurred during the mid-late stage leads to a significant reduction in the weight of the canes and the content of sucrose, affecting the Brix%, Pol% cane, and Purity% (Mendonça et al.

Table 3.1 Lepidopteran borers recorded to feed on sugarcane in the world

Species	Type of borer	World distribution
Family: Castniidae		
Telchin licus licus (Drury)	Stem borer	Americas
Family: Crambidae		
Bissetia steniellus Hamp	Stem borer	India, Pakistan
Chilo auricilius Dudgeon	Stem borer	Central and Southeast Asia
Chilo infuscatellus Snellen	Shoot borer	Northern, Central, and Southeast Asia
Chilo orichalcociliellus (Strand)	Stem borer	Sub-Saharan Africa
Chilo partellus Swinhoe	Stem borer	Ethiopian
Chilo sacchariphagus (Bojer)	Stem borer	Central to Southeast Asia, Indian ocean islands, Mauritius, Reunion, Madagascar, Mozambique
Chilo terrenellus Pagenstecher	Top borer	Papúa Nueva Guinea
Chilo tumidicostalis (Hampson)	Stem borer	India, Southeast Asia
Eoreuma loftini (Dyar)	Stem borer	Mexico, southern Texas
Diatraea albicrinella Box	Stem borer	Colombia, Brazil
Diatraea busckella Dyar and Heinrich	Stem borer	Colombia, Venezuela, Panamá, Ecuador
Diatraea centrella (Moschulsky)	Stem borer	West Indies, Guyana, Surinam, French Guiana, Venezuela, Colombia
Diatraea considerata Heinrich	Stem borer	Mexico
Diatraea dyari Box	Stem borer	Argentina
Diatraea flavipennella Box	Stem borer	Brazil, Colombia, Venezuela
Diatraea guatemalella Schaus	Stem borer	Mexico, Guatemala, Costa Rica
D. indigenella Dyar and Heinrich	Stem borer	Colombia
Diatraea rosa Heinrich	Stem borer	Venezuela
Diatraea saccharalis (Fabricius)	Stem borer	Central and South America, the United States
Diatraea tabernella Dyar	Stem borer	Central America, Colombia

(continued)

Table 3.1 (continued)

Species	Type of borer	World distribution
Diatraea veracruzana Box	Stem borer	Mexico
Scirpophaga nivella Fabricius	Top shoot borer	China
Family: Noctuidae		
Busseola spp.	Stem borer	Ethiopian
Pseudaletia unipuncta	Shoot borer	America
Sesamia calamistis Hampson	Pink stem borer	Ethiopian
Sesamia inferens Walker	Pink stem borer	Japan, Central and Southeast Asia, Indonesia and PNG
Sesamia nonagrioides Lefebvre	Stem borer	Southern Europe to West Asia, West Africa to Sudan
Sesamia poephaga Tams and Bowden	Stem borer	West Africa to Sudan, Comoros, Madagascar
Family: Olethreutidae		
Argyroploce schistaceana Snellen	Stem borer	China
Family: Pyralidae		
Eldana saccharina Walker	Stem borer	Sub-Saharan Africa
Elasmopalpus lignosellus	Shoot borer	Argentina, United States and Mexico
Emmalocera depressella (Swinhoe)	Root borer	India, Pakistan, Bangladesh
Proceras venosatus Walker	Stem borer	China
Scirpophaga excerptalis	Top shoot borer	Bangladesh, India
Scirpophaga nivella	Top shoot borer	Bangladesh

Adapted from Sallam (2006); Solis and Metz (2016)

1996; Parra 1993; Salvatore et al. 2009), and the increase of wind broken stalks (Zeping et al. 2016).

Control strategies have not been effective, since soon after hatching the larvae of stem borers produce galleries in the sugarcane stalks and complete its development inside of them. In that sense, once the larvae have penetrated the stem, they are out of

reach of insecticides. Systemic insecticides are also largely useless due to the poor translocation within the plant. Management of moth stem borers in sugarcane is multi-tactic, and several strategies should be applied for appropriate pest reduction and sustainable production of canes. In this chapter, we reviewed control strategies of moth stem borers based on agroecological management tactics.

3.2 Changes in Planting Date

Although the change in the planting date in order to escape the insect attack can be used in some agricultural crops, this case is a bit unusual in relation to sugarcane. The date of planting cane is limited in time and varies from country to country. For example, in Louisiana, planting dates are initiated from 1 August to 15 October, and sometimes until November. Typical sugarcane planting recommendations proposed that early sugarcane planting provides ideally greater root development and establishment and increase in total yields (Viator et al. 2005). The sugarcane plants cultivated in August are strongly attractive to the *D. saccharalis* infestations, and the percentage of damaged sugarcane varieties planted in the summer is high. Early-summer-planted sugarcane was more suitable for moths' oviposition, and the availability of sugarcane stubbles to stem borers in late summer and autumn is much higher in early-planted or early-harvested cultivars (Beuzelin et al. 2011; Charpentier and Mathes 1969). In a 2-year field experiment in Louisiana, Beuzelin et al. (2011) assessed four planting dates in relation to sugarcane damage by *D. saccharalis*. Sugarcane varieties were planted on 6 August, 5 September, 10 October, and 21 November. The results of this study clearly showed that sugarcane farms planted in 6 August provide an extended time duration of crop availability for sugarcane stem borer's incidence and damage than varieties which were planted at late summer or early autumn. Sugarcane cultivars planted in early summer produced higher shoot density and become taller in height which leads to increase of *D. saccharalis* damage. In China, to reduce damage of *C. sacchariphagus* on sugarcane, it is recommended that the planting date should be considered in early summer (Huang 2018). In Iran, Nikpay et al. (2015) evaluated effects of planting date on incidence and damage severity of pink stalk borers *Sesamia* spp. on five commercial sugarcane varieties. The authors showed that there were significant differences on planting date and stalk borer infestation on each variety. Sugarcane varieties planted at mid-August had more percentage of "dead hearts" and autumn populations of *Sesamia* spp. larvae. In sugarcane farms where moth stem borers are in high density and susceptible varieties are planted, optimization of planting date may help decrease stem borers' population buildup.

3.3 Interaction of Weeds and Sugarcane Pests

Annual and perennial weed species in sugarcane production systems may cause considerable and economic yield losses. Severe damage of weeds can affect sugarcane biomass and tonnage of raw sugar. Because of wide space between sugarcane rows and relative slow rate of seed germination, weeds can compete with sugarcane more significantly than other agricultural crops. Moreover, weeds are considered as second hosts of diseases such as sugarcane mosaic virus and yellow leaf syndrome and nematodes and insect pests such as stem borers and whiteflies (Showler 2013; Singh et al. 2019a, b). Notwithstanding weed species can compete strongly with sugarcane, conservation of weeds where they will not decrease crop yield (create a weedy line or patch) might supply natural habitats for conservation of predators' population (Showler 2013). In United States and Brazil, the red fire ant, *Solenopsis invicta*, is a common predator of sugarcane borer *D. saccharalis*. In weedy sugarcane plots, colonization of *S. invicta* was denser, and better control of *D. saccharalis* was achieved in comparison with weed-free trial plots. The results showed that in plots where weeds are completely eliminated, the damage caused by *D. saccharalis* was significantly greater than weedy plots (Showler and Reagan 1991). In India, Srikanth et al. (2002) conducted research trials and evaluated weedy and weed-free sugarcane plots in different periods on incidence and damage of early shoot borer *C. infuscatellus*. Weeds were eliminated by manual weeding practice in 2-week intervals. The authors reported that the incidence of shoot borer was significantly lower in weedy sugarcane plots than in weed-free treatment. Although weeds are highly competitive with sugarcane in terms of space, water, and nutrients, maintaining weeds in places where they would not affect and decrease sugarcane tonnage may prepare natural surroundings for support populations of predators and parasitoids (Showler 2013). Conservation of weeds and flowering plants along field borders in a limited area (describe as "weedy islands") can be a useful and practical technique which provides nectars for adults of parasitoids such as tachinid flies, ichneumonid, and braconid wasps and could be considered as a natural shelter for predators such as ants and spiders (Ali and Reagan 1985; Showler 2013).

3.4 Intercropping

Intercropping is the cultivation of several crops with no similarity in height, growth, development time, and crop management in the same farm to obtain utmost the use of space in the same field. Sugarcane has long duration period until harvest, wider space, and slow-growing time at early stage of growth, and these characteristics lead to considering sugarcane as a potential candidate for intercropping (Parsons 2003). Intercropping with sugarcane is a popular topic and strategy among small-scale farmers in developing countries, and it can be exploited by farmers in sugarcane-producing countries such as Pakistan (Ahmed et al. 2008; Rehman et al. 2014), Sri Lanka (Rodrigo et al. 2000), India (Geetha et al. 2015; Srikanth et al. 2000), Bangladesh (Rahman et al. 2016), South Africa (Barker et al. 2006; Berry et al.

Fig. 3.1 Intercropping of sugarcane with pepper in Vietnam. (Photo credit: C.A. Duong)

2009; Parsons 2003), Mauritius (Govinden 1990), Vietnam (C.A. Duong, personal communication), and China (Li et al. 2013). Intercropping has been widely exploited by sugarcane smallholder farmers to enhance crop tonnage, increase soil fertility, suppress annual weeds, reduce insect pests' populations, and have greater beneficial arthropod biodiversity (Li et al. 2013; Srikanth et al. 2000). A wide range of monocotyledons and dicotyledons including cereals, beans, pepper, coriander, potato, onion, soybean, lentil, peanut, and oilseeds have been pinpointed by scientists and used by local farmers as intercropped with sugarcane (Berry et al. 2009; Bokhtiar et al. 2003; Geetha et al. 2015; Li et al. 2013; Srikanth et al. 2000) (Fig. 3.1).

Intercropping in sugarcane production systems may lead to increasing biodiversity of beneficial arthropods such as parasitoids and predators and reduction of sugarcane pests including stem borers, termites, and parasitic nematodes (Ahmed et al. 2008; Berry et al. 2009; Srikanth et al. 2000). In South Africa, Beje (1998) evaluated the effects of intercropping beans with sugarcane on populations of predators of stem borer *Eldana saccharina* (Lepidoptera: Pyralidae). The results of trials have shown significant increase in population of predatory ant *Dorylus helvolus* (Formicidae) and predatory mites. Another study conducted in South Africa revealed that intercropping of sugarcane with *Melinis minutiflora* (Poaceae) at a spacing of 20 rows had a positive role in minimizing *E. saccharina* incidence and level of damage to sugarcane varieties. Interestingly, this

intercropping pattern could reduce weed biomass due to displacement in experimental plots (Barker et al. 2006). The results of experiments in India provided different views of positive, neutral, or negative effects of intercropping on sugarcane stem borers' damage and abundance of natural enemies. To give instances, intercropping of sugarcane with onions, garlic, coriander, soybean and green gram, black gram, and cowpea decreased incidence and damage of early shoot borer *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae) and top borer *Scirpophaga excerptalis* Walker (Lepidoptera: Pyralidae) (Chaudhary 2008; Misra and Hora 1982; Rao et al. 2010). On the other hand, some researches indicated that intercropping of sugarcane with wheat led to a significant increase on infestation level of the pink stalk borer *Sesamia inferens* Walker (Lepidoptera: Noctuidae) (Misra and Hora 1982). Srikanth et al. (2000) found that intercropping of sugarcane with cowpea, green gram, and soybean has no significant differences on borer *C. infuscatellus* and its predator's population in comparison with sugarcane monoculture.

3.5 Pheromones

Pheromones are chemical signals that are secreted by one gender of a species. They appear to be species specific (Bruce 1970). In moths, sex pheromones are released mostly by females to attract males. Female sex pheromone lure and pheromone traps are some of the appropriate devices for detecting and monitoring population levels of stem borers. Pheromone traps are one of the main component and concept of area-wide integrated pest management (IPM) for the reason that they are nontoxic and eco-friendly, act at very low moth borers' density, are species specific, have high level of compatibility with all the procedures of IPM, and do not cause resistance among pests (Mukunthan et al. 2003). Also, pheromone traps are applied for controlling moths by mass trapping and mating disruption. Mating disruption performance of pheromone traps has been more evaluated with interesting results, and it is obtained in controlling pests of sugarcane grown under plantation conditions (Chand et al. 2018; David et al. 1985). Application of pheromone traps is a common practice in management of sugarcane stem borers, and there are several reports in the case of using pheromones in sugarcane fields (Chand et al. 2018; David et al. 1985; Kumar et al. 2016; Li et al. 2018; Mukunthan et al. 2003; Way et al. 2004; Wilson et al. 2017) (Fig. 3.2).

In India, David et al. (1985) recommended mass trapping in management of internode borer, and mating disruption method was possibly applicable in the matter of stalk borer. The authors found that the total damage of *Chilo sacchariphagus* Bojer (Lepidoptera: Pyralidae) in plots covered with pheromones was significantly lower and cane tonnage was greater than in treated plots compared to control. Several progresses including indigenization of pheromone synthesis, maximizing the efficacy of pheromone traps and design, and evolving of multielement pheromone lures were achieved (Mukunthan et al. 2003). In Marromeu sugar state in Mozambique, Way et al. (2004) carried out experiments to assess the efficacy of pheromone traps in monitoring of *C. sacchariphagus*. Three traps including bottle

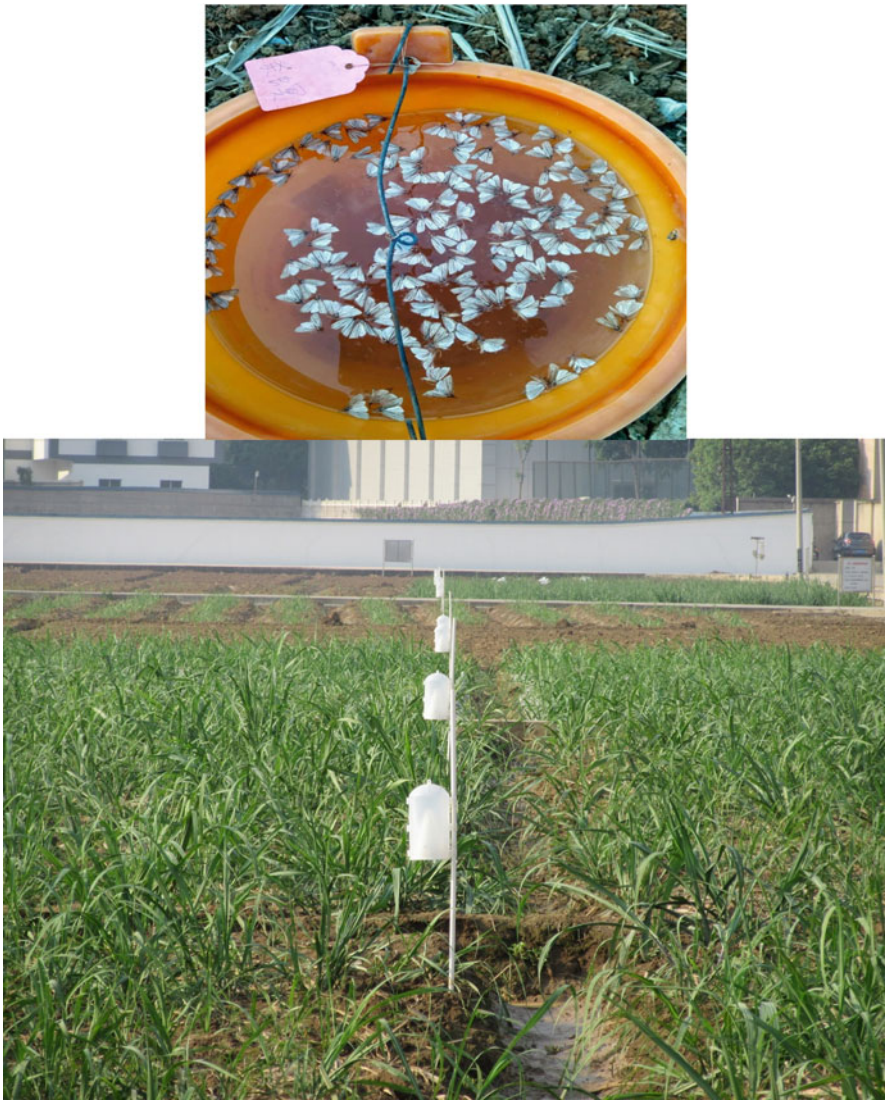

Fig. 3.2 Mass trapping of *Chilo sacchariphagus* and sex pheromone traps in sugarcane fields in Guangxi Province and Yunnan Province, China. (Photos credit: ZQ. Qin and Y.K. Huang)

trap, dry drip tray trap, and double-funnel trap were used. The results clearly showed that the pheromone-baited traps successfully and efficiently attracted internode borer moths. In India, Chand et al. (2018) investigated the effectiveness of pheromone traps for management of different sugarcane moth stem borers in three consecutive year's trials. They used 21 traps per hectare as mass trapping of adult moth borers, and finally, the authors found that pheromone traps could reduce the incidence and

severity damage of *C. infuscatellus*, *S. excerptalis*, and *Chilo auricilius* Dudgeon compared to control plots. The authors concluded that mass trapping of moth borers may be added as one part of integrated stem borers' management. Using pheromone traps in sugarcane IPM is considered as green control techniques in China. In Yunnan sugarcane plantations in China, Li et al. (2018) stated that application of sex pheromone trap (installed in March) in combination with tebufenozide (1.5 L per hectare) had good results in reducing both percentage of dead heart and internode bored compared to check plots. In major sugarcane production states in the United States, pheromone traps are the key tool for monitoring of damaging moth stem borers. In a research study, Wilson et al. (2017) evaluated the effectiveness of two types of pheromone trap. They used electronic automated pheromone traps and conventional traps for monitoring the population of Mexican rice borer *Eoreuma loftini* Dyar (Lepidoptera: Crambidae) in sugarcane fields. The results illustrated that electronic automated pheromone traps could approximately capture threefold moths than conventional pheromone traps. In Brazil, live female moths are used in traps and every 2 or 3 days the adult moths replaced with new females (personal communications with J.A. Rossato; Fig. 3.3).

To sum up, pheromone traps can be used effectively and efficiently in detection, monitoring, population dynamics and mass trapping of moth stem borers in sugarcane agroecosystems.

3.6 Light Traps

Light trapping is the most efficient, popular, and widespread method of collecting and controlling nocturnal insect pests throughout the globe. This method of survey and control is classified as physical control of insect pests which can be a subset of cultural management strategy. The mentioned procedure has not been promoted significantly in insect control although light traps have been employed efficiently as survey and sampling methods for some species of Coleoptera and Lepidoptera. In the context of sugarcane, application of light traps has been used frequently for controlling of various insect pests such as stem borers, white grubs, and leafhoppers (Carnegie and Leslie 1991; Duong 2014; Huang 2018; Nikpay and Goebel 2016; Perez-Perez 1971; Thein et al. 2011) (Fig. 3.4).

In the United States, Perez-Perez (1971) set up a series of experiments to determine effectiveness of pheromone and light traps for adult catching of *D. saccharalis*. The results showed that both male and female adults of sugarcane borer were more caught in traps with blacklight lamp than baited trap contained with virgin female. During application of blacklight traps in sugarcane fields, the average of infestation and damage incidence by *D. saccharalis* was decreased in comparison with control. There were more males attracted to the light traps than females, and the sex ratio of males and females on trapping area was 10:1 (Perez-Perez 1971). In South Africa, the light traps have been considered as inexpensive and effective tools for monitoring of sugarcane moth stem borers including *E. saccharina* and *Sesamia calamistis* Hampson. In Southeast Asian sugarcane-producing countries such as

Fig. 3.3 Pheromone trap with live adult moths in Brazil. (Photo credit: J.A. Rossatto)

Vietnam and China, light traps are used for monitoring and controlling of different moth borer species. In Vietnam, Duong (2014) conducted a series of laboratory and field trials to assess the efficacy of light traps on attractiveness and efficacy on borers. The light traps could attract giant borer *Phragmataecia castanea* Hübner, *S. excerptalis*, *Sesamia* sp., *C. infuscatellus*, and *C. sacchariphagus*. The ratio of caught moth species was significantly different, and the giant borer was the most collected species by light traps. In a large area of sugarcane plantation in Tay Ninh, Vietnam, Nuoc Trong Joint Stock Company applied field trials with light traps for control of giant borer. Five hundred hectares of sugarcane fields were covered with 500 light traps and the other 500 hectares considered as control. The light traps were operated from planting to harvesting time, and the items of percentage of stalk

Fig. 3.4 A solar light trap for trapping of adult moth borers in Longtan Experimental Station, Guangxi Province, Nanning, China. (Photo credit: A. Nikpay)

damage, percentage of internode bored, tonnage of sugarcane, and percentage of commercial cane sugar (CCS) were recorded. The obtained results of these field trials clearly showed that light traps were highly efficient on giant borer and could reduce the incidence and damage of borer in comparison with control. In China, light traps are recommended as a cultural method for moth stem borers. In sugarcane fields in Yunnan Province, at the peak of infestation of *S. inference*, *C. sacchariphagus*, *C. infuscatellus*, *Tryporyza intacta* Snellen, and *Argyroploce schistaceana* Snellen, one light trap per 2–4 hectares is used (Huang 2018). Several factors including trap design, borer species, and environmental conditions may affect the efficacy of light traps on collecting moth borers. However, application of light traps may be successfully used in sugarcane fields and should be integrated with other IPM techniques.

3.7 Eliminating Stem Borers in Sugarcane Trashes and Stubbles

The population of stem borer larvae is low in spring, as many of them are killed during the winter. Top canes, stalks, and leftovers from harvesting operations and newly planted canes are the most important sources of overwintering insects that infest farms in the spring (Leslie 2004; Long and Hensley 1972). Reducing the number of overwintering populations is one of the most satisfactory methods for reducing the number of larvae for the year ahead. There are several methods for reducing stem borer larvae in leftovers of harvesting operations. Early harvesting is

Fig. 3.5 Cane pile leftovers in the field as a source of stem borers' infestation in Indonesia. (Photo credit: F.R. Goebel)

an effective method that can greatly decrease the population of stem borers' larvae in highly infested fields. This method avoids increasing damage and provides more time for the destruction of sugarcane stubbles in the fields, which results in more mortality of the overwintering larvae. Another method is to remove the sugarcane residues from the farm surface. Remained stems after harvesting are the source and shelter for overwintering larvae (Fig. 3.5).

Birds also act as a natural enemy of stem borers' larvae and feed on remained larvae in cane residues and stubbles (Fig. 3.6).

All residues and cane leftovers should be completely burned after harvest, since the remaining cane leaves on the fields protect the remaining larvae in sugarcane stubbles. Long and Hensley (1972) in the United States showed that the burning of plant remains causes deaths of nearly 75% of overwintering larvae. The value of this method is to reduce the population of the first generation of overwintering larvae. In the fields that are considered as ratoons for the following year, the burned residues must be crushed and returned to soil as soon as possible by plowing. The soil that remains on cane leftovers prevents larvae from escaping to form adult moth borers. In India, researchers have shown that the survival of young canes in postharvest farms provides suitable habitat for the survival of larvae of *Chilo auricilius* stem borer. In the United States in 2011, Sandhu et al. showed that the survival of sugarcane residues after harvest in the field could lead to an increase in the

Fig. 3.6 Presence of birds which feed on stem borer larvae in Panama. (Photo credit: R. Atencio)

population of *Elasmopalpus lignosellus*. Also, the results showed that the amount of stem damage was higher in treatments containing postharvest residues. Detrashing sugar cane in the presence of labor force is one of the methods of agronomic practices, and by detrashing of sugarcanes, stem borers could not lay their eggs, and it is useful for management of stem borers in late generations. This method is applicable in sugarcane-producing countries such as India, Bangladesh, Sri Lanka, and Reunion Island (Fig. 3.7).

3.8 Push-Pull Strategies in Management of Moth Stem Borers

Push-pull is a strategy that uses the plant diversity to control pests, by attracting them and sometimes killing them (push) or attracting parasitoids (pull) and predators to kill the pest. By integrating new plant species (service plants) into the agroecosystem, it is possible to mitigate the impact of insect pests through several methods which can also be combined. These service plants can thus be used to develop a push-pull system, which can become a useful part of agroecological crop protection (Goebel and Nikpay 2017) (Fig. 3.8).

One main objective of the push-pull strategy is to promote/preserve the presence of natural enemies. It involves taking the interactions between insects and their

Fig. 3.7 Detrashing of sugarcane leaves as a source of stem borers' egg-laying reduction in Reunion Island. (Photo credits: F.R Goebel)

natural or cultivated habitats into account in order to then shape these habitats to increase the effectiveness of biological control (Fig. 3.9).

Push-pull focuses first and foremost on insects and plants. It is strongly linked with chemical ecology. For example, by identifying the chemical messages (volatile compounds) released by plants when they are attacked or by specifying the services provided by plants, it will be possible to identify the plants to be grown in and around cultivated fields with the aim of attracting or repelling insect pests or their natural enemies. Research also focuses on ecological processes both within and beyond agroecosystems, in order to rethink farming practices based on an agroecological approach, to characterize and promote ecosystem services, and also to identify local knowledge and facilitate its use. Finally, it deals with stakeholder strategies with a view of ensuring the coordination for a more effective and sustainable crop protection. To illustrate this approach, several examples are described below.

In South Africa, *Eldana saccharina* is a major pest of sugarcane, and the borer larvae cause economic losses in terms of biomass and sugar. This pest as the other moth borers is difficult to control due to its behavior and cryptic biology, damaging the internodes. Biological control has never been a success and remains difficult with local parasitoids, and the South African Sugarcane Research Institute (SASRI) has tried different other ways to control this pest, including the use of insecticides and

Fig. 3.8 *Canavalia* spp. as companion plant to increase biodiversity on sugarcane fields in Reunion Island. (Photo credit: F.R. Goebel)

sterile insect technique (SIT). In terms of agroecological management of *Eldana*, the researchers have identified several companion plants to use or introduce around fields in order to stimulate the natural control of the sugarcane borer: wild plants such as *Cyperus*, *Erianthus*, *Pennisetum*, or *Desmodium* and cultivated plants such as maize or sorghum, which attract parasitoids and trap or repel pests (Rutherford 2015). Another example is the vetiver grass (*Chrysopogon zizanioides* (L.)) which has demonstrated the potential as trap crop component of an overall "push-pull" strategy to concentrate *C. partellus* oviposition away from the maize crop and reduce subsequent population development. The research gave interesting results to be used by small-scale farmers. To sum up, push-pull strategy reduces stem borers' damage

Fig. 3.9 Intercropping of sugarcane with *Desmodium* spp. (push plant) to repel adult moth borers from sugarcane fields. (Photo credit: F.R. Goebel)

and infestations to sugarcane. Also, this method efficiently decreases the development of resistance to chemical pesticides and may increase biodiversity support of natural enemies in sugarcane fields.

3.9 Burning Fields

Burning sugarcane fields before harvest is a traditional and ancient practice that has been carried out worldwide with both favorable and unfavorable implications, which are discussed by Davies (1998), Legendre (2000), Chaves Solera and Bermúdez

Loría (2006), Ortiz Laurel et al. (2012), and Rugeles (2015). One of the side effects of burning sugarcane fields is the impact on biodiversity of insect populations, in both pests and their natural enemies (Goebel and Nikpay 2017). When a sugarcane field is burned, the fire reaches in a short time a temperature of 400 °C, enough to cause the elimination of microorganisms and insects that live both aboveground and underground (first centimeters) (Foster 1979). On the other hand, the arthropod populations in the burned fields recover again after harvest and continue their development although in a low species diversity compared to unburned fields (Araújo and Macedo 1998; Araújo et al. 2005). Stem borers have been a case of interest because most of their life they live inside the stalk (Smith et al. 1993; Wiedenmann 2004), and when the cane is ready to harvest, larvae and pupae of stem borers remain in stalks aboveground and underground (Vejar-Cota et al. 2009). The sugarcane burnt has a detrimental effect on stem borer populations and its natural enemies, those that are found in the stalk prior to harvest. Vejar-Cota et al. (2009) in Sinaloa, Mexico, found that the crambid *Diatraea considerata* Heinrich were higher in the stalk tops than in underground stalks (8–12 times). Besides, they also found that larvae and pupae of stem borer deep in stalk tops did not survive to reach adulthood after burning, whereas individuals found inside stalks underground were protected from heat with 47.1% pupating, while 39.5% emerged as adults, suggesting that the underground stalks serve as reservoirs for the first generation in ratoon crops. In contrast, some studies indicated that the practice of burning does not always eliminate borers deep inside the stalks, as indicated by Rochat et al. (2001) in *C. sacchariphagus* in Reunion Island and Capinera (2001) in *D. saccharalis* in the United States. Araújo and Macedo (1998) evaluated the arthropod populations in areas with burned and unburned "green" harvest, finding that arthropod populations are more abundant and diverse in areas not burned, concluding that the burning fields were detrimental to the natural enemies of *D. saccharalis* resulting in an increase in the populations of this pest. Likewise, Goebel and Nikpay (2017) reported that biodiversity is severely affected by burning causing a total biological imbalance, while ban of cane burning in areas heavily infested by *C. sacchariphagus* reduced the damage to 50% in Reunion Island. Macedo and Araujo evaluated the effect of cane burning on larvae and egg parasitoids of *D. saccharalis* in two consecutive cultivation cycles, concluding that cane burning negatively affects the populations of the larval parasitoids *Metagonistylum minense* Townsend, *Paratheresia claripalpis* Wulp, and *Cotesia flavipes* (Cameron), especially the last species, as well as its egg parasitoid *Trichogramma* spp. On the other hand, Vejar-Cota et al. (2009) found that the parasitoid braconid *Macrocentrus prolificus* Wharton was recovered from larvae that survived after fire inside underground stalk (18.4%) and also from larvae inside stalk tops (0.5%), which explains the appearance of this parasitoid in the next ratoon crop. Apparently, unburned sugarcane fields favor conservation of natural enemies and decrease damage caused by stem borers (Araújo and Macedo 1998; Goebel and Nikpay 2017). However, Dinardo-Miranda and Fracasso (2013) found in Brazil a strong relationship between the increase in the area with harvest of unburned cane and the rate of infestation of *D. saccharalis*; the infestation of the borer was increased from $<4\%$ in 2006 to $>8\%$ in 2011 (reaching ca. 50% through 5 years),

concluding that the harvest of green cane was proportionally more beneficial to the pest. On the other hand, in Colombia, Gómez-Laverde and Lastra-Borja (1998) did not observe significant differences in the populations of the borers *Diatraea* spp. and *Valentinia* sp. in fields with harvest of burned cane and not burned. Due to environmental regulations, the trend of cane burning is to disappear in the future (Amit 2015; Goebel and Nikpay 2017), which forces us to look for a new approach for sugarcane management strategies for the new worldwide conditions for this crop.

3.10 Mechanically Hand Removing Dead Hearts

One of the recommended cultural agronomic practices around the world for sugarcane stem borer control is manually hand removing dead hearts caused by borer larvae (Ingram and Bynum 1941; Leslie 2004; Najarro 2009; Nikpay and Sharafizadeh 2018; Pérez et al. 1993; Ram et al. 2011). Dead heart symptom is observed with the brown-yellowish, dying condition of the inner whorl of leaves, which contrasts with the healthy, green appearance of the outer and lower leaves (Ingram and Bynum 1941; Smith et al. 1993). This symptom is caused by most of the stem borer species worldwide because they share similar biology and ecology (Smith et al. 1993; Wiedenmann 2004). Dead hearts may occur during different sugarcane growing stages: (a) during the tillering stage (first 3 months), (b) during the grand growth period (4–10 months), if the attack is severe, the plant stops growth and produces lateral shoots showing the "top dead" symptom (Ingram and Bynum 1941; Mendoza 1996; Ram et al. 2011), and (c) in the case of sugarcane zones where winters are defined, dead hearts appear 1–3 months after harvest. This last condition is originated by the larvae feeding on underground stalk before harvest, and it is considered as one important part of the first generation in the next ratoon (Vejar-Cota et al. 2009). The tillering stage is recommended to perform dead hearts removing practice, before stalks are observed (Najarro 2009; Nikpay and Sharafizadeh 2018; Pérez et al. 1993). Dead heart removal is not recommended after tillering stage (Ram et al. 2011). Dead hearts affect both young and mature plants, and they cause a delay in development, lateral buds sprouting, aerial rooting, weight loss, decrease of sucrose in mature stalks, internodes deterioration, and plant death (Ingram and Bynum 1941; Mendoza 1996).

During dead hearts removing practice, people walk through the sugarcane field during the tillering stage (Fig. 3.10) and cut with machetes or knives all the shoots which show dead heart symptom described above. If the larvae from the dead hearts are not killed, they will continue their development inside underground stalk or in adjacent plants and finally in emerging moths. Care should be taken to cut off the shoots below the lower end of the borer tunnel in order that the larva or pupae may be included in the cut portion (Ingram and Bynum 1941). Dead hearts removal has been evaluated in different countries around the world in order to eliminate the first borer generation (Vejar-Cota et al. 2008) with different results. For example, in Guatemala, Najarro (2009) mentions that dead hearts removal is a feasible cultural practice to reduce damages caused by *D. saccharalis* and *D. crambidoides* Grote

Fig. 3.10 Manually removing dead hearts of infested sugarcane at Sinaloa, Mexico. (Photo credit: G. Vejar-Cota)

(Lepidoptera: Crambidae), recommending it in combination with other control strategies and at least twice during tillering stage. In Sinaloa, Mexico, Vejar-Cota et al. (2008) evaluated the effectiveness of dead hearts removing practice twice during tillering stage. The results showed that this practice is not effective in preventing damage caused by *Diatraea considerata* Heinrich (Lepidoptera: Crambidae). On the other hand, Nikpay and Sharafizadeh (2018), in Iran, found that four times hand removing of dead hearts was necessary in fortnightly intervals, to obtain a significant reduction in damage caused by the borer *Sesamia* spp. (Lepidoptera: Noctuidae), recommending it to reach appropriate results that it should be incorporated with other control methods such as biological control, varietal resistance, and chemical control. Khaliq et al. (2005) in Pakistan evaluated the effectiveness of several control methods including varietal resistance, mechanical control, and chemical control by integrating them in all possible combinations on different varieties of sugarcane for the control of *Scirpophaga nivella* F. (Lepidoptera: Crambidae). The results showed that dead hearts removal was the third best treatment with a protection level close to 55% with respect to control treatment in fortnightly intervals during tillering. It is difficult to determine losses from dead hearts, because most of the injured plants survive by producing new shoots, although they may be less mature at harvest time which may represent some losses in sucrose (Ingram and Bynum 1941). There is little information regarding the costs involved in the practice of dead hearts removal; for example, in the sugarcane region of Sinaloa, Mexico, the cost of performing twice dead hearts removal in commercial fields was 30 dollars per hectare (Vejar-Cota et al. 2008).

Dead hearts practice effectiveness to reduce borers' populations probably depends on diverse factors such as bore larvae per hectare, annual generations, susceptibility of the variety, field size, frequency of dead hearts removal, people training for larvae extraction, tool extraction type, immigration pressure, climate (seasons defined), and biological control agents (mainly predators and egg parasitoids), so they must be considered before starting this agronomic practice. One of the side effects of dead hearts removal is the elimination or reduction of larvae and pupae parasitoids of stem borers, which could affect its natural enemies' augmentative population and prevent the establishment of other parasitoids within classical biological control (Smith et al. 1993; Vejar-Cota et al. 2008).

In some regions of the world, manual dead hearts removal has been discontinued due to high operating costs, lack of trained personnel, and the negative impact of larvae and pupae parasitoid of stem borers (Flores 2007; Khaliq et al. 2005; Rodríguez-del-Bosque et al. 2014; Vejar-Cota et al. 2008). The feasibility of performing manually removing of dead hearts alone or combined with other control strategies in a sugarcane region should be evaluated before commercial implementation to avoid unnecessary costs.

3.11 Transgenic Sugarcane as a Strategy for Management of Moth Stem Borers

A primary control strategy for management of moth stem borers in sugarcane would be the election of the most resistant sugarcane cultivars for commercial cultivation (Reagan and Martin 1987) considering that sugarcane varieties have different resistance traits against the sugarcane borer (Tomaz et al. 2018). However, variations in weather parameters, insect population dynamics, and long duration of the crop impose several difficulties in precisely determining the level of resistance in commercial fields (Srikanth et al. 2011). Even more, due to the biological complexity of sugarcane, the valuable traditional approach of conventional breeding has also had its limitations to obtain these insect-resistant varieties. This may be due to the labor-intensive assessment of sugarcane borer damage required in order to characterize elite clones by measuring several resistance traits (i.e., percentage of bored internodes, internodes with moth exit holes, damage rating, larval recovery) (Tomaz et al. 2018).

To overcome the limitations of available control strategies, a recent and efficient method of control consists of the incorporation of one or a few genes from any source, which could confer characteristics of interest to an "elite" variety in a relatively short period of time. Successful genetic engineering requires an efficient transformation method. There are multiple transformation systems to incorporate genes into sugarcane, including *Agrobacterium tumefaciens*, bioballistic, electroporation, and polyethylene glycol, by using protoplasts, leaf rolls, or embryogenic callus as transformation materials (Aftab and Iqbal 2001; Arencibia et al. 1997; Islam et al. 2016). Nevertheless, electroporation or polyethylene glycol treatments are limited by severe difficulties with plant regeneration from protoplasts for most

cultivars. In addition, many elite varieties are recalcitrant to the transformation mediated by *Agrobacterium*. Bower and Birch (1992) proposed the bioballistic system to introduce exogenous genes into sugarcane cells. This approach is highly susceptible to transform sugarcane and has greater reproducibility and less recalcitrance depending on the genotype, in comparison with the other methods (Ramasamy et al. 2018). Even more, it allows the introduction of not linked multiple minimum expression cassettes in order to stack genes (Altpeter et al. 2005). For the aforementioned reasons, this technique is one of the most frequently used for sugarcane transformation (Altpeter and Oraby 2010).

Development of vigorously performing transgenic plants with minimal somaclonal variation depends on several factors out of which tissue culture duration contributes significantly, and it is the most amenable to modification for minimizing undesirable effects (Taparia et al. 2012). There are two major regeneration routes for the production of transgenic sugarcane: indirect somatic embryogenesis (ISE) (Bower and Birch 1992; Gallo-Meagher and Irvine 1996) and direct somatic embryogenesis (DSE) (Snyman et al. 2006; Taparia et al. 2012; Van Der Vyver 2010). The process of ISE involves the establishment of dedifferentiated cell cultures that are capable of regenerating in plants after the stable integration of transgenes into their genome (Vasil and Vasil 1980, 1981). This intermediate callus phase can be accomplished through the exogenous application of auxins and cytokinins (Garcia et al. 2007; Lakshmanan et al. 2006). As callus is formed through uncontrolled cell divisions (Vázquez 2001), a greater chromosomal variability is observed in this phase (Saravanan et al. 2011). In DSE, the dedifferentiation step (callus) is absent, and the cultures give rise to normal plants identical to those of the mother plant (Peschke and Phillips 1992). For that reason, DSE is the preferred way to obtain genetically uniform plants in sugarcane (Manchanda and Gosal 2012).

Advances in genetic transformation technology and knowledge of gene expression have led to rapid progress in genetic engineering of crop plants for protection against insect pests (Romeis et al. 2006). In this sense, different molecules like proteinase inhibitors (PI), plant lectins, ribosome-inactivating proteins, secondary plant metabolites, delta endotoxins and vegetative insecticidal protein from *Bacillus thuringiensis* (Bt) have been successfully used (Bates et al. 2005). Among them, the use of transgenic plants expressing Cry protein from *Bacillus thuringiensis* (Bt) is one of the most common strategies.

Bacillus thuringiensis is a gram-positive bacterium that produces toxins with activity against mites, protozoa, nematodes, and insects. During sporulation, it produces a parasporal inclusion formed by one or more crystalline bodies of a protein called Cry that are toxic to different invertebrates, especially larvae of lepidopteran, dipteran, and coleopteran insects (Palma et al. 2014). While in the vegetative stage of bacterial growth, other toxins called vegetative insecticidal proteins (Vips) are produced. The Vip1 and Vip2 proteins act as binary toxins against coleopteran, whereas the Vip3 toxin is active against lepidopteran (Chakroun et al. 2016). Insecticidal proteins from Bt are widely used to produce transgenic plants to control insects because they are not toxic to vertebrates and nontarget insects (Baranek et al. 2017).

Knowledge of the mode of action of Bt toxins is essential to maintain and improve the effectiveness against pests. This has been mostly studied for Cry1A protein; however, it is assumed no essential changes for other Cry and Vip proteins. It is generally accepted that Cry toxins are pore-forming toxins that exert their toxic activity by causing an osmotic imbalance in the epithelial cells where they are inserted into the membrane (Soberón and Bravo 2007). Most models of Bt mode of action agree that the inactive full-length forms of Cry1Ab and Cry1Ac proteins called protoxins are converted to activated toxins by insect midgut proteases and then bind to insect midgut receptors and exert toxic effects. Nevertheless, Tabashnik et al. (2015) suggest that both the protoxin and activated toxin forms can kill insects, with each form exerting its toxic effect via a different pathway. The protoxins could also bind gut receptor molecules leading to oligomerization, membrane insertion, and pore formation. As the final pores induced by protoxin or by the toxin have different characteristics, a dual mode of action is suggested (Soberón et al. 2018). The symptoms that are observed when the larvae of susceptible insects ingest the Bt crystals are cease of the ingestion, paralysis of the intestine, diarrhea, total paralysis, and finally death.

The first insect-resistant transgenic crop with the *cry1Ab* gene was described by Vaeck et al. (1987) in tobacco plants. After that, the Bt genes were introduced in several crops including cotton (Polanía et al. 2008), corn (Zhang et al. 2015), rice (Breitler et al. 2001), and tomato (Delannay et al. 1989) with one or several stacked genes: *cry1Ab*, *cry1Ac*, *cry1Fa*, *cry2Ab*, and *vip3Aa*. Even more, some sugarcane varieties have already been engineered with Bt genes. In 1997, Arencibia et al. (1997) described the first transgenic sugarcane resistant to *D. saccharalis* including the *cry1Ab* gene. Then, several works where different genes such as *cry1Ab*, *cry1Ac*, *cry2A*, *skti*, and *sbbi* (PI) were incorporated in order to improve stem borer resistance were carried out. In the last 22 years, significant progress has been made toward the development of a transgenic sugarcane resistant to stem borer (Table 3.2) (Fig. 3.11).

Currently, there is a growing concern regarding the generation of insect populations resistant to transgenic plants that express a single protein. Key among the different mechanisms is the binding of toxins to receptors in the membrane of the midgut of insects. In addition, toxins that share a receptor could cause cross-resistance (Ferré et al. 2008). In that sense, in the reports about Cry1 that shared receptors in different insect species, Hernández-Martínez et al. (2013) suggested a possible development of cross-resistance with a single Cry protein. Indeed, Cry1Ab and Cry1Ac sharing a receptor in *D. saccharalis* have been reported (Rang et al. 2004).

For the aforementioned reason, successful commercial development of an insect-resistant transgenic crop requires consistent and strong expression of Bt proteins and an integrated pest management (IPM) strategy that includes a refuge system and the use of competitive crop varieties (Carrière et al. 2016; Peng et al. 2014). This strategy called high-dose/refuge (HDR) is used to delay the evolution of resistance to Bt proteins by susceptible insects (Carrière et al. 2016). It combines transgenic lines expressing high levels of the toxin with planting refuges of non-transgenic plants (Carrière et al. 2010; Tabashnik et al. 2013). The refuges are areas of non-Bt

Table 3.2 List of transgenic sugarcane with insect resistance

Variety	Type of explant	Promoter	Candidate gene	Target pest	Method of transformation	Reference
Ja 60-5	Calli	CaMV35S	*cry1Ab*	*D. saccharalis*	Electroporation	Arencibia et al. (1997)
SP80-1842	Calli	Maize PEPC	*cry1Ab*	*D. saccharalis*	Particle bombardment	Braga et al. (2001), Braga et al. (2003)
CP65-357		Maize Ubi-1	Snowdrop lectin	*D. saccharalis*	Paint-sprayer delivery	Sétamou et al. (2002)
SP80-1842 and SP80-3280	Calli	Maize Ubi-1	Soybean Kunitz trypsin inhibitor (*skti*) and soybean Bowman-Birk inhibitor (*sbbi*)	*D. saccharalis*	Particle bombardment	Falco and Silva-Filho (2003)
YT79-177 and ROC16	Calli	Maize Ubi-1	synthetic-*cry1Ac*	*P. venosatus*	Particle bombardment	Weng et al. (2006)
ROC25	Calli	Ubi-1	Fusion of *Amaranthus viridis* agglutinin and *skti* genes	*D. saccharalis*	*Agrobacterium*	Deng et al. (2008)
Gui94-119	Calli	Ubi	*cry1Ac*	*D. saccharalis*	Particle bombardment	Xu et al. (2008)
CoC671	Leaf roll	CaMV35S	*cry1Aa3*	*C. infuscatellus*, *C. sacchariphagus*, and *S. excerptalis*	*Agrobacterium*	Kalunke et al. (2009)
Co 86032 and CoJ 64	Calli	Maize Ubi-1	*cry1Ab*	*C. infuscatellus*	Particle bombardment and *Agrobacterium*	Arvinth et al. (2010)
CoC 92061 and Co 86032	Calli	Maize Ubi-1	*Aprotinin*	*S. excerptalis*	Particle bombardment	Christy et al. (2009)

YT79-177 and ROC16	Calli	Maize Ubi-1	Modified *cry1Ac*	*P. venosatus*	Particle bombardment	Weng et al. (2011)
FN15	Calli	CaMV35S	*cry1Ac*	*D. saccharalis*	Particle bombardment	Gao et al. (2016)
LK 92-11	Calli	CaMV35S	*cry1Ab*	*D. saccharalis*	*Agrobacterium*	Islam et al. (2016)
ROC22	Calli	Ubi-1	*cry1Ab*	*D. saccharalis*	*Agrobacterium*	Wang et al. (2017)
CTC20	Calli	PEPC	*cry1Ab*	*D. saccharalis*	Particle bombardment	da Silveira (2017)
FN15 and ROC22	Calli	CaMV35S	*cry1Ac*	*D. saccharalis*	Particle bombardment	Zhou et al. (2018)
SP 803280	Calli	CaMV35S and FMV	*cry1Ab* and *cry2Ab*	*D. saccharalis*	*Agrobacterium*	Cristofoletti et al. (2018)
ROC22	Calli	ST-LSI	*cry2A*	*C. sacchariphagus*, *S. nivella*, *C. infuscatellus*, *A. schistaceana*, and *S. inferens*	Particle bombardment	Gao et al. (2018)

Adapted from Srikanth et al. (2011)

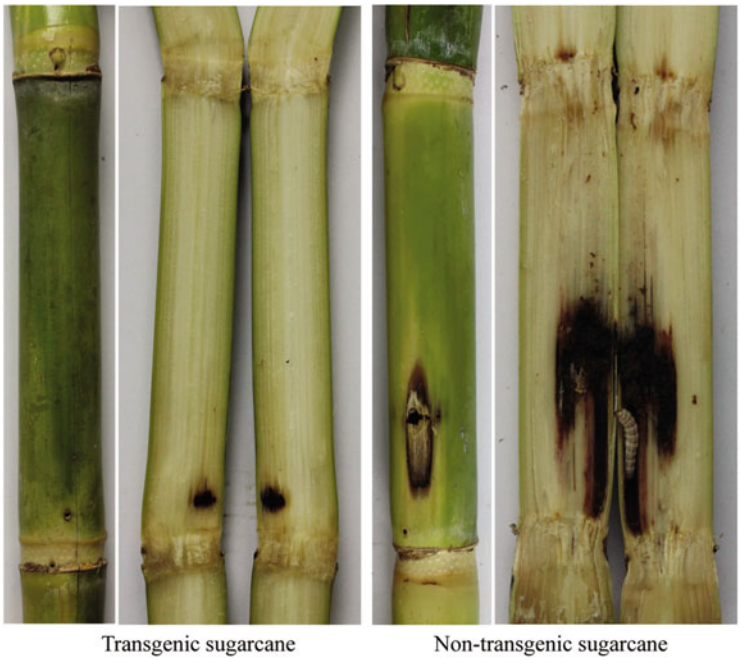

Fig. 3.11 Damage of *Diatraea saccharalis* comparing between transgenic and non-transgenic sugarcane stalks. (Gao et al. 2016; PLoS One 11: e0153929)

crops, adjacent to the Bt crops, that promote the survival of susceptible individuals that mate with the resistant (rare) individuals who survived the Bt crop. It must be highlighted that the use of alternative crops as refuge should be determined for each plague. In the specific case of *D. saccharalis*, a study revealed the existence of gene flow barriers among populations from different locations and the presence of cryptic signals of host adaptation that would indicate that the use of alternative crop hosts such as maize may decrease random mating (Fogliata et al. 2019). The refuge area suggested by the Environmental Protection Agency (EPA) of the United States seeks to balance economic and environmental considerations and could vary depending on the kind of the refuge: structured or seed blend and the transgenic crop (https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/insect-resistance-management-bt-plant-incorporated#refuges).

Another more recent strategy to delay the evolution of the resistance in the plagues involves the development of stacked Bt crops. They produce two or more Bt toxins that kill the pests and act over different membrane receptors. In fact, there must be a synergistic effect between the toxins, that is, higher concentration of individual toxins in the pyramid compared to their concentration in plants of a single toxin (Carrière et al. 2015). If the concentration of each toxin in a pyramid is high enough to kill all susceptible insects and no cross-resistance occurs between toxins, complete redundant killing occurs. Considering that the sugarcane borer is capable

of completing up to five generations per year (Salvatore et al. 2009), it is expected that the development of a multi-toxin transgenic sugarcane will be able to control 100 generations, extending the commercial life of the crop (Cristofoletti et al. 2018). When these pyramidal crops emerged, requirements on refuges became less stringent (Alyokhin 2011; EPA 2007). Consequently, by reducing the area sown with refuges, the environmental and economic benefits of Bt crops increase. Pyramided Bt genes have been rapidly adopted in several crops and are expected to be even more frequent in the future, especially for sugarcane.

Regarding deregulation of single or pyramidal transgenic sugarcane, an extensive field testing is essential to properly evaluate potential impact on agricultural environments and food safety (Noguera et al. 2015). The transgenic variety would be essentially the same of the non-transgenic one in agronomical and industrial parameters, where the only difference should be the consequence of the characteristic introduced. Besides, molecular and biochemical studies are required to ensure stable expression of the introduced gene(s) in the transgenic plant that should retain its genetic integrity, chemical composition, and agronomic characteristics (Noguera et al. 2015). This is especially important in species like sugarcane, where major concerns relating to genomic changes during callus transformation and in vitro micropropagation procedures have been raised in previous studies (Arencibia et al. 2000, 1999; Gallo-Meagher and Irvine 1996; Gilbert et al. 2009; Sala et al. 1999). So, considering the large genetic variability inherent in transgenic sugarcane populations, the aforementioned evaluations require extensive field assessment of a large population of independent transgenic events (Nerkar et al. 2018).

In the specific case of Bt crops, additional studies are required in order to test their effectiveness against the pest. Plants can be phenotyped by measuring insect populations or their effects on plants. Indicators of infestation on sugarcane plants include direct evidence of insect activity, such as damaged sheath number, number of perforations, total tunneling length, and dead heart symptoms (Goggin et al. 2015). For this purpose, several bioassays could be performed at different plant growth stages in laboratory, greenhouse, or field trials with different number and instars of larvae. Initial bioassays are performed with artificial diet mixed with transgenic leaf (Huang et al. 2006). Another common lab test is the detached leave assay where larvae are inoculated on leave segments and maintained at optimal conditions. After a period of time, larval mortality and foliar damage could be measured in order to compare transgenic vs. non-transgenic plants. In a more advanced stage, infestation of seedlings is a useful method for an initial and rapid screening to analyze transgenic Bt plants. After that, resistance is evaluated through field trials (Wang et al. 2017). This kind of trials has some inherent problems that may affect the assessment of resistance related to unmanaged insect populations that inflict a consistent level of damage (Tomaz 2014). For that reason, a thoughtful characterization is required to assure the level of resistance of transgenic plants.

Regarding commercial release of transgenic sugarcane around the world, there are only a few reports. The first one would be a drought-tolerant variety (http://www.thejakartapost.com/news/2013/05/20/development-underway-first-transgenic-sugarcane-plantation.html) released in Indonesia in 2013. This variety contains a bacterial

gene responsible for the production of betaine, a compound that stabilizes the plant cells under water stress in the field. In 2015, the official approval of commercial release of the first transgenic sugarcane variety resistant to glyphosate was issued by the corresponding regulatory bodies in Argentina. However, due to economic reasons, the commercial release was put on hold by the industrial sugar sector of the country. In 2017, the Comissão Técnica Nacional de Biossegurança (CTNBio, or National Commission of Biosafety) from Brazil approved the commercialization of CTC20 developed by Centro de Tecnologia Canavieira (CTC, or Sugarcane Technology Center) containing the *cry1Ab* gene (da Silveira 2017). Studies conducted at CTC showed that the *cry1Ab* gene is eliminated during the obtention of sugar or ethanol, so it is absent in the final products. The approved Bt sugarcane has already been planted on 400 hectares by about 100 mills in Brazil, but would not be milled in the 2019 season since mills will first multiply this variety while awaiting approval from sugar importers (http://www.dextrainternational.com/fda-approves-sugar-made-from-brazils-first-bt-sugarcane-variety/). In the United States (Food and Drug Administration) and Canada (Health Canada), its main importers had already declared that sugar produced from genetically modified sugarcane is safe for consumption.

In summary, the development of Bt transgenic crops has been a major breakthrough in the substitution of costly and laborious spraying of chemical insecticides by environment friendly alternatives (Qamar et al. 2015). It must be highlighted that in order to assure the durability of this technology and delay the evolution of resistance, it is necessary to properly manage the refuges and pyramided genes with different modes of action. In that sense, some pioneer countries in transgenic crop adoption like Argentina are working in this subject.

References

Aftab F, Iqbal J (2001) Peg-mediated somatic hybridization studies in sugarcane (*Saccharum* spp. Hybrid cvs. Col-54 and cp-43/33). Pak J Bot 33:233–238

Ahmed S, Khan RR, Hussain G, Riaz MA, Hussain A (2008) Effect of intercropping and organic matters on the subterranean termites population in sugarcane field. Int J Agric Biol 10:581–584

Ali A, Reagan T (1985) Vegetation manipulation impact on predator and prey populations in Louisiana sugarcane ecosystems. J Econ Entomol 78:1409–1414

Altpeter F, Oraby H, (2010) Sugarcane. Genetic modification of plants. In: Kempken, Frank, Jung, Christian Springer, Berlin, pp 453-472.

Altpeter F, Baisakh N, Beachy R, Bock R, Capell T, Christou P, Daniell H, Datta K, Datta S, Dix PJ (2005) Particle bombardment and the genetic enhancement of crops: myths and realities. Mol Breed 15:305–327

Alyokhin A (2011) Scant evidence supports epa's pyramided Bt corn refuge size of 5%. Nat Biotechnol 29:577

Amit G (2015) El fin de las quemas en los cultivos de caña. Caña de azúcar, trabajadores, salud y ecosistemas, revista mensual sobre la actualidad ambiental:37–38

Anonymous (2007) Us environmental protection agency. Pesticides news story: Epa approves natural refuges for insect resistance management in Bollgard II cotton. http://www.epa.gov/oppfead1/cb/csb_page/updates/2007/bollgard-cotton.html

Araújo JR, Macedo N (1998) Effect of sugarcane burning on sugarcane borer Diatraea saccharalis (Fabr. 1794) and its major natural enemies. STAB - Açúcar, Álcool e Subprodutos 16:30–34
Araújo RA, Araújo MS, Gonring AH, Guedes RN (2005) Impact of controlled sugarcane straw burning on local insect community. Neotrop Entomol 34:649–658
Arencibia A, Vázquez RI, Prieto D, Téllez P, Carmona ER, Coego A, Hernández L, Gustavo A, Selman-Housein G (1997) Transgenic sugarcane plants resistant to stem borer attack. Mol Breed 3:247–255
Arencibia AD, Carmona ER, Cornide MT, Castiglione S, O'relly J, Chinea A, Oramas P, Sala F (1999) Somaclonal variation in insect-resistant transgenic sugarcane (*saccharum* hybrid) plants produced by cell electroporation. Transgenic Res 8:349–360
Arencibia A, Carmona E, Cornide M, Menendez E, Molina P (2000) Transgenic sugarcane (*Saccharum* spp.). In: Bajaj YPS (ed) Transgenic crops I. Springer, Berlin, pp 188–206
Arvinth S, Arun S, Selvakesavan R, Srikanth J, Mukunthan N, Kumar PA, Premachandran M, Subramonian N (2010) Genetic transformation and pyramiding of aprotinin-expressing sugarcane with cry1ab for shoot borer (*Chilo infuscatellus*) resistance. Plant Cell Rep 29:383–395
Baranek J, Konecka E, Kaznowski A (2017) Interaction between toxin crystals and vegetative insecticidal proteins of *Bacillus thuringiensis* in lepidopteran larvae. Bio Control 62:649–658
Barker A, Conlong D, Byrne MJ (2006) Habitat management using *Melinis minutiflora* (poaceae) to decrease infestation of sugarcane by *Eldana saccharina* (Lepidoptera: Pyralidae). In: Proceedings of South African Sugar Technologists' Associations, pp 226–235
Barrera GP, Villamizar LF, Espinel C, Quintero EM, Belaich MN, Toloza DL, Ghiringhelli PD, Vargas G (2017) Identification of *Diatraea* spp.(Lepidoptera: Crambidae) based on cytochrome oxidase II. PLoS One 12:e0184053
Bates SL, Zhao J-Z, Roush RT, Shelton AM (2005) Insect resistance management in GM crops: past, present and future. Nat Biotechnol 23:57
Beje S (1998) The effect of intercropping beans on *Eldana saccharina* walker (Lepidoptera: Pyralidae) arthropod predator populations in sugarcane. Dissertation, University of Durban, South Africa, p 96
Berry S, Dana P, Spaull V, Cadet P (2009) Effect of intercropping on nematodes in two small-scale sugarcane farming systems in south Africa. Nematropica 39:11–34
Beuzelin J, Mészáros A, Akbar W, Reagan T (2011) Sugarcane planting date impact on fall and spring sugarcane borer (Lepidoptera: Crambidae) infestations. Fla Entomol 94:242–253
Bleszynski S (1969) The taxonomy of the crambine moth borers of sugar cane. In: Williams JR, Metcalfe JR, Mungomery RW, Mathes R (eds) Pests of sugar cane. Elsevier, Amsterdam, pp 11–59
Bokhtiar S, Hossain M, Mahmud K, Paul G (2003) Site specific nutrient management for sugarcane-potato and sugarcane-onion in intercropping systems. Asian J Plant Sci 2:17–24
Bower R, Birch RG (1992) Transgenic sugarcane plants via microprojectile bombardment. Plant J 2:409–416
Braga D, Arrigoni E, Burnquist W, Silva-Filho M, Ulian E, Hogarth D (2001) A new approach for control of *Diatraea saccharalis* (Lepidoptera: Crambidae) through the expression of an insecticidal cryia (b) protein in transgenic sugarcane. Proc Intl Soc Sugar Cane Technol 24:331–336
Braga DP, Arrigoni ED, Silva-Filho MC, Ulian EC (2003) Expression of the cry1ab protein in genetically modified sugarcane for the control of *Diatraea saccharalis* (Lepidoptera: Crambidae). J New Seeds 5:209–221
Breitler JC, Cordero MJ, Royer M, Meynard D, San Segundo B, Guiderdoni E (2001) The −689/+197 region of the maize protease inhibitor gene directs high level, wound-inducible expression of the cry1b gene which protects transgenic rice plants from stemborer attack. Mol Breed 7:259–274
Bruce HM (1970) Pheromones. Br Med Bull 26:10–13
Capinera JL (2001) Sugarcane borer, *Diatraea saccharalis* (Fabricius) (Insecta: Lepidoptera: Pyralidae). Dissertation, University of Florida Cooperative Extension Service, Institute of Food, Pub. EENY-217

Carnegie A, Leslie G (1991) Trends shown by light trap catches of some sugarcane pests. In: Proceedings of the South African Sugar Technologists' Association, pp 87–91
Carrière Y, Crowder DW, Tabashnik BE (2010) Evolutionary ecology of insect adaptation to BT crops. Evol Appl 3:561–573
Carrière Y, Crickmore N, Tabashnik BE (2015) Optimizing pyramided transgenic BT crops for sustainable pest management. Nat Biotechnol 33:161
Carrière Y, Fabrick JA, Tabashnik BE (2016) Can pyramids and seed mixtures delay resistance to bt crops? Trends Biotechnol 34:291–302
Chakroun M, Banyuls N, Bel Y, Escriche B, Ferré J (2016) Bacterial vegetative insecticidal proteins (vip) from entomopathogenic bacteria. Microbiol Mol Biol Rev 80:329–350
Chand H, Kumari R, Minnatulah ME, Paswan S (2018) Management of borers complex through pheromone traps in sugarcane agro ecosystem of Bihar. Int J Curr Microbiol App Sci 7:158–167
Charpentier L, Mathes R (1969) Cultural practices in relation to stalk moth borer infestations in sugar cane. In: Williams JR, Metcalf JR, Mungomery RW, Mathes R (eds) Pests of sugarcane. Elsevier publishing Co., New York, NY, pp 163–174
Chaudhary J (2008) Control of different borers in sugarcane crop. Cooperat Sugar 40:29–39
Chaves Solera M, Bermúdez Loría A (2006) Motivos y razones para quemar las plantaciones de caña de azúcar en Costa Rica. In: XVI Congreso de ATACA (Asociación de Técnicos Azucareros de Centro América), Costa Rica, San José, pp 248–253
Christy LA, Arvinth S, Saravanakumar M, Kanchana M, Mukunthan N, Srikanth J, Thomas G, Subramonian N (2009) Engineering sugarcane cultivars with bovine pancreatic trypsin inhibitor (aprotinin) gene for protection against top borer (*Scirpophaga excerptalis* walker). Plant Cell Rep 28:175–184
Cristofoletti PT, Kemper EL, Capella AN, Carmago SR, Cazoto JL, Ferrari F, Galvan TL, Gauer L, Monge GA, Nishikawa MA (2018) Development of transgenic sugarcane resistant to sugarcane borer. Trop Plant Biol 11:17–30
David H, Nesbitt BF, Easwaramoorthy S, Nandagopal V (1985) Application of sex pheromones in sugarcane pest management. Proc Indian Acad Sci 94:333–339
Davies J (1998) The causes and consequences of cane burning in Fiji's sugar belt. J Pacific Stud 22:5–12
Delannay X, LaVallee BJ, Proksch RK, Fuchs RL, Sims SR, Greenplate JT, Marrone PG, Dodson RB, Augustine JJ, Layton JG (1989) Field performance of transgenic tomato plants expressing the *Bacillus thuringiensis* var. Kurstaki insect control protein. Biotechnology 7:1265
Deng Z-N, Wei Y-W, Lü W-L, Li Y-R (2008) Fusion insect-resistant gene mediated by matrix attachment region (mar) sequence in transgenic sugarcane. Sugar Tech 10:87–90
Dinardo-Miranda LL, Fracasso JV (2013) Sugarcane straw and the populations of pests and nematodes. Sci Agric 70:305–310
Duong CA (2014) Light traps: an effective method for controlling the sugarcane giant borer Phragmataecia castaneae hubner in Vietnam. In: Proceedings of 5th IAPSIT International Sugar Conference, Nanning, China, pp 464–467
Falco MC, Silva-Filho MC (2003) Expression of soybean proteinase inhibitors in transgenic sugarcane plants: effects on natural defense against *Diatraea saccharalis*. Plant Physiol Biochem 41:761–766
Ferré J, Van Rie J, MacIntosh SC (2008) Insecticidal genetically modified crops and insect resistance management (IRM). Integration of insect-resistant genetically modified crops within IPM programs. Springer, New York, NY, pp 41–85
Flores S (2007) Las plagas de la caña de azúcar en México, 2nd edn. Colegio de posgraduados, México
Fogliata SV, Herrero MI, Vera MA, Castagnaro AP, Gastaminza G, Murúa MG (2019) Host plant or geographic barrier? Reproductive compatibility among *Diatraea saccharalis* populations from different host plant species and locations in Argentina. Entomol Exp Appl 167:129–140
Foster DH (1979) Trends in burnt cane processing. In: Proceedings of the Australian Society of Sugar Cane Technologists, pp 11–17

Gallo-Meagher M, Irvine J (1996) Herbicide resistant transgenic sugarcane plants containing the bar gene. Crop Sci 36:1367–1374
Gao S, Yang Y, Wang C, Guo J, Zhou D, Wu Q, Su Y, Xu L, Que Y (2016) Transgenic sugarcane with a Cry1ac gene exhibited better phenotypic traits and enhanced resistance against sugarcane borer. PLoS One 11:e0153929
Gao S, Yang Y, Xu L, Guo J, Su Y, Wu Q, Wang C, Que Y (2018) Particle bombardment of the Cry2a gene cassette induces stem borer resistance in sugarcane. Int J Mol Sci 19:1692
Garcia R, Cidade D, Castellar A, Lips A, Magioli C, Callado C, Mansur E (2007) In vitro morphogenesis patterns from shoot apices of sugar cane are determined by light and type of growth regulator. Plant Cell Tissue Organ Cult 90:181–190
Geetha P, Sivaraman K, Tayade A, Dhanapal R (2015) Sugarcane based intercropping system and its effect on cane yield. J Sugarcane Res 5:1–10
Gilbert R, Glynn N, Comstock J, Davis M (2009) Agronomic performance and genetic characterization of sugarcane transformed for resistance to sugarcane yellow leaf virus. Field Crop Res 111:39–46
Goebel FR, Nikpay A (2017) Integrated pest management in sugarcane cropping systems. In: Rapisarda C, Massimino-Cocuzza GE (eds) Integrated pest management in tropical regions. CAB International, Wallingford, pp 113–133
Goggin FL, Lorence A, Topp CN (2015) Applying high-throughput phenotyping to plant–insect interactions: picturing more resistant crops. Curr Opin Insect Sci 9:69–76
Gómez-Laverde LA, Lastra-Borja LA (1998) Insectos y caña verde. Carta Trimestral Cenicaña 4:8–10
Govinden N (1990) Intercropping of sugar-cane with potato in mauritius: a successful cropping system. Field Crop Res 25:99–110
Hernández-Martínez P, Hernández-Rodríguez CS, Van Rie J, Escriche B, Ferré J (2013) Insecticidal activity of vip3aa, vip3ad, vip3ae, and vip3af from *Bacillus thuringiensis* against lepidopteran corn pests. J Invertebr Pathol 113:78–81
Huang Y-KL (2018) Color illustration of diagnosis and control for modern sugarcane diseases, pests, and weeds. China Agriculture Press; Springer Nature Singapore Pte Ltd., Beijing; Singapore
Huang F, Leonard BR, Gable RH (2006) Comparative susceptibility of European corn borer, southwestern corn borer, and sugarcane borer (Lepidoptera: Crambidae) to cry1ab protein in a commercial *Bacillus thuringiensis* corn hybrid. J Econ Entomol 99:194–202
Ingram JW, Bynum EK (1941) The sugarcane borer. Farmer's bulletin. US Department of Agriculture, Washington, DC
Islam N, Laksana C, Chanprame S (2016) Agrobacterium-mediated transformation and expression of bt gene in transgenic sugarcane. J ISSAAS 22:84–95
Junior JADSR, Fernandes OA, Mutton MJR, Higley LG, Madaleno LL (2010) Sugarcane response to two biotic stressors: *Diatraea saccharalis* and *Mahanarva fimbriolata*. In: Proceedings of International Society of Sugar Cane Technologists (ISSCT), pp 1–5
Kalra A, Sidhu A (1955) Studies on biology of sugarcane top borer, *Scirpophaga nivella* fabr. in the Punjab. Indian Sugar 15:37–43
Kalunke RM, Kolge AM, Babu KH, Prasad DT (2009) Agrobacterium mediated transformation of sugarcane for borer resistance using cry 1aa3 gene and one-step regeneration of transgenic plants. Sugar Tech 11:355–359
Khaliq A, Ashfaq M, Akram W, Choi JK, Lee JJ (2005) Effect of plant factors, sugar contents, and control methods on the top borer (*Scirpophaga nivella* F.) infestation in selected varieties of sugarcane. Entomol Res 35:153–160
Kumar A, Chand H, Kumar N (2016) Population dynamics of borers complex on sugarcane through pheromone traps. Int J Sci Environ Technol 5:4242–4248
Lakshmanan P, Geijskes RJ, Wang L, Elliott A, Grof CP, Berding N, Smith GR (2006) Developmental and hormonal regulation of direct shoot organogenesis and somatic embryogenesis in sugarcane (*saccharum* spp. Interspecific hybrids) leaf culture. Plant Cell Rep 25:1007–1015

Legendre B (2000) Louisiana sugarcane burning. Louisiana State University Agricultural Center, Baton Rouge, LA
Leslie G (2004) Pests of sugarcane. In: James GS (ed) Sugarcane. Blackwell Science, Oxford, pp 78–100
Li X, Mu Y, Cheng Y, Liu X, Nian H (2013) Effects of intercropping sugarcane and soybean on growth, rhizosphere soil microbes, nitrogen and phosphorus availability. Acta Physiol Plant 35:1113–1119
Li W, Yin J, Luo Z, Shan H, Zhang R, Wang X, Cang X, Huang Y (2018) Research on the green control techniques for sugarcane borers. J Yunnan Agric Univ 33:168–171
Long W, Hensley S (1972) Insect pests of sugar cane. Annu Rev Entomol 17:149–176
Manchanda P, Gosal S (2012) Effect of activated charcoal, carbon sources and gelling agents on direct somatic embryogenesis and regeneration in sugarcane via leaf roll segments. Sugar Tech 14:168–173
Mendonça AF, Moreno JA, Risco SH, ICBR (1996) As brocas da cana-de-açúcar. In: Mendonça AF (ed) Pragas da cana-de-açúcar. Insetos e Cia, Maceió, pp 51–82
Mendoza AF (1996) Pragas da cana-de-açúcar. Insetos e Cia, Maceió-Al, Brasl, p 239
Misra S, Hora B (1982) Note on the incidence to top-borer (*Tryporyza nivella* Fabr.) and pink-borer (*Sesamia inferens* wlk.) on sugarcane grown in companion cropping pattern. Indian J Agric Sci 52:45–46
Mukunthan N, Easwaramoorthy S, Singaravelu B (2003) Pheromone research in sugarcane moth borers of India. Sugar Tech 5:45–49
Najarro DGR (2009) Trabajo de graduación realizado en el departamento de investigación agrícola de la caña de azúcar *Saccharum* spp., del ingenio magdalena, s.A., la democracia, escuintla. Dissertation, Universidad de San Carlos de Guatemala, p 58
Nerkar G, Thorat A, Sheelavantmath S, Kassa HB, Devarumath R (2018) Genetic transformation of sugarcane and field performance of transgenic sugarcane. Biotechnol Crop Improv 2:207–226
Nikpay A, Goebel FR (2016) Major sugarcane pests and their management in Iran. In: Proceedings of the International Society of Sugar Cane Technologists, Chiang Mai, Thailand, pp 103–108
Nikpay A, Sharafizadeh P (2018) Effectiveness of mechanically hand-removing of dead-hearts as a part of cultural management of stalk borers *Sesamia* spp. in sugarcane fields. In: Proceedings of 6th IAPSIT International Sugar Conference (IAPSIT), Udon Thani, Thailand
Nikpay A, Ziaee M, Sharafizadeh P (2015) Impact of planting date on *Sesamia* spp. (lepidoptera: Noctuidae) stalk borers' damage on five sugarcane varieties. In: Proceedings of 1st Iranian International Congress of Entomology, Tehran, Iran, p 278
Noguera A, Enrique R, Perera MF, Ostengo S, Racedo J, Costilla D, Zossi S, Cuenya MI, Filippone MP, Welin B (2015) Genetic characterization and field evaluation to recover parental phenotype in transgenic sugarcane: a step toward commercial release. Mol Breed 35:115
Ortiz Laurel H, Salgado García S, Castelán Estrada M, Córdova Sánchez S (2012) Perspectivas de la cosecha de la caña de azúcar cruda en México. Revista Mexicana de Ciencias Agrícolas 3:767–773
Palma L, Muñoz D, Berry C, Murillo J, Caballero P (2014) *Bacillus thuringiensis* toxins: an overview of their biocidal activity. Toxins 6:3296–3325
Parra J (1993) Controle das principais pragas da cana-de-açúcar. Produção da cana-de-açúcar. FEALQ, Piracicaba, pp 184–197
Parsons MJ (2003) Successful intercropping with sugarcane. In: Proceedings of South African Sugar Technologists' Associations, pp 77–98
Peng T, Sun X, Mumm RH (2014) Optimized breeding strategies for multiple trait integration: I. Minimizing linkage drag in single event introgression. Mol Breed 33:89–104
Pérez A, Caro A, Hernández C (1993) Introducción del parasitoide *Cotesia flavipes* (Cam.) en el control integrado del gusano barrenador de la caña de azúcar del ingenio de los mochis, sin. In: Simposium Nacional La Capacitación y Desarrollo Tecnológico en el Campo Cañero Mexicano. Tomo I. Xalapa, Veracruz, México, pp 515–524

Perez-Perez R (1971) Sex pheromone and blacklight trap studies with the sugarcane borer, *Diatraea saccharalis* (F.). Dissertation, The Louisiana State University, p 54

Peschke VM, Phillips RL (1992) Genetic implications of somaclonal variation in plants Advances in genetics. Elsevier, Amsterdam, pp 41–75

Polanía I, Rodríguez JAA, Maldonado HA, Cruz RM, Bayona-R M (2008) Susceptibilidad de cuatro noctuidos plaga (lepidoptera) al gene cry1ac del *Bacillus thuringiensis* incorporado al algodonero. Revista Colombiana de Entomología 34:41–51

Qamar Z, Riaz S, Nasir IA, Ali Q, Husnain T (2015) Transformation and transgenic expression studies of glyphosate tolerant and cane borer resistance genes in sugarcane (*Saccharum officinarum* L.). Mol Plant Breed 6:1

Rahman MS, Khatun S, Rahman MK (2016) Sugarcane and sugar industry in Bangladesh: an overview. Sugar Tech 18:627–635

Ram B, Karuppaiyan R, Pandey S (2011) Sugarcane cultivation in subtropical india-farmer's guide. Extension Bulletin. No. SBIRCKNL01/2011. Sugarcane Breeding Institute, Regional Centre, Haryana

Ramasamy M, Mora V, Damaj MB, Padilla CS, Ramos N, Rossi D, Solís-Gracia N, Vargas-Bautista C, Irigoyen S, DaSilva JA (2018) A biolistic-based genetic transformation system applicable to a broad-range of sugarcane and energycane varieties. GM Crops Food 9:211–227

Rang C, Bergvingson D, Bohorova N, Hoisington D, Frutos R (2004) Competition of *Bacillus thuringiensis* cry1 toxins for midgut binding sites: a basis for the development and management of transgenic tropical maize resistant to several stemborers. Curr Microbiol 49:22–27

Rao NV, Rao CVN, Bhavani B, Naidu N (2010) Influence of intercrops on incidence of early shoot borer, *Chilo infuscatellus* Snellen in sugarcane. J Entomol Res 34:275–276

Reagan TE, Martin FA (1987) Breeding for resistance to *Diatraea saccharalis*. In: Naidu MK, Sreenivasan TV, Premachandran MN (eds) Sugarcane varietal improvement. Sugarcane Breeding Institute, Coimbatore, pp 313–330

Rehman A, Qamar R, Qamar J (2014) Economic assessment of sugarcane (*Saccharum officinarum* L.) through intercropping. J Agric Chem Environ 3:24

Ricaud C, Egan B, Gillaspie A, Hughes C (2012) Diseases of sugarcane: major diseases. Elsevier Science Publishers B.V, Amsterdam

Rochat J, Goebel R, Tabone E, Begue LJ-M, Fernandez E, Tibere R, Gauvin J-C, Vercambre B (2001) Integrated control of the sugarcane spotted stalk borer *Chilo sacchariphagus* (Lep: Pyralidae) in Réunion Island. In: Proceedings of the South African Sugar Technologists' Association, pp 253–254

Rodrigo V, Nugawela A, Sivanathan A, Witharama W, Jayasinghe W (2000) Rubber cum sugarcane intercropping a suitable cropping system for the farmers in the intermediate zone of Sri Lanka. J Rubber Res Inst Sri Lanka 83:62–74

Rodríguez-del-Bosque LA, Vejar-Cota G, Vásquez-López I, Villanueva-Jiménez JA, López-Collado J, Alatorre-Rosas R, Hernández-Rosas F (2014) Plagas. In: Mata-Vázquez H, Rodríguez-del-Boque LA, Rodríguez-Morelos VH, V-G E (eds) Manejo integral de caña de azúcar. SAGARPA-INIFAP-UANL, Mexico, pp 147–186

Romeis J, Meissle M, Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat Biotechnol 24:63

Rugeles C (2015) Letalidad del fuego y el humo en la zafra cañera. Caña de azúcar, trabajadores, salud y ecosistemas, revista ambientico 252:34–36

Rutherford S (2015) IPM for Eldana control. South African Sugarcane Research Institute, Mounte Edgecombe. 86 p

Sala F, Arencibia A, Castiglione S, Christou P, Zheng Y, Han Y (1999) Molecular and field analysis of somaclonal variation in transgenic plants. In: Altman A, Ziv M, Izhar S (eds) Plant biotechnology and in vitro biology in the 21st century. Kluwer Academic Publishers, Dordrecht, pp 259–262

Sallam MNS (2006) A review of sugarcane stem borers and their natural enemies in Asia and Indian Ocean Islands: an Australian perspective. Annales de la Societe Entomologique de France 42:263–283

Salvatore AR, López G, Willink E (2009) Capítulo 11: Plagas ene l cultivo d ela caña de azúcar. In: Cañero Manual

Saravanan S, Sarvesan R, Vinod M (2011) Identification of DNA elements involved in somaclonal variants of *Rauvolfia serpentina* (L.) arising from indirect organogenesis as evaluated by ISSR analysis. Indian J Sci Technol 4:1241–1245

Sétamou M, Bernal J, Legaspi J, Mirkov T, Legaspi B Jr (2002) Evaluation of lectin-expressing transgenic sugarcane against stalkborers (Lepidoptera: Pyralidae): effects on life history parameters. J Econ Entomol 95:469–477

Showler A (2013) Beneficial and detrimental interactions between weeds and other pests of sugarcane. In: Taab A (ed) Weeds and their ecological impacts. Nova, New York, NY, pp 153–188

Showler A, Reagan T (1991) Effects of sugarcane borer, weed, and nematode control strategies in Louisiana sugarcane. Environ Entomol 20:358–370

da Silveira E (2017) Canaviais mais resistentes. Biotecnologia. Pesquisa FAPESP:80–81

Singh SP, Singh RP, Tiwari AK (2019a) Efficacy of weedicides on growth, yield a quality of sugarcane. Agrica 8(2):88–91

Singh SP, Singh P, Tiwari AK (2019b) Factors associated with sugarcane germination. Agrica 8 (2):57–60

Smith JWJ, Wiedenmann RN, Overholt WA (1993) Parasites of lepidopteran stemborers of tropical gramineous plants. ICIPE Science Press, Nairobi

Snyman S, Meyer G, Richards J, Haricharan N, Ramgareeb S, Huckett B (2006) Refining the application of direct embryogenesis in sugarcane: effect of the developmental phase of leaf disc explants and the timing of DNA transfer on transformation efficiency. Plant Cell Rep 25:1016–1023

Soberón M, Bravo A (2007) Las toxinas cry de *Bacillus thuringiensis*: Modo de acción y consecuencias de su aplicación. Biotecnología 14:303–314

Soberón M, Monnerat R, Bravo A (2018) Mode of action of cry toxins from *Bacillus thuringiensis* and resistance mechanisms. Microb Toxins:15–27

Solis AM, Metz MA (2016) An illustrated guide to the identification of the known species of Diatraea Guilding (Lepidoptera, Crambidae, Crambinae) based on genitalia. ZooKeys 565:73–121

Srikanth J, Easwaramoorthy S, Kurup NK (2000) Borer and predator incidence in sugarcane intercropped with pulses. Sugar Tech 2:36–39

Srikanth J, Salin K, Easwaramoorthy S, Kailasam C (2002) Incidence of sugarcane shoot borer under different levels of weed competition, crop geometry, intercropping and nutrient supply systems. Sugar Tech 4:149–152

Srikanth J, Subramonian N, Premachandran M (2011) Advances in transgenic research for insect resistance in sugarcane. Trop Plant Biol 4:52

Tabashnik BE, Brévault T, Carrière Y (2013) Insect resistance to bt crops: lessons from the first billion acres. Nat Biotechnol 31:510

Tabashnik BE, Zhang M, Fabrick JA, Wu Y, Gao M, Huang F, Wei J, Zhang J, Yelich A, Unnithan GC (2015) Dual mode of action of bt proteins: protoxin efficacy against resistant insects. Sci Rep 5:15107

Taparia Y, Fouad WM, Gallo M, Altpeter F (2012) Rapid production of transgenic sugarcane with the introduction of simple loci following biolistic transfer of a minimal expression cassette and direct embryogenesis. In Vitro Cell Dev Biol Plant 48:15–22

Thein MM, Jamjanya T, Hanboonsong Y (2011) Evaluation of colour traps to monitor insect vectors of sugarcane white leaf phytoplasma. Bull Insectol 64:S117–S118

Tomaz AC (2014) Genetic divergence and resistance of sugarcane genotypes to *Diatraea saccharalis*. Federal University of Viçosa, Brazil

Tomaz A, Coutinho A, Soares B, Peternelli L, Pereira E, Barbosa M (2018) Assessing resistance of sugarcane varieties to sugarcane borer *Diatraea saccharalis* Fab. (Lepidoptera: Crambidae). Bull Entomol Res 108:547–555

USDA (2018) United States Department of Agriculture, Foreign Agricultural Service. Sugar: world markets and trade. USDA, Washington, DC

Vaeck M, Reynaerts A, Höfte H, Jansens S, De Beuckeleer M, Dean C, Zabeau M, Van Montagu M, Leemans J (1987) Transgenic plants protected from insect attack. Nature 328:33

Van Der Vyver C (2010) Genetic transformation of the euploid *Saccharum officinarum* via direct and indirect embryogenesis. Sugar Tech 12:21–25

Vasil V, Vasil I (1980) Isolation and culture of cereal protoplasts. Theor Appl Genet 56:97–99

Vasil V, Vasil IK (1981) Somatic embryogenesis and plant regeneration from tissue cultures of *Pennisetum americanum*, and *p. Americanum* x *p. Purpureum* hybrid. Am J Bot 68:864–872

Vázquez AM (2001) Insight into somaclonal variation. Plant Biosyst 135:57–62

Vejar-Cota G, Rodríguez-del-Bosque L, Sahagún D (2008) Economic and ecological impacts of hand removing dead hearts caused by Diatraea considerata (Lepidoptera: Crambidae) on sugarcane in Mexico. Southwest Entomol 33:157–159

Vejar-Cota G, Rodriguez-del-Bosque LA, Caro A (2009) Impact of sugarcane burning on the stalkborer *Diatraea considerate* (Lepidoptera: Crambidae) and its parasitoid *Macrocentrus prolificus* (Hymenoptera: Braconidae) in Western Mexico. Southwest Entomol 34:213–217. 215

Viator RP, Richard EP Jr, Garrison DD, Dufrene EO Jr, Tew TL (2005) Sugarcane cultivar yield response to planting date. J Am Soc Sugar Cane Technol 25:78–87

Wang WZ, Yang BP, Feng XY, Cao ZY, Feng CL, Wang JG, Xiong GR, Shen LB, Zeng J, Zhao TT (2017) Development and characterization of transgenic sugarcane with insect resistance and herbicide tolerance. Front Plant Sci 8:1535

Way MJ, Goebel FR, Conlong DE (2004) Trapping Chilo sacchariphagus (Lepidoptera: Pyralidae) in sugarcane using synthetic pheromones. In: Proceedings of South African Sugar Technologists' Associations, pp 291–296

Weng LX, Deng H, Xu JL, Li Q, Wang LH, Jiang Z, Zhang HB, Li Q, Zhang LH (2006) Regeneration of sugarcane elite breeding lines and engineering of stem borer resistance. Pest Manag Sci 62:178–187

Weng L-X, Deng H-H, Xu J-L, Li Q, Zhang Y-Q, Jiang Z-D, Li Q-W, Chen J-W, Zhang L-H (2011) Transgenic sugarcane plants expressing high levels of modified cry1ac provide effective control against stem borers in field trials. Transgenic Res 20:759–772

Wiedenmann RN (2004) Taller internacional sobre barrenadores del tallo de caña de azúcar. Sociedad Mexicana de control biológico. In: Rodríguez-del-Bosque LA, Vejar-Cota G, Cortéz ME (eds) Ecology of lepidopteran stemborers, Los Mochis, Sinaloa, México, pp 23–28

Wilson BE, Beuzelin JM, VanWeelden MT, Reagan TE, Way MO (2017) Monitoring Mexican rice borer (Lepidoptera: Crambidae) populations in sugarcane and rice with conventional and electronic pheromone traps. J Econ Entomol 110:150–156

Xu J-S, Gao S, Xu L, Chen R (2008) Construction of expression vector of cryia (c) gene and its transformation in sugarcane. Sugar Tech 10:269–273

Zeping W, Lu L, Hongtao J, Gemin Z, Weihua H, Qiang L, Weixing D, Yijie L, Jinju W, Zhenqiang Q (2016) Field efficacy test of 22% fipronil fs against sugarcane stem borers and thrips. Plant Dis Pests 7:23

Zhang Y, Liu Y, Zhang J, Wang G, Wang J, Liu Y (2015) Assessment of transgene copy number and zygosity of transgenic maize overexpressing cry1ie gene with sybr® green qrt-pcr. In Vitro Cell Dev Biol Plant 51:125–134

Zhou D, Liu X, Gao S, Guo J, Su Y, Ling H, Wang C, Li Z, Xu L, Que Y (2018) Foreign cry1ac gene integration and endogenous borer stress-related genes synergistically improve insect resistance in sugarcane. BMC Plant Biol 18:342

Sugarcane White Leaf and Grassy Shoot Management for Healthy Seed Production in Vietnam

4

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Abstract

Sugarcane white leaf disease (SWLD) and grassy shoot disease (SGSD) are caused by phytoplasma transmission between plants through insect vectors and infected cane setts, resulting in severe reduction of global yield and quality. Vietnam has large areas of sugarcane cultivation, and huge losses are caused by SWLD and SGSD, which impact on the sugar industry and local farmers. The current situation is summarized, and strategies to control white leaf and grassy shoot phytoplasma-associated sugarcane diseases in Vietnam are presented.

Keywords

Phytoplasma · SWLD · SGSD · Vietnam · Sugarcane · Vectors

4.1 Introduction

Based on the symptoms, four types of phytoplasma-associated diseases are recognized including sugarcane white leaf (SWL), sugarcane grassy shoot (SGS), sugarcane green grassy shoot (SGGS), and sugarcane Ramu stunt (SRS). Of these, SWL, SGS, and SGGS diseases are more common and show external symptoms that are easily confused with each other (Rott et al. 2000; Marcone 2002). Sugarcane

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diseases impact on production in Asian countries, causing loss rates of 5–20% and in some severe cases up to 100% of sugarcane yield (Nasare et al. 2007; Tiwari et al. 2012). Sugarcane grassy shoot disease (SGSD) was first identified in India in 1958 (Chona 1958) and has subsequently been recorded in many other countries including Bangladesh, Malaysia, Nepal, Pakistan, Sri Lanka, and Vietnam (Rishi and Chen 1989; Sdoodee et al. 1999; Viswanathan 2001; Singh et al. 2002; Ariyarathna et al. 2007; Hoat et al. 2012). Infection rates of phytoplasma causing SGSD have been recorded at 60–100%, resulting in huge losses in productivity and sugar content (Rao et al. 2005; Srivastava et al. 2006). Sugarcane white leaf disease (SWLD) is also phytoplasma-associated and causes severe damages and losses in the sugarcane industry. SWLD was first discovered in Taiwan in 1958 and later in India and Thailand in 1964 and 1972, respectively (Chen 1974). Currently, SWLD has been detected in many sugarcane-growing countries including Thailand, Laos, India, Sri Lanka, and Australia (Ling 1962; Matsumoto et al. 1968; Pisitkul et al. 1989; Rishi and Chen 1989; Nakashima and Murata 1993; Nakashima et al. 1994, 1996, 2001; Sarindu and Clark 1993; Wongkaew et al. 1997; Kumarrasinghe and Jones 2001; Rao and Ford 2001; Hanboonsong et al. 2002, 2006; Ariyarathna et al. 2007).

SWLD was first reported in Dong Nai and Binh Thuan, southern provinces of Vietnam, in the mid-1990s due to the increase in importing ROC sugarcane varieties from Taiwan. The area of sugarcane affected by white leaf disease in Dong Nai Province and the surrounding areas in 1997 amounted to 2000 ha, and one of the most severely damaged sugarcane varieties was ROC10. Phytoplasma particles were found in sugarcane using electron microscopy, but SWLD in the south of Vietnam was thought to be caused by infected cuttings due to the lack of transmission vectors such as *Matsumuratettix hiroglyphicus*, which is found in Taiwan. The disease did not reappear until 2–3 years ago when an outbreak of SWLD was recorded with a wider range and infection spreading speed, due to uncontrolled development of Thai sugarcane varieties in southern Vietnam provinces. Recently, SWLD has been recorded in almost all Vietnamese sugarcane-growing areas especially the southeast, south-central, and central highland provinces (Fig. 4.1). This reoccurrence of SWLD in the southern provinces needs to be seriously addressed by the authorities, professionals, and sugarcane enterprises. Severe damage from sugarcane green grassy shoot disease (SGGSD) has also been reported in Nghe An Province. Although it first appeared in 2005 in the growing areas of Nghe An and Tale and Lyle Joint Venture Company, this disease had spread to an area covering almost 10,000 ha by 2010, and most sugarcane varieties grown in Nghe An Province were affected including My55-14 previously known as nonsusceptible to phytoplasma causing SGGSD. The damage caused by SGGSD to the Nghe An sugar industry is enormous and estimated at hundreds of billions of Vietnamese Dong (VND).

Fig. 4.1 Sugarcane growing areas infected by SWLD and SGSD in Vietnam

4.2 Symptoms of SWLD and SGSD

Initial symptoms of SWLD are the appearance of long, narrow, cream-colored or white stripes running from the base to the top of the leaf and parallel to the main vein. As the disease progresses, these blisters spread and merge causing the entire leaf to turn white (Fig. 4.2). Sugarcane green grassy shoot (SGGS) and grassy shoot (SGS) diseases (Fig. 4.3) show typical symptoms such as grass bushes. The leaves are usually small and narrow with blue and white stripes (Pliansinchai and Prammanee 2000). Leaves of sugarcane with these two diseases are usually short, small, and narrower than in healthy plants. Sugarcane affected by white leaf disease gradually loses its ability for photosynthesis, with loss of chlorophyll causing the plant to grow stunted, weak, and eventually rot, dry, and die. Although SWLD can be harmful at the tillering phase, the infection is mainly observed during the sugarcane germination phase. At the tillering phase, infected sugarcanes have short stems; axillary buds are more developed and most axillary shoots show symptoms of white leaves or white stripes. The sugarcanes grow poorly, producing low harvest at the end of the season.

Fig. 4.2 Symptoms of sugarcane white leaf disease in Tay Ninh Province, Vietnam

Fig. 4.3 Symptoms of sugarcane grassy shoot disease in Nghe An Province, Vietnam

4.3 Infection Conditions and Damages Caused by SWLD and SGSD

Sugarcane white leaf disease (SWLD) often arises and causes more damage to sugarcane grown on sandy soil in dry and hot conditions, which are appropriate for insect pests to transmit disease, reproduce, and develop. Alternatively, sugarcane green grassy shoot disease (SGGSD) usually arises and causes more harm for sugarcane grown in cool, high humidity conditions. Sugarcane grassy shoot disease (SGSD) is more prevalent during dry and hot summers. Damage caused by SWLD can vary greatly depending on cultivar varieties, weather, and other environmental conditions affecting growth. Sugarcane cultivation in summer-autumn is usually more prone to infection than crops grown in winter-spring. Severe damages have been reported in ratoon canes with poor management in highland and dry areas compared with well-managed crops grown in low areas with irrigation. Ling and Chuang-Yang (1962) stated that sugarcane yield and sugar content reduced by up to 75% and 30% from summer-autumn sugarcane fields infected by SWLD, with over 90% chlorophyll content reduction.

4.4 Vectors and Disease Transmission

To date, up to 12 species of leafhopper including *Balclutha rubrostriata* Melichar, *Balclutha* sp., *Bhatia olivacea* Melichar, *Exitianus indicus* Distant, *Macrosteles striifrons* Anufriew, *Matsumura tettixhiroglyphicus* Matsumura, *Recilia distincta* Motschulsky, *Recilia dorsalis* Motschulsky, *Recilia* sp., *Thaiaoryzivora* Ghauri, *Yamatotettix flavovittatus* Matsumura, and *Xestocephalus* sp. have been identified as capable of carrying phytoplasma causing SWLD, with prevalence of pathogens ranging from 5% in *Bhatia olivacea* Melichar to 35% in *Xestocephalus* sp. (Hanboonsong et al. 2006). However, only two species of aphids, i.e., *Matsumura tettixhiroglyphicus* and *Yamatotettix flavovittatus*, are capable of phytoplasma transmission causing SWLD at 55% and 45%, respectively (Matsumoto et al. 1968; Hanboonsong et al. 2002, 2006). *Deltocephalus vulgaris*, *E. indicus*, *C. unimaculata*, *Pyrilla perpusilla*, and *M. portico* are identified as causal agent of SGSD in India (Singh et al. 2002; Srivastava et al. 2006; Rao et al. 2014; Tiwari et al. 2016, 2017). In Vietnam, these insects have not yet been recorded; therefore, other species play a role in the spread of phytoplasma-associated diseases in sugarcane.

Phytoplasma is transmitted from one plant to another through grafting, infected cuttings, and insect vectors (McCoy et al. 1989). Nested PCR (polymerase chain reacion) or real-time PCR can be used to detect phytoplasma in psyllids. Srivastava et al. (2006) used nested PCR to detect phytoplasma in *Deltocephalus vulgaris*. By contrast, some studies indicated that *Proutista vulgaris* was capable of transmitting phytoplasma causing SGSD in India (Rishi and Chen 1989). However, *P. moesta* has not been recorded in Australia or Vietnam, so another insect acts as a vector for the transmission of phytoplasma causing SGSD (Tran-Nguyen et al. 2000).

4.5 Management Approach

To date, the most effective method to prevent phytoplasma-associated diseases in sugarcane such as SWLD and SGSD is tissue culture of pathogen-free plant stocks, together with prevention of phytoplasma transmission by insect vectors in the field (Lee et al. 2000). Advancements in biotechnology and molecular techniques now allow rapid detection of phytoplasma 16SrXI causing SWLD and SGSD in sugarcane fields at an early stage, resulting in successful disease prevention and spread (Marcone and Rao 2008; Rao et al. 2012; Hoat et al. 2013). In Vietnam, SWLD and SGSD are common phytoplasma-associated diseases. However, various approaches to control SWLD and SGSD such as treatment of cuttings with hot water (50 °C for 2 h and 54 °C for 30 min), chemical treatment of cuttings with tetracycline or Ledermycin at a concentration of 500 ppm, mechanized methods (cutting and digging out diseased plants), and reducing insect vectors in sugarcane fields still have limitations. Effective management of SWLD and SGSD is not possible using chemical and mechanical methods, while vector management is difficult to implement and less feasible for treatment of cutting with hot water and antibiotics. Some novel approaches such as cryotherapy, UV light treatment, and endophytic bacteria

have been developed and suggested for efficient elimination of phytoplasma and production of pathogen-free plant stocks; however, the use of moist hot air or a hot water approach is still considered the recommended method to reduce grassy shoot and white leaf disease transmitted by cane setts (Frison and Putter 1993; Kaewmanee and Hanboonsong 2011; Rao et al. 2012). Therefore, for effective, long-lasting management of sugarcane phytoplasma in Vietnam, many approaches can be applied in sugarcane-growing areas as follows:

1. Set up a system of production and propagation of healthy sugarcane with a three-tier seed program that is capable of providing enough commercial varieties for new cultivation every year (Fig. 4.4).
2. Apply cane setts with hot water at 54 °C for 2.5 h or antibiotic solutions such as tetracycline and Ledermycin, at a concentration of 500 ppm, to eliminate pathogens in cuttings before planting in breeder seed cane fields, followed by growing foundation seed cane and certified seed cane in fields to meet the needs of new cultivation every year. The three-tier seed program should be optimized to produce healthy sugarcane suitable for the conditions of each sugarcane-growing region in Vietnam. This will be highly effective in the long term both technically and economically.
3. Establish training courses to educate farmers in the dangers of using cuttings from SWLD- and SGSD-infected fields for breeding. At the same time, make it widely known that rootstock for all new sugarcane fields should be obtained from designated addresses and recommended as a three-tier seed program. Farmers should be prohibited to source seed canes from material fields.
4. Coordinate with specialized research agencies to identify the vectors transmitting phytoplasma causing SWLD and SGSD in Vietnam. Studies should be conducted to better understand the reproduction and development of insect vectors and instigate effective control methods.
5. Regular visits should be made to sugarcane fields to discover and destroy root canes showing infected phytoplasma-associated diseases with damage rate over 20%.
6. Evaluate SWLD/SGSD resistance for all domestic and imported sugarcane varieties before releasing for mass production.
7. Minimize imports of foreign varieties, particularly from Thailand, which do not comply with quarantine regulations of post-imported sugarcane varieties set by the United Nations Food and Agriculture Organization (FAO) and the International Board for Plant Genetic Resources (IBPGR), following technical guidelines for safe movement of sugarcane germplasm in 1993.
8. Strictly control the removal of cuttings from infected areas.

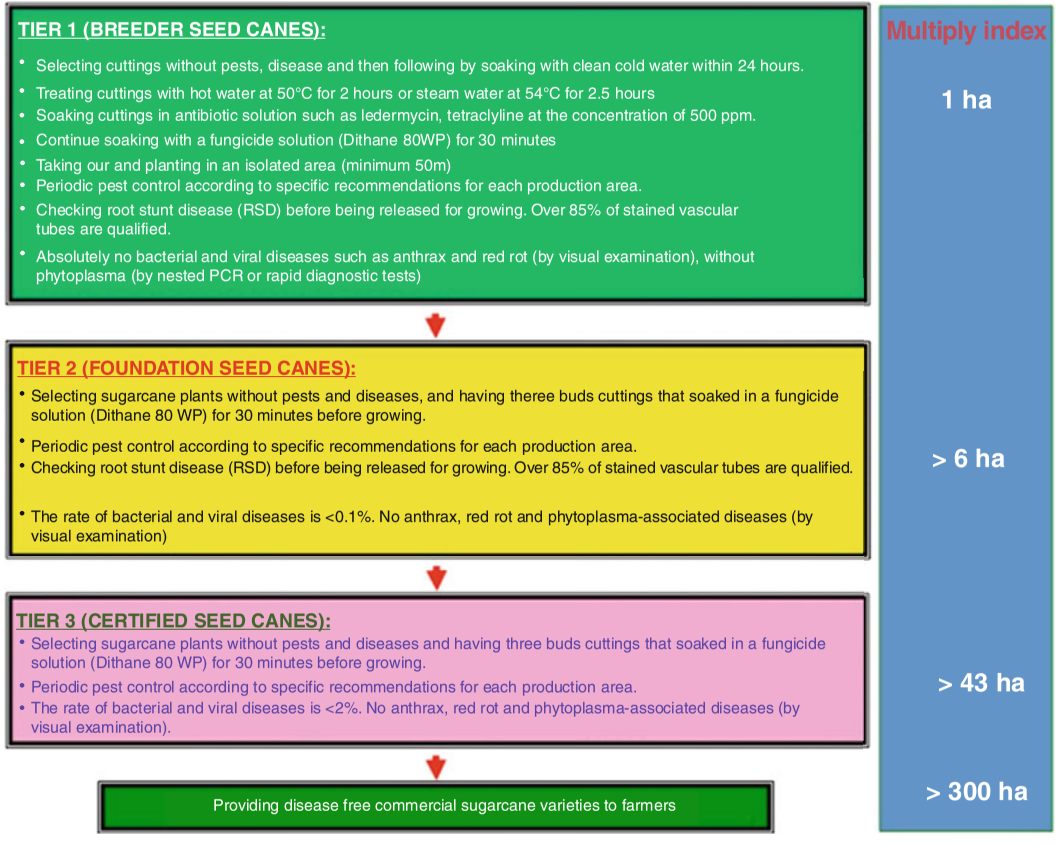

Fig. 4.4 Three-tier program for healthy sugarcane production in Vietnam

4.6 Conclusions

Sugarcane white leaf and grassy shoot diseases spread very quickly, resulting in rapid increase of infected sugarcane-growing areas, causing severe productivity losses and reduced quality of sugarcane. This directly affects the lives of sugarcane farmers and impacts the sugar industry not only in Vietnam but also in South East Asia and the Asia-Pacific region. Recent advances in biotechnological methods can now rapidly detect phytoplasma at an early stage in the field. Methods must be implemented to better understand the infection mechanisms of phytoplasma and vectors of disease transmission and devise effective ways to control SWLD and SGSD. Presently, the best available solutions to control SWLD and SGSD are being applied throughout sugarcane-growing areas in Vietnam, but some immediate approaches can be implemented to help farmers identify the symptoms, understand the risks of these diseases in their fields, and follow the habit of selecting pathogen-free cuttings from healthy cane fields while also collecting and destroying infected material. Effective control of sugarcane white leaf and grassy shoot diseases in the field requires integrated management of pathogen-free cuttings through water and chemical treatments, cultivation of resistant varieties, pest management, appropriate farming systems, and the use of disease-free planting areas to focus on minimizing the symptoms and risks caused by phytoplasma.

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References

Ariyarathna HACK, Everard JMDT, Kurunanayake EH (2007) Diseased sugarcane in Sri Lanka is infected with sugarcane grassy shoot and/or sugarcane white leaf phytoplasma. Aust Plant Dis Notes 2:123–125

Chen CT (1974) Sugarcane white leaf disease in Thailand and Taiwan. Sugarcane Pathol Newslett 11/12:23

Chona BL (1958) Some disease of sugarcane reported from India in recent year. Indian Phytopathol 11:1–9

Frison EA, Putter CAJ (1993) FAO/IBPGR technical guidelines for the safe movement of sugarcane germplasm. Food and Agriculture Organization of the United Nations/International Board for Plant Genetic Resources, Rome

Hanboonsong Y, Choosai C, Panyim S, Damak S (2002) Transovarial transmission of sugarcane white leaf phytoplasma in the insect vector *Matsumura tettixhiroglyphicus* (Matsumura). Insect Mol Biol 11:97–103

Hanboonsong Y, Ritthison W, Choosai C, Sirithorn P (2006) Transmission of sugarcane white leaf phytoplasma by *Yamatotettix flavovittatus*, a new leaf-hopper vector. J Econ Entomol 99:1531–1537

Hoat TX, Bon NG, Quan MV, Hien VD, Thanh ND, Dickinson M (2012) Detection and molecular characterization of sugarcane grassy shoot phytoplasma in Vietnam. Phytoparasitica 40:351–359

Hoat TX, Nhung LTT, Thanh DVT, Bon NG, Duong CA, Ha TN, Kumasaringhe NC (2013) Molecular detection and identification of sugarcane white leaf phytoplasma in Vietnam. Int Sugar J 115:505–511

Kaewmanee C, Hanboonsong Y (2011) Evaluation of the efficiency of various treatments used for sugarcane white leaf phytoplasma control. Bull Insectol 64(Suppl):S197–S198

Kumarrasinghe NC, Jones P (2001) Identification of white leaf disease of sugarcane in Sri Lanka. Sugar Tech 3:55–58

Lee IM, Davis RE, Gundersen-Rindal DE (2000) Phytoplasmas: phytopathogenic mollicutes. Annu Rev Microbiol 54:221–255

Ling KC (1962) White leaf disease of sugarcane. Taiwan Sugar 9:1–5

Ling KC, Chuang-Yang C (1962) A preliminary study on the white leaf disease of sugarcane. Rep Republ Taiwan Sugar Exp Stat 28:139–172

Marcone C (2002) Phytoplasma diseases of sugarcane. Sugar Tech 4(3-4):79–85

Marcone C, Rao GP (2008) "*Candidatus* Phytoplasm cynodontis": the causal agent of Bermuda Grass White Leaf disease. In: Harrison NA, Rao GP, Marcone C (eds) Characterization, diagnosis and management of phytoplasma. Studium Press LLC, Houston, TX, pp 353–364

Matsumoto T, Lee CS, Teng WS (1968) Studies on sugarcane white leaf disease of Taiwan with special references to transmission by leafhopper, *Epitettix higroglyphicus* Mats. Proc Int Soc Sugarcane Technol 13:1090–1098

McCoy RE, Caudwell A, Chang CJ et al (1989) Plant disease associated with mycoplasma-like organisms. In: Whitcomb RF, Tully JG (eds) The mycoplasma. Volume 5. Spiroplasmas, acholeplasmas, and mycoplasmas of plants and arthropods. Academic Press, San Diego, CA, pp 545–640

Nakashima K, Murata N (1993) Destructive plant diseases caused by mycoplasma-like organisms in Asia. Outlook Agric 22:53–58

Nakashima K, Chalepron W, Wongkaew P, Sirithorn P (1994) Detection of mycoplasma like organism associated with white leaf disease of sugarcane in Thailand using DIVA probes. JIRCAS J 1:1–57

Nakashima K, Hayashi T, Chaleeprom W, Wongkaew P, Sirithron P (1996) Complex phytoplasma flora in Northeast Thailand as revealed by 16S rDNA analysis. Ann Phytopathol Soc Jpn 62:57–60

Nakashima K, Wungkaew P, Sirithorn P (2001) Molecular detection and characterization of sugarcane white leaf phytoplasmas. In: Rao GP, Ford RE, Tosic M, Teakle DS (eds) Sugarcane pathology. Volume II. Virus and phytoplasma diseases. Science Publishers Inc, Enfield, NH, pp 157–175

Nasare K, Yadav A, Singh AK, Shivasharanappa KB, Nerkar YS, Reddy VS (2007) Molecular and symptom analysis reveal the presence of new phytoplasmas associated with sugarcane grassy shoot disease in India. Plant Dis 91:1413–1418

Pisitkul S, Kanta C, Wongkaew S, Neera P, Chaloy P, Chetarash C (1989) Studies on the insect vector of sugarcane white leaf and their control. KhonKaen Agric 17:164–172

Pliansinchai U, Prammanee S (2000) Green grassy shoot. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (eds) A guide to sugarcane diseases. CIRAD Publication Service, Montpellier, pp 221–225

Rao GP, Ford E (2001) Vectors and phytoplasma diseases of sugarcane-an overview. In: Rao GP, Ford RE, Tosic M, Teakle DS (eds) Sugarcane pathology. Volume II. Virus and phytoplasma diseases. Science Publishers Inc, Enfield, NH, pp 267–318

Rao GP, Singh A, Singh HB, Sharma SR (2005) Phytoplasma diseases of sugarcane: characterization, diagnosis and management. Indian J Plant Pathol 23(1-2):1–21

Rao GP, Mall S, Marcone C (2012) Recent biotechnological approaches in diagnosis and management of sugarcane phytoplasma diseases. Funct Plant Sci Biotechnol 6(2):19–29

Rao GP, Madhupriya, Tiwari AK, Kumar S, Baranwal VK (2014) Identification of sugarcane grassy shoot-associated phytoplasma and one of its putative vectors in India. Phytoparasitica 42:349–354

Rishi N, Chen CT (1989) Grassy shoot and white leaf disease. In: Ricaud C, Egan BT (eds) Disease of sugarcane: major diseases. Elsevier, Amsterdam, pp 289–300

Rott P, Bailey RA, Comstock JC, Croft B, Saumtally AS (2000) A guide to sugarcane diseases. CIRAD Publication Service, Montpellier. 339 p

Sarindu N, Clark MF (1993) Antibody production and identification of MLOs associated with sugarcane white leaf disease and Bermuda grass white leaf disease from Thailand. Plant Pathol 42:396–402

Sdoodee R, Schneider B, Padovan A, Gibbs K (1999) Detection and genetic relatedness of phytoplasmas associated with plant diseases in Thailand. J Biochem Mol Biol Biophys 3:133–142

Singh V, Baitha A, Sinha OK (2002) Transmission of grassy shoot disease of sugarcane by a leafhopper (*Deltocephalus vulgaris*). Indian J Sugarcane Technol 17:60–63

Srivastava S, Singh V, Gupta PS, Sinha OK, Baitha A (2006) Nested PCR assay for detection of sugarcane grassy shoot phytoplasma in the leafhopper vector *Deltocephalus vulgaris*: a first report. Plant Pathol 22:25–32

Tiwari AK, Vishwakarma SK, Rao GP (2012) Increasing incidence of sugarcane grassy shoot disease in Uttar Pradesh, India and its impact on yield and quality of sugarcane. Phytopathogen Mollicut 2:63–67

Tiwari AK, Madhupriya, Srivastava VK, Pandey KP, Sharma BL, Rao GP (2016) Detection of sugarcane grassy shoot phytoplasma (16SrXI-B subgroup) in *Pyrilla perpusilla* Walker in Uttar Pradesh, India. Phytopathogen Mollicut 6:56–59

Tiwari AK, Kumar S, Mall S, Jadon V, Rao GP (2017) New efficient natural leafhopper vectors of sugarcane grassy shoot phytoplasma in India. Sugar Tech 19:191. https://doi.org/10.1007/s12355-016-0455-9

Tran-Nguyen L, Blanche KR, Egan B, Gibb KS (2000) Diversity of phytoplasma in northern Australian sugarcane and other grasses. Plant Pathol 49:666–679

Viswanathan R (2001) Different aerated steam therapy regimes on the development of grassy shoot disease symptoms in sugarcane. Sugar Tech 3(3):83–91

Wongkaew P, Hanboonsong Y, Sirithon P, Choosai C, Boonkrongs S, Tinnangwattana T, KitchareonPanya R, Damak S (1997) Differentiation of phytoplasmas associated with sugarcane and gramineous weed white leaf disease and sugarcane grassy shoot by RFLP analysis and sequencing. Theor Appl Genet 95:660–663

Vegetable Seed Production: Prospects and Challenges: The Case of Ghana

5

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Abstract

Quality seed is a prerequisite for a profitable vegetable industry. The climate in Ghana is conducive for the production of a wide range of vegetables. Seeds of various local crops marketed in Ghana are developed, released and registered by the Ghanaian research institutions. However, exotic vegetable seeds are imported and supplied by private seed sector. All seeds marketed in Ghana have to meet the minimum standard stipulated in the Plants and Fertilizers Act 2010. Vegetable seed production is normally done in special seed production fields or structures. Seed production requires the application of appropriate agronomic principles necessary for the production of good-quality seeds. Seeds need to be genetically and physically pure, physiologically viable and free from weeds, insect pests and diseases. To achieve this, trained and skilled personnel have to be included in the production and postharvest activities prior to the seed reaching the end user. Modern commercial vegetable production systems are based on crop uniformity. To obtain the required crop uniformity and for precision drilling, vegetable seeds are normally graded. A good packaging and labelling are also important in seed business. The packaging material should be designed to create an airtight condition within the seed environment. Seed storage preserves the viability and vigour of seeds until marketing and protects the seed marketer's profits and reputation.

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Through seed distribution and marketing, vegetable seeds are made available to the farmer who is the end user. Changes in temperature and relative humidity, which normally contribute to deterioration of the containers, labelling system and seeds not in vapour-proof cans should be minimized during transportation. There are prospects for vegetable seed production in Ghana based on factors including the high demand for local and exotic vegetable seeds, favourable climatic conditions, availability of manpower and government policies supporting the vegetable seed industry. This is not to forget the challenges that are associated with vegetable seed production including the maintenance of genetic purity, pests and diseases, storage and marketing.

Keywords

Vegetable seeds · Seed production · Agronomic principles · Crop uniformity

5.1 Introduction

Vegetables provide food, employment and raw materials for industrial purposes and foreign exchange. They are used for cooking soups and stews or grounded and eaten raw with other staples. As food, vegetables are important sources of essential vitamins, minerals and fibres for human growth and development. Ghana occupies the 149th position among vegetable-producing countries in the world with China, India, Vietnam, Nigeria and the Philippines in the lead. The quantity of vegetables produced in Ghana increased from an estimated 8200 tonnes in 2007 to 12,453 tonnes in 2017 (FAOSTAT 2019). However, local production of some vegetables like tomato and onion (dry and fresh) is supplemented by imports from Burkina Faso and Togo, respectively (Netherlands-African Business Council 2014; Melomey et al. 2019). Both rural and urban farmers engage in vegetable production in Ghana. It provides food, employment and raw materials for industrial purposes and foreign exchange. Vegetables are used for cooking soups and stews or grounded and eaten raw with other staples. The growing middle class also eats vegetables as salads. Vegetable production in Ghana can be classified into three distinct components, namely, commercial/market gardening, medium-scale production for contractors/middlemen and small-scale domestic/backyard farming (Saavedra et al. 2014). Production is carried out in the rural and urban areas for urban market and exports. Generally, vegetable production is rain-fed; however, irrigated agriculture is increasing around the Volta River (Netherlands-African Business Council 2014) as well as urban areas in and around Accra and Kumasi. Production is seasonal, and the types of vegetables produced vary from one agroecological zone to the other. Tomato (*Solanum lycopersicum* L.), chilli pepper (*Capsicum annuum*), hot pepper (*C. frutescens*), okra (*Abelmoschus esculentus*) and eggplant (*Solanum melongena*) are grown across most of the agroecological zones. Shallot (*Allium cepa* var. *aggregatum*) is produced in the coastal savannah zone, whilst onion (*Allium cepa* L.) is produced in the Sudan savannah zone (Badiane et al. 1992; Gerken et al. 2001; Seini and Nyanteng 2003). Leafy vegetables such as ayoyo (*Corchorus olitorius*),

alefu (*Amaranthus caudatus*) and bra or roselle (*Hibiscus sabdariffa*) are cultivated in various parts of the country (Drechsel and Keraita 2014). Exotic vegetables are usually cultivated in urban areas, and these include lettuce, cauliflower, carrots, mints, cucumber, spring onion, radish, green pepper, Asian vegetables (cabbage, marrow, tinda and ravaya), French beans and butternut squash (Sinnadurai 1973; Saavedra et al. 2014; MOFA 2016).

The vegetable market in Ghana is wide including supermarkets, hotels and restaurant within the country as well as regional market and EU market (Saavedra et al. 2014). The vegetable market has great potential given the high-value domestic market and export opportunities. The domestic market is increasing at more than 10% per annum, and the export market is estimated at US$250 million. In order to tap the full potential of this market, the sector needs to be improved through investment and innovations as well as improvement in the business climate. This will involve improvement on credit availability, quality inspection services, export logistics and availability of agricultural input (Netherlands-African Business Council 2014).

Quality seed is a prerequisite to a vibrant and profitable agricultural industry. The seed industry in Ghana began in 1958 and was managed by the public sector until 1989 when it was privatized. The seed sector is made up of both formal and informal delivery systems. Local vegetable seeds in Ghana are developed, released and registered by the Ghanaian research institutions. Nonetheless, most of the vegetable seeds are imported and supplied by private seed sector. The key private companies that import vegetable seed into the country are Dizengoff, Wienco and AgriServ (field interviews, NASTAG, GSID-2016 cited in the early generation seed report). The seed law frowns on importation of certified seeds into the country but encourages the multiplication of foundation seeds to certified seeds locally by multinational seed companies. The seed sector is plagued with many challenges such as weak institutional linkages and unclear mandates, inadequate collaboration among participating partners, poor oversight arrangements and inadequate resources to support both public servicing agencies and inexperienced private seed entities. Commercial seed production is in its infancy, and therefore, production and distribution of certified seed are limited. This has resulted in the informal seed sector supplying about 80% of seed used in the country (National Seed Plan 2015). This chapter looks at the current state of the vegetable seed system in Ghana and the prospects and challenges militating against realising the full potential of the system.

5.2 Vegetable Seed Systems

Ghana has two distinct seed systems, namely, traditional (informal) and formal. However, there is an emerging integrated system which combines the two systems (Sperling and Cooper 2003). This integrated system is mostly operated under the community seed production, farmer-based organizations and non-governmental organizations.

5.2.1 Traditional Vegetable Seed System

The traditional or informal seed system is not regulated by law (Etwire et al. 2013). The traditional vegetable seed system is characterized by farmer-to-farmer seed exchange, purchase of grain or seed extraction from fruit purchased at local markets and seeds from previous savings, barter and gift. This forms the major source of vegetable seeds planted by farmers and vegetable crop seeds farmers save for planting, and regularly purchased or exchanged are tomato, pepper, garden eggs and okra (Addo-Quaye and Djokoto 2013). The delivery of these seeds especially in the remote areas is done through raising of seedlings by selected farmers who nurse the seeds of common vegetables for cash sales at the beginning of the farming season. This affords local farmers the opportunity of selecting healthy and uniform seedlings for timely planting. Farmer-to-farmer spread is usually informed by farmers' observation of new varieties in other farmers' fields or by learning about them from friends or relatives or observing the fresh fruit on the market. The quality of seeds delivered through this system is usually low; however, through participatory breeding, establishment of demonstration and farmer field days, farmers have been trained on how to improve the seeds through extraction, sorting, grading and treatment using local materials.

The traditional seed systems have both merits and demerits. The problem of the three "As" (availability, accessibility and affordability) does not exist since in most cases farmers are able to readily buy or exchange enough seeds to plant their smallholdings of cultivation (Pousseu et al. 2014). Crop adaptation is not a challenge as crops selected are locally grown which are also resistant to pests and diseases. This system continues to play a major role in biodiversity conservation and provide germplasm for crop improvement even under the formal seed system. Dissemination and adoption of new varieties are faster under this system compared to the formal system. On the other hand, low seed quality is a major problem. Purity, both genetic and physical, is poor which cumulates in poor yield. Seed-borne diseases are easily transmitted from one farm to the other as sorting and seed treatments are rarely carried out (Ayana et al. 2014). Vegetable seeds under this system also have problem of low viability as a result of poor packaging and storage practices leading to low field establishment.

5.2.2 Formal Vegetable Seed System

The formal seed system is regulated by laws enforced by state agencies (Herpers et al. 2017), and seed development is handled by research institutions including universities and private breeders. Large proportion of vegetable seeds demand in Ghana is met through commercial seed imports mainly from Europe and Asia by private seed importers, and it includes cabbage, cucumber, carrot and lettuce. This is partly due to the unfavourable climatic conditions required for the reproductive development of these exotic vegetables cultivated in Ghana. Unavailability of certified seeds of crops like the chillies, tomatoes and okra which can be produced

in the country is another reason accounting for the importation of the seeds. Linked to this is the non-involvement of local vegetable seed producers who are largely interested in the cereal and legume crops.

Vegetable seeds delivered under this system whether imported or developed locally will have to meet the minimum standard stipulated in the Plants and Fertilizers Act 2010 (Act 803). Furtherance to this, seed legislation in Ghana restricts persons to the following: "Subject to the Exports and Imports Act 1995 (Act 503), a person shall not produce, condition or market any seed unless (a) the seed is of a registered variety, (b) is of a standard prescribed by this Act or its Regulations, (c) it is multiplied in a seed multiplication farm, conditioned in a seed conditioning plant or tested in a registered laboratory, and (d) packaged and labelled as prescribed by this Act or its Regulations."

Three chilli pepper varieties have been released officially in Ghana under this system, namely, Legon 18 by the University of Ghana and "mako ntose" and "shito adope" by Crops Research Institute. Unlike the traditional seed system, seeds delivered under the formal system are of high quality in terms of genetic and physical purity, uniformity, high germination percentage resulting in higher field establishment and improved yield. However, since over 80% of the vegetable seeds are imported, availability, accessibility and affordability become a challenge. Another challenge is lack of storage facilities for these exotic vegetable seeds as importers, wholesalers and retailers do not have good storage conditions leading to loss of viability especially carry-over seeds. Also, adaptability of the exotic vegetable crop varieties disseminated under this system sometimes faces harsh environmental conditions.

5.2.3 Regulation of the Vegetable Seed System

Regulating the seed industry requires seed law which establishes a legal framework by which all activities from seed development up to utilization are governed. Ghana's seed regulatory system just like the entire industry is undergoing transformation to meet both local farmers' needs and broader regional harmonization within the ECOWAS (ECOWAS 2008) and other international bodies such as the International Union for the Protection of New Varieties of Plants (UPOV), International Seed Testing Association (ISTA) and World Trade Organization's (WTO) Sanitary and Phytosanitory (SPS) Agreement. In the last decade, Ghana's seed industry in general has released four important documents. These include the Plants and Fertilizer Act 2010 (Act 803), the Ghana Seed Policy (2013), the National Seed Plan (2015) and the National Catalogue (2015). The Ministry of Food and Agriculture (MOFA) through its directorates has primary regulatory responsibility over the seed sector and exercises responsibility over the formal seed sector. The Ghana Seed Inspectorate Division under the Plant Protection and Regulatory Services Directorate of MOFA is responsible for implementing most of the quality standards from production to distribution, whereas the Crops Services Directorate is in charge of variety development, release and registration.

Part two of the Plants and Fertilizer Act 2010 (Act 803) talks about the regulation, monitoring of exportation, importation and commercial transaction of seeds and related matters (sections 30–47). Any person or organization engaged in vegetable seed production or commercialization is expected to register with MOFA to get the required license/permit to operate. For instance, any person who wants to import vegetable seeds into the country is expected to apply for import permit to bring samples for testing by an accredited research organization or entity before commercial quantities can be imported or otherwise based on the recommendation after testing (Plants and Fertilizer Act 2010 (Act 803)). For variety development and release, a researcher/institution is required to apply to the Variety Release and Registration Committee for a minimum of two inspections to be conducted after which a recommendation is submitted to the National Seed Council (NSC) for approval or otherwise. Sections 65 and 66 of the Plants and Fertilizer Act 2010 (Act 803) stipulate the offences and penalties for any persons or organizations that go contrary to the regulations.

Ghana also belongs to the African Regional Intellectual Property Organization (ARIPO), a regional organization dedicated to intellectual property rights (IPR) (Kuhlmann and Yuan 2016). Another important bill which is very vital in the development and release of vegetable crop varieties is the Plant Breeder's Bill (PBB) which is currently before parliament. When this bill is passed, it will provide full plants variety protection in the country.

5.3 Vegetable Seed Production

5.3.1 Why Start with a Good Seed?

The fundamental input in crop production is "seed." Accessibility to affordable quality seed with superior characteristics is a means to increasing agricultural production and productivity. Sowing high-quality seed is very necessary in commercial crop production. Poor-quality seeds result in low field emergence and weak seedlings that are usually susceptible to biotic and abiotic stresses which reduced the quality and yield of the crops produced. Good seed gives better results with less effort. Quality seed is always characterized with high analytical purity devoid of other species, varieties and weed seeds. Good seed always results in good germination, good emergence and high vigour.

5.3.2 Source of Vegetable Seeds

Seeds used in vegetable seed production must be of high quality in terms of its purity and also from the right class (breeder seed, foundation seed and certified seed) obtained from authorized official quarters. Some features must be critically looked at whilst acquiring the seeds:

- The seed is from the recommended class. It is always important to note that for the purpose of raising a foundation seed, seed from the breeder's seed class is used, and for the purpose of certified seed production, seed from the foundation seed class is used.
- The seeds are tagged with the right colour tag indicating the class of seed and labelled with the producer's name, address and characteristics of the seed as in germination percentage and the percentage of other materials rather than the specified seed on the label.
- The validity period of the seed is indicated on the seed packaging material and has not expired its validity.

There are basically two main sources of vegetable seed. They are local and foreign sources:

- *Local source*
  This type of seed is characterized by farmer's own way of getting their seed. The local source is a form of an informal seed system where farmers either save their own seed or access seeds through exchange, gift and barter and from the local market. With the local source of seed, farmers use their own saved seed and recycle the planting materials year after year. This is a cheap and easy source of accessing planting materials. However, they are characterized by purity problems, that is, the seeds are usually not true to type and may contain some admixtures like weed seeds, stones and seeds of other plants and varieties and low mean germination rate.
- *Imported seed*
  Until recently, vegetable farmers in Ghana rely on their own source of seed for planting. With the passing of the harmonized seed legislation in West Africa and free trade among other African countries, farmers can now trade among themselves for seed especially vegetable seeds. Imported seed is easily accessed by farmers from the agro dealers and from grocery shops, especially plants that do not produce seeds under tropical/temperate condition. Imported seeds despite its advantages have some disadvantages which include unknown source, failure to germinate as it might not be properly stored and lack of available information on its genetic quality.

5.3.3 Principles of Vegetable Seed Production

High technical know-how is required in producing genetically pure and quality pure-bred vegetable seed. Production of such seeds must be conducted under strict and well-structured conditions. Vegetable seed growers must be familiar with the genetic and agronomic principles of vegetable seed production and also attention given to the maintenance of the genetic purity so as to achieve the full potential of the superior seed being introduced.

5.3.3.1 Genetic Deterioration

In seed production, it is always essential to ensure that the seed is true to type. This is achieved by following the necessary steps listed in the production guide of the particular variety. Number of factors affects genetic purity of a variety during the phase of production; these include the following:

- Developmental variation: This type of variation happens when seeds are cultivated or grown in different (difficult) environment, different soil and climate condition or under different photoperiods. This variation can be reduced when crops are grown in their areas of adaptation and growing season.
- Mechanical mixtures: This is very critical during seed production. These mixtures occur during planting, when different varieties are used in sowing with one seed drill. These seeds act as volunteer plant, thereby resulting in varietal mixtures. To avoid these mechanical mixtures, there is the need to rogue the seed fields and practice extreme care during seed production, harvesting, threshing and other handling.
- Natural crossing: this deterioration usually occurs when there is natural crossing with diseased plant, off-type plants or undesirable types.
- Mutation: this type of deterioration is normally not detected when minor mutation occurs.
- Minor genetic variation: These variations occur later in production cycle due to selective elimination by the environment. Yield trials are done to offset these problems in case it happens.
- Selection influence of diseases: New varieties of crop often become susceptible to new races of diseases which are often caused by obligate parasites that were not previously considered in the seed programme. Also, vegetatively propagated seeds deteriorate very fast if affected by viral, fungal and bacterial diseases.
- Breeder's technique: This happens as a result of cytogenetical irregularities not correctly assessed in the elite varieties before their release. Also, factors like male sterility breakdown, adverse environmental conditions and heritable variation may significantly affect the genetic purity.

As indicated by Kadam in 1942, genetic deterioration of seed, mechanical mixtures, selection influence of disease and natural crossing are possibly the main reasons for genetic deterioration of genetic lines (pure seed) during seed production. Genetic shift and developmental variations may also occur when seeds are grown or raised outside the area of their adaptation.

5.3.3.2 Maintenance of Genetic Purity

Maintenance of genetic purity in seed production is very vital for every country's seed system. Genetic purity of seeds is maintained if the following measures are considered:

- Use only approved seed in seed multiplication. In maintaining the genetic purity of any seed source, it is very important to ensure that only the right classes of

seeds are used. For example, in the production of foundation seeds, the breeder seed class is used, whilst in the production of certified seeds, the foundation seed class is used.

- Inspect and approve field prior to planting.
- Crop rotation. Adequate intervals between related and similar crops are required to minimize the risk of planting materials or dominant seeds remaining from the previous season which are likely to contaminate the planned seed crop. Also in crop rotation putting in mind planting crops that will take care of the nutrition of the soil, maintain the soil physical structure and condition, whilst minimizing the risk of soil-borne pathogens and diseases is also considered.
- Timely inspection of growing crops at crucial stages of the plants for authentication of genetic purity and detection of weed and admixtures and also to ensure the fields are free from noxious weed and viral and seed-borne diseases.
- Sample and seal cleaned seed lots.
- Grow samples of potentially approved seeds for evaluation and confirmation with authentic seeds.
- Ensure adequate isolation to prevent contamination by natural crossing and mechanical mixtures.
- Timely rouging of off-type plant at various stages to prevent contaminations.
- Periodic testing of varieties to sure genetic purity.
- Grow crops in their area of adaptation to avoid genetic shift.
- Seed certification to maintain quality and genetic purity.
- Adopting the generational system. That is strictly adhering to the production from breeder seed where the seed can only be grown in three generations (foundation seeds, registered seeds and certified seeds).
- Grow-out test. Different seeds grown for seed production must be tested periodically for genetic purity by grow-out tests to ensure that their genetic purity is maintained in its original form.

5.3.3.3 Agronomic Principles

- Selection of a suitable agroclimatic condition
  For any crop variety to be grown for seed production, it must be adaptable to the temperature and photoperiodic condition prevailing in that particular environment.
- Selection of seed and field for planting
  Site selected for planting must be free from volunteer plants, fallowed for some time and free from noxious and related weeds that might act as alternate host for insect pest of the intended plant and have good soil structure and texture and high nutrient content to support plant growth, and the soil should also be free from any soil-borne disease and insect pest.
- Isolation of seed crop
  Field ear marked for seed production must be adequately isolated either by time or by distance from other nearby fields of the same crops and other contaminating crops depending on the requirement of the certification standards.
- Land preparation

Better land preparation improves seed germination, good stand establishment and destruction of seed bank of potential weeds. Good land preparation ensures good aeration in the soil and helps in water management and uniform irrigation.

- Varietal selection
  The variety of seed for seed production must be cautiously selected, i.e. varieties with superior qualities like disease tolerant/resistant, early maturing, quality grain, higher yielding and climate resilient and in high demand.
- Seed treatment
  Depending on the type of seed and the requirement, the following seed treatment could be given: chemical seed treatment, bacterial inoculation for legumes and seed treatment for breaking dormancy.
- Time of planting
  Planting of seed should be consistent with their normal time of planting. Unless there is incidence of diseases and pests, then some adjustment could be made to offset those perceived problems.
- Seed rate
  In commercial seed production, lower seed rate is preferable to facilitate rouging operation and inspection of seed fields.
- Method and depth of sowing
  The use of mechanical drillers for sowing seed is the most efficient. Bigger or lager seeds are sown at a greater depth, whilst smaller seed is sown in a lower depth; this ensures good plant stand.
- Rouging
  Adequate and timely rouging is very important in seed production to prevent contamination. It is necessary to rouge at all stages of the plant: vegetate or preflowering stage, flowering stage and at maturity.
- Supplementary pollination
  Construction of honeybee hives in very close proximity to the seed production field is very beneficial, as the bees aid in pollination. This ensues good seed setting, thereby increasing the seed yields.
- Weed control
  Good weed control is a very basic requirement in seed production. This could be done either by using hand weeding or by the use of herbicides/weedicides.
- Diseases and insect/plant protection
  Insect- and disease-free seed production fields are very critical in commercial seed production. Infected and infested seed fields not only reduce yield but also reduce the quality of the seed produced.
- Plant nutrition
  Some essential nutrients like nitrogen, phosphorus, potassium and other minor nutrients are required in seed production for proper plant development. It is very important to know and identify the nutritional requirement of seed crop in production and apply the fertilizer adequately for good establishment.
- Irrigation
  Irrigation can be important at planting for seed crops on dry soils to ensure good uniform germination and adequate crop stands. Note that excess moisture or

prolonged drought adversely will affect germination and frequently results in poor crop stands.

- Harvesting of seed crops
  Harvesting at the right time in seed production is very critical as it ensures maximum yield and best quality of the seed.
- Drying of seeds
  In order to preserve seed viability and vigour, it is necessary to dry seeds to safe moisture content levels (9–12%).
- Storage and packaging of seeds
  It is always advisable to store seeds in airtight sacks or bags when they are to be stored for a short period of time not exceeding 1 year. And for long-term storage, it is recommended to store it in refrigerator where constant electricity is assured.

5.3.4 Agronomic/Field Requirements

In seed production, similar principles and practices are practiced. But the ultimate objective is to get seed that is of good quality to be used for the production of further crop generations. So it is always important to apply the best possible agronomic practices for raising healthy seeds. Best agronomic practices for tomato are used here as an example for vegetable seed production.

- Timely seed sowing
  Optimum plant population and optimum irrigation are some of the agronomic practices that need to be considered in obtaining higher yields and better-quality seeds. For a successful tomato seed production, it is always advisable to plant tomatoes for the early season around July/August, and for the main season, it is advisable to plant in mid-September, whilst for the late season, planting is done in November. All these are subject to change depending on the variety.
- Fertilization/plant nutrition:
  Tomatoes due to their rapid growth in a long production period require high amount of nutrient and a soil pH which is slightly acidic to neutral (6.0–7). When the soil pH is slightly below 5.5, then blossom-end rot may occur. Nitrogen, potassium and phosphorus as the main element together with other minor elements like boron, molybdenum, sulphur and zinc play a very important role in seed and crop production. It is therefore important to know and identify the nutritional requirement of your seed crop and apply the recommended rate and fertilizer. Good nutrition of the seed results in increased yield and better expression of the plant type which facilitates in rouging and thereby helps in maintaining higher genetic purity and good quality of seed.
- Disease and insect pest control
  Insect pest infestation and disease infection in seed and on plant not only reduce the seed yield but also negatively affect the quality of the seeds. Therefore, successful control of this problem is very critical in raising healthy seed crops. Insecticides and pesticides differ from country to another, but only recommended

and approved ones should be used in seed production with the necessary precautions taken into consideration. Note that frequent weeding and ensuring good sanitation on the field help to prevent pest and disease. It is important to note that the use of these pesticides may adversely affect some pollinating insects and also modify the seed germination potential.

- Supplementary pollination
  Tomatoes are naturally self-pollinated by the virtue of its male and female structure enclosed in the flower. Pollination in tomatoes is done with the aid of wind that vibrates the flower to enable pollination. Although tomatoes are self-pollinated, research has indicated that aiding pollination in tomato seed production with electric vibrator increased the yield and good seed set and fruit size. To obtain a good seed set and fruit size in tomato seed production, it is necessary to plant tomatoes in an open field where strong wind is assured.
- Harvesting, drying and storage of seed
  Time of harvesting is of importance in seed crop production as different seeds are harvested using their physiological characteristics. It is very important to harvest the seed at the right time to ensure maximum yield and the best quality of seed. Generally, seeds are harvested when their moisture content is about 15–20%. In vegetables like tomato, well-ripen fruits are harvested. The fruits are cut or smashed under minimum pressure to extract seeds. The fruits are made to ferment for 1 or 2 days for fast and easy extraction of seeds. Seeds are then cleaned and dried to safe moisture content to ensure that the viability and vigour of the seed are preserved.

Drying of seeds can be done by using sunlight, chemical desiccants and mechanical driers with the air temperature of the drier not exceeding 38 °C in order to maintain good vigour and viability. For a short storage period, dry clean seed should be stored in an airtight bag or container, and for a long-period storage, dry clean seed can be stored in cold room with temperatures below 5 °C, and also, seed can be stored in nitrogen liquid for long-period storage.

5.4 Orthodox and Recalcitrant Species

Orthodox seeds normally undergo a period of drying during their maturation and are harvested at low water content in equilibrium with the existing relative humidity determined by the composition of the seeds. All orthodox seeds can withstand dehydration and can be dried to a lower moisture content of about 2–5% and tolerate freezing temperatures. Liquid nitrogen (−196 °C) has been used to store orthodox seed for a long time without deterioration. Usually, orthodox seed requires low moisture content to enable it to store for a longer period. Generally, orthodox seeds are cheap and easy to store as compared to recalcitrant seeds. Most orthodox seeds are from annual species grown in the open fields. Morphologically, orthodox seeds differ from recalcitrant seed not only in size but also in its complexity and viability.

Recalcitrant seeds are seeds that usually undergo little or no maturation drying and are very sensitive to desiccation during development and at storage. There exists a wide range of variability among recalcitrant seeds of different species and individual species under different conditions. Recalcitrant seeds are short-lived and can be viable from days to a few weeks and are difficult to store. Many tropical and subtropical trees are known to produce recalcitrant seeds. Recalcitrant seeds have high moisture content, which is estimated to be in the range of 30–70% at maturity. Recalcitrant seed germinates very fast when sown fresh; this attribute makes them difficult to store.

5.4.1 Technologies in Vegetable Seed Production

Like other areas of agriculture, seed production and handling practices have advanced. For instance, farmers used to save their own vegetable seeds and maintained their own cultivars for some time in the past. More recently, seeds were often obtained from local agricultural retailers. Currently, vegetable growers have access to a wide range of seed cultivars and treatments that can be purchased from sources all over the world. There are many companies involved in the vegetable seed trade most of which are multinationals. Whilst few develop new cultivars and grow their own seeds, others specialized in seed treatment and retailing. To ensure quality, the vegetable seed industry is regulated by specialized laws. These laws, which mostly vary from countries, specify minimum standards for vegetable seed quality and labelling. Many countries undertake seed certification programmes which are supported by standard seed testing laboratories to ensure that seeds meet minimum germination percentage and genetic and physical purity.

Vegetable seed production is normally done in special seed production fields or structures. Most seed production is undertaken in screen houses with special irrigation systems in a more controlled environment. Under such controlled conditions, a well-designed integrated pest management (IP) system is used to enhance the availability of insect pollinators. Well-designed crop rotation systems are mostly in place to control some soil-borne pest such as nematodes. Seed treatment has also become an integral part of the vegetable seed production system. Some companies only specialize in seed treatment of seeds produced by other companies. Similarly, grading and pelleting of seed lots to facilitate their use by growers in conjunction with precision drills or machines for sowing in soil blocks to raise transplants are done by some seed trade and their related agencies.

For some time in the past, open-pollinated (OP) cultivars used to dominate the vegetable seed market. Currently, most seed companies have now developed F_1 hybrid cultivars which through the phenomenon of heterosis yield higher than their OP counterparts. These F_1 hybrid seeds are more expensive than the OPs. An F_1 hybrid is produced by crossing two distinct lines which are known to have higher heterosis. The hybrid system normally helps the seed companies to recoup their investment since the plants segregate for different traits when harvest from the F_1 hybrids is replanted. These force farmers to buy F_1 hybrid seeds from the seed

companies for each season's planting. Details on F_1 hybrid vegetable seed development is provided by Kumar and Singh (2005).

5.4.2 Processing, Packaging and Storing of Vegetable Seeds

5.4.2.1 Processing

The term processing is used to include a wide range of operations to improve or upgrade the seed lot after threshing or extraction. The major objectives are usually to remove plant debris, non-plant materials such as soil and stones, seeds of other crops and weeds, seed appendages, seeds which are outside the accepted size and damaged and discoloured seeds. The common cleaning processes normally include winnowing where the dried seeds are separated from less dense or lighter debris by air movement (George 2009). Some species also require scalping where the bulk of plant debris and other non-seed materials and seed clusters are separated by vibrating or rotating sieves. There are various machine types which combine winnowing and sieving together which are well elaborated by George (2009). Modern commercial vegetable production systems are based on crop uniformity. To obtain the required crop uniformity and for precision drilling, vegetable seeds are normally graded. There are various grading machines which put the seeds into size categories which are normally defined in the seed catalogues and packages. After grading, the machine leaves behind only specific contaminants which were not achieved by the screen and air-cleaning operations. These are further removed by special techniques which exploit the differences in some physical properties between the desired seed and the undesirable materials. It should be ensured that the cleaning process is well managed to avoid contamination. It is advisable that machines are cleaned between seed lots. This is usually done by vacuum-cleaning of the removable machine parts such as screens and the interiors. Floors and other areas around the processing machines must be clean and free from debris.

Currently, untreated seeds are rarely used by commercial vegetable growers. As part of processing, most vegetable seeds are treated. The seeds are treated for different reasons including the following:

1. Pesticide applications where a light coating of fungicide is usually applied to the seed surface. In such cases, a brightly coloured dye is normally added as a reminder that a fungicide has been applied.
2. Inoculation with the right rhizobium species (mostly legumes) to improve its nitrogen-fixing ability after germinations. Sometimes, some small-seeded species are coated or pelleted to make seed handling easier.
3. Coating or pelleting seeds for easy handling and planting, particularly when planters that utilize belts with prepunched holes of a specific size are to be used.

5.4.2.2 Packaging

Before seeds are packaged, it should be ensured that the seeds have dried to the required moisture content. Drying is normally done to reduce the moisture content to

the required level. Some seeds require drying right from the field at the processing centre. Others also require drying after extraction from the fruit or possibly after processing. Different natural and artificial means of drying vegetable seeds have been elaborated by George (2009). It is recommended that the seed moisture content is checked with a laboratory moisture-determining instrument to confirm that it is well dried before packaging.

Seeds should always be packaged into desirable containers and labelled properly with the right certification tags which carry all relevant seed quality information. This information usually has to meet the legal requirements of the country in which the seeds are to be marketed. Most seed companies also utilize the opportunity to provide additional information according to the market outlet. It is always recommended that vegetable seeds are packaged in weights (kg) or sizes desired by the target market or correspond to farm size units. Seeds are packaged in many types of containers. A good packaging material should be used since it influences seed quality in storage. The packaging material should be designed to create an airtight condition within the seed environment. Containers made of paper or cloth should be avoided since they do not prevent moisture uptake by the seeds and are more easily broken than other materials. Foil or plastic hermetically sealed packets are recommended because they keep seed moisture content low. Self-sealing packets are also easy to use as they protect the seeds from changes in moisture content. Larger quantities of seeds are often sold in metal cans. Such cans provide effective protection from rodents and moisture until opened. In such cases, the cans should be supplied with resealable lids. Most modern seed companies have very sophisticated packaging lines attached to their warehouses that are capable of automatically filling containers by machines. These machines deliver predetermined quantities of seeds into packets and seal and apply labels to the completed vapour-proof packets.

5.4.2.3 Storage

Seed storage preserves the viability and vigour of seeds until marketing and protects the seed marketer's profits and reputation. For successful seed storage, the determinants of seed quality in storage and the process of seed deterioration need to be well understood. The moisture content and temperature are the main determinants of seed longevity in storage. As a general rule, seed storage life decreases by half for every 5 °C rise in temperature from 0 to 50 °C or for every 1% increase in moisture content from 5% to 14% (Achaab 2018). Another helpful rule is that the percent relative humidity plus the temperature in degree Fahrenheit should be kept below 100 during seed storage (George 2009). Seeds are hygroscopic and can gain or lose moisture from the air. If seeds are stored at high moisture content (>18%), damage can occur from heat build-up due to seed respiration. Fungi, mould growth and insect infestation also result from storing seed with high moisture content. It is recommended that starchy seeds in general should be stored at less than 12% moisture content, whilst oily seeds such as watermelon are maintained at moisture contents less than 9% (George 2009). Although the lower the moisture content, the better the storage, some seeds, when stored at less than 4% moisture content, can be damaging due to auto oxidation of lipids. It is advised that

warehouses/seed stores should be located at geographical areas with reasonably favourable climate for storage (low temperature and low RH). If not, then some environmental modifications such as in cold rooms are required.

The period of storage is also critical and depends mostly on the seed type and the storage environment. As a general rule, the longer the storage period, the drier the seeds should be in addition to a cooler environment. Although most seed companies store seeds from harvesting until the next planting, there should be plans for medium-term storage for carry-over or excess seeds. Only seed companies involved in crop variety development may require long-term storage facility for germplasm maintenance and stock seed (breeder and foundation). During seed storage, there should be regular inspection of seed lots to check out germination ability and if necessary fumigation to control pest infestation. Seeds in storage should also be well labelled and proper records taken.

5.4.3 Marketing and Distribution of Vegetable Seeds

Through seed distribution and marketing, vegetable seed is made available to the farmer who is the end user. Most vegetable seed production is carried out at specialized locations or regions due to some specialized flowering or seed setting requirements. The resulting product has to be moved closer to small-scale dealers or farmers. A commercial company marketing seeds internationally usually has a marketing manager for each region. Within a country, there are usually distributive agents, or the company may have its own direct sales outlets. It is recommended that vegetable seeds are transported in vapour-proof containers and packages (George 2009). Changes in temperature and relative humidity, which normally contribute to deterioration of the containers, labelling system and seeds not in vapour-proof cans should be minimized during transportation. For success as a company, the seeds should be delivered in adequate quantities, at the right time, in suitable units (weight) and in attractive packaging materials at an affordable price. It is also advised that seeds should be delivered very close to the farmer for accessibility.

A seed marketing company should be strategic in conducting market research to assess needs and gather market intelligence. There should also be strategically positioned seed stores and logistics for easy movement whenever necessary. The marketing company should promote and publicize their products. This is normally done through promotional sales, field days and field demonstrations. The company should also be strategic in pricing. Seeds should be delivered to the farmer in a realistic price that will help the company to recoup investment. Sometimes, it is necessary to employ discriminatory pricing to address customers with different strengths. In such cases, prices of the same quantity of seed could differ depending on location. Prices could also vary on time bases in areas where demand is extremely high. Companies also could adopt promotional pricing where seeds are priced low to attract customers and later increase the price to recover all costs.

5.5 Maintenance of Vegetable Seed Purity and Viability

5.5.1 Seed Formation and Maturation

Seed is defined as the product of a fertilized ovule made up of an embryo, cotyledon and seed coat (Singh et al. 2014). Seed formation is preceded by pollination and fertilization. Pollination is the transfer of pollen grains from the anther to the receptive stigma of a flower. Fertilization on the other hand is the union of the male and female gametes resulting in the development of a zygote (McCormack 2004). Pollination and fertilization in vegetables are mostly influenced by environmental conditions particularly temperature. High temperatures have been reported to kill and damage pollen tube growth. High night temperatures are the single most important factor reducing seed set in most vegetable crops, for example, tomato (McCormack 2004).

Seeds are physiologically mature when all developmental processes are completed. In most fruit vegetables, physiological maturity of seed is attained when natural fruit ripening begins. For example, in eggplant seed production, seeds are extracted from ripe yellow fruits (Chen 2001). Fruit dehiscence can be used as an indicator of seed physiological maturity in some vegetables such as okra, roselle and jute mallow.

5.5.2 Seed Dormancy and Viability

Seed dormancy refers to the failure of a viable seed to germinate under suitable environmental conditions due to the presence of inherent inhibitors in the seed (Nee et al. 2017). Seed viability is defined as the ability of a viable seed to germinate under favourable conditions. A viable seed may be in a state of dormancy in which case the dormancy has to be released before the seed can germinate. Seed dormancy, a result of immature embryo or impermeable seed coat, is controlled by plant hormones and dormancy proteins during seed maturation and storage. The initiation and breakdown of dormancy is influenced by environmental factors (Nee et al. 2017). Although high seed dormancy is not desirable in most vegetable crops, low seed dormancy is important in inhibiting preharvest sprouting and germination of seeds in storage. Seed dormancy may be broken by drying seed to the recommended moisture content, mechanical abrasion of the seed coat, soaking seed in water, hot water treatment of seed and the application of gibberellic acid.

5.5.3 Recommended Seed Multiplication Processes

Vegetable seed production requires the application of appropriate agronomic principles necessary for the production of good-quality seeds (Singh et al. 2010). In order to produce genetically and physically pure, physiologically viable seeds that are free from weeds, insect pests and diseases, expertise of highly trained and skilled

personnel who are familiar with the genetics of the crop as well as the recommended agronomic principles is required (Moharana et al. 2016). Quality vegetable seed production and distribution are labour intensive and require high investments and the application of modern technologies (Bradford 2006). The recommended agronomic principles required for producing quality vegetable seed include the following:

1. Selection of suitable seed production fields: The site for vegetable seed production should be able to support the growth of the crop. The climatic conditions in the area should be able to provide conducive environment for flower formation and fruit development (Chen 2001).
2. Varietal selection and source of seed: The choice of a variety is very important and depends on the interest and goal of the seed producer. The class of seed to be grown should be obtained from reputable institutions and organizations with the track record of producing that class of seed.
3. Seed treatment: Seeds of vegetable crops are relatively expensive especially hybrid seed. Therefore, protect seeds from soil-borne pathogens and insect pests by applying seed dressers (fungicide and insecticide) to seeds before sowing.
4. Field management practices: The recommended agronomic principles required for producing vegetable seeds depend on the particular crop. However, some activities are mandatory irrespective of the crop. Depending on the type of vegetable crop, the seed is either raised at the nursery or planted direct in the field. Follow recommended nursery management practices to ensure that healthy seedlings are produced. Cultural practices such as optimum plant density, weeding, plant nutrition, staking and pruning, disease and insect pest control and supplementary irrigation should be carried appropriately to obtain higher yield and quality seeds. Harvesting should be done at the right time when seeds are fully mature. Harvest seed plots carefully to avoid mechanical mixtures.
5. Postharvest practices: Seeds should be carefully extracted from fruits with no mechanical damage to the seed. Wash and clean seeds with clean water. Air-dry seeds under shade to the right moisture content to avoid deterioration (Chen 2001). Drying can also be done using an electric dehydrator. Sort out immature, malformed and discoloured seeds as well as foreign material from the seed lot. Package seeds in clean sacks or airtight plastic bags, and store in clean well-ventilated room on raised platform. Label seeds before storage.

5.5.4 Maintenance of Genetic Purity

Maintaining the genetic purity of a variety is one of the most important criteria required for seed certification (Bradford 2006). The genetic purity of a variety can break down due to factors such as natural crossing, mechanical mixture, mutations and minor genetic variation (Bradford 2006). However, the genetic purity of a variety can be preserved through the following:

Table 5.1 Isolation distances of some vegetable crops

Crop	Scientific name	Isolation distance (m)
Amaranth	*Amaranthus cruentus*	1000
Eggplant	*Solanum melongena*	100–200
Tomato	*Solanum lycopersicum*	3–5
Nightshade	*Solanum scabrum*	5–30
Jute mallow	*Corchorus olitorius*	1000
Pepper	*Capsicum* sp.	500–1000
Okra	*Abelmoschus esculentus*	50–1000

Source: McCormack (2004) and Chen (2001)

1. Control of seed source: The class of seed of a variety should be handled with care to avoid genetic deterioration. Growers should avoid using any class of seed from unapproved sources.
2. Crop rotation: Rotate crops on the seed production field, and ensure adequate intervals between planting the same crop or different varieties of a particular crop to minimize the risk of cross-contamination.
3. Isolation: Avoiding cross-pollination between crops of the same species (compatible crops) is one key requirement in seed production which is a prerequisite for maintenance of varietal purity (Chen 2001). Vegetable seed production fields can be isolated by time or distance. Isolation by time involves producing seeds off season or ensuring that the flowering of two species of the same crop does not coincide. Isolation by distance involves separating seed production fields of the same crop species by a minimum isolation distance. Ensure that the minimum isolation distance of a crop is adhered to especially for cross-pollinated crops (Moharana et al. 2016). Seed production of self-pollinated crops does not require much isolation distance; however, natural out-crossing and mechanical mixtures during harvesting can affect seed purity (Singh et al. 2014). Cross-pollinated crops require high isolation distance to prevent genetic deterioration (Table 5.1).
4. Roguing of seed field: Roguing refers to the removal of off-type plants and diseased and abnormal plants from the seed production field. It is done to ensure that seeds are not collected from off-type plants. Roguing should be done before and after flowering and at harvest to ensure phenotypically different plants are removed (Chen 2001). In cross-pollinated vegetable crops, the seed production field should be thoroughly rogued before flowering to avoid cross-contamination.
5. Harvesting and handling of seeds: The purity of a seed can be compromised through physical contamination from other varieties during harvesting and seed processing. Proper and efficient execution of these activities would reduce the possibility of seed contamination.

5.5.5 Seed Storage

The basic aim of seed storage is to ensure that the physiological purity of the seed is not compromised. Seed moisture content is one key factor affecting seed health in storage. Improperly dried seeds deteriorate faster in storage. Dry seeds to the recommended moisture content. Dry orthodox seeds to a moisture content of 8% or below for long-term storage. Recalcitrant seeds are, however, sensitive to drying to a low moisture content of below 15% (Singh et al. 2014). Seeds should be disinfected with fungicides and insecticides to protect them against storage pests and soil-borne pathogens. Store seeds in clean sacks or airtight plastic bags or containers. Seeds should be stored on raised platforms in clean well-ventilated or cold rooms. Environmental conditions in the storage room influence the longevity, viability and vigour of the seeds. In general, seeds store longer in cool dry conditions than warm humid conditions.

5.6 Prospects of Vegetable Seed Production

There are prospects for vegetable seed production in Ghana. Factors or circumstances that point to success in vegetable seed production in the country include the following. First of all, there is a high demand for local and exotic vegetable seeds in the country. Secondary, the climatic conditions are favourable for the cultivation of a wide variety of vegetables. Also, the available manpower, both skilled and unskilled, can be employed to go into the business of vegetable seed production. In addition, government policies support the vegetable seed industry. Last but not the least, available technologies in crop husbandry can make vegetable seed production very lucrative. Vegetable seed production thus has the potential to create jobs, improve farmers' income and contribute to economic growth of Ghana.

Unlike the major cereals and legumes such as rice, maize, cowpea and groundnut, commercial vegetable seed production is mainly informal in Ghana (IFRI 2013). The leading seed companies in Ghana are "Heritage," "M&B," "WEKU," "Yonifah" and "PAG." Unfortunately, none of these companies are producing vegetable seeds at the moment (Diallo 2018). This means that the needed seeds for vegetable cultivation in Ghana are mostly farmer-saved or imported. Seeds are saved from vegetables, mainly land races that produce viable seeds under the local conditions, by farmers. Seeds of exotic vegetables such as cabbage and sweet pepper on the other hand are imported. There are some crops with both farmer-saved and imported seeds. Tomato, okra and hot pepper are among the farmer-saved and imported vegetable seeds. The absence of significant commercial vegetable seed production in Ghana despite the high demand gives a general indication for big opportunity in the sector.

There is high demand for vegetable seeds in Ghana. This market availability is the first and most important stimulus that will encourage anyone to go into vegetable seed production on commercial scale. Currently, a major constraint identified in the vegetable industry is unavailability of quality seeds (Djokoto et al. 2015). Many farmers are compelled to use their own saved seeds for their vegetable cultivation

because of lack of seeds. However, seed production itself needs skills that the farmer may not have. This unavailability of vegetable seeds, therefore, creates opportunity in the seed industry.

Whilst farmer-saved seeds are mostly used for local vegetables, high-valued exotic vegetable seeds are imported and reach farmers through formal system. Exotic vegetables with high demand in Ghana include cabbage, lettuce, sweet pepper, tomato and cucumber. They are sold to farmers through agro input dealers. Importation and sale of exotic vegetable seeds are described as lucrative (Diallo 2018). Although it is possible to venture into the production of these seeds, big capital investment and skill labour including plant breeders are needed. However, it may be a profitable venture as these seeds are all imported. There is, therefore, a big prospect for both local and exotic vegetable seed production in Ghana.

There are seven different agroecological zones in Ghana. All of these zones have their unique advantages and suitability for the cultivation of a variety of vegetables. The diverse agroecological zones and the various crop varieties also create opportunity for seed production. Seed businesses can be set up at the different zones creating small-to-medium-scale businesses. Closely associated with the ecological zones and conducive environments for crop production are climatic factors including sunshine, rainfall and temperature for the cultivation of diverse crops across Ghana. The natural endowment makes vegetable seed business a manageable task. One does not need to manipulate the environmental conditions in most cases before producing crops in Ghana. Even in the case of water, rainfall is adequate for the cultivations of most crops in the country.

Scientific skills and knowledge in seed production are key to the success of vegetable business in general and the seed industry in particular as was the case in India (Moharana et al. 2016). In the first place, quality and viability issues have to be dealt with using scientific knowledge. For example, hybrid vigour contributes to better performance and is the reason for developing F_1 hybrids. Most if not all of the imported vegetable seeds in the market are hybrids. Venturing into the seed industry will therefore require this scientific knowledge and skills. Farmers with higher levels of scientific orientation will, therefore, understand hybrid principles better and are more likely to succeed in the sector (Singh and Kaur 2014). As people of higher education are expected to enter into vegetable farming, one can predict better future for vegetable seed industry with enlarging market.

Ghana government's programmes in promoting agriculture including vegetable production will positively affect the vegetable sector including seeds. Vegetables are included in the "planting for food and job" programme (Mabe et al. 2018). This will surely increase the need for seeds of vegetables. The "one village one dam" policy is another programme that will positively affect the vegetable sector. The fertilizer subsidy among other programmes will also increase demand for vegetable seeds. There is, therefore, more prospects for vegetable seed production because of the government's policies.

Going back, the current situation of farmers saving their own seeds is a great opportunity for commercial production of vegetable seeds. Clue can be drawn from the cereals sector which until recently was mainly informal. The changing situation

brought by emerging seed companies for the major crops like maize can be replicated with vegetables. Seed companies in Ghana are growing fast, but their focus is on the major cereals. Desire for better crops and their seed is the major driving force that will bring evolution of commercial vegetable seed production in Ghana. Commercial vegetable seed production needs scientific and business professionals. These calibres of people will lead to the production of high-quality seeds of improved varieties of vegetables to increase yields and incomes.

Other areas where scientific knowledge is crucial are the production under greenhouse conditions and the use of soilless media (Sanwal et al. 2004). Available technology can be used to produce seeds of crops that are normally difficult to produce under Ghanaian climatic conditions. Technology continues to advance, and advantage can be taken of the new development to produce vegetable seeds in Ghana at least for the local market. Fortunately, in this direction, Ghanaians are receiving education and training all over the world and can use their expertise in the vegetable seed industry. Although some challenges will have to be dealt with, there are prospects for vegetable seed production in Ghana.

5.7 Challenges of Vegetable Seed Production

The choice of good-quality seed is the beginning of every productive farming business. Seed is a very important component among all the inputs that are required for bumper harvest (Mekbib 2008). This was realized and practiced by farmers long time ago and is evident in the selection and preservation of good and attractive cobs, pods and other planting materials at the time of harvest. Local farmers would select these prospective planting materials and preserve them in areas where pests and other disease pathogens would not get access to. In some cultures, selection and utilization of good-quality seed were seen as key to the preservation of the human race. It has been estimated in some studies that the quality of seeds used in production could account for between 15% and 25% of the final crop productivity (Poonia 2013; Koundinya and Kumar 2014). This therefore underlines the importance of good-quality seed in production. Unlike conventional staple crops, vegetable seed production is faced with several challenges that include genetic and environmental factors, pests and diseases, storage and marketing.

5.7.1 Production Challenges (Genetics vs. Environmental Effects)

Vegetable production requires special techniques, care and maintenance for best results. One key consideration in seed production is the maintenance of genetic purity. A good genetic seed stock ensures that the actual original genetic materials are maintained from year to year and place to place (Bradford 2006). Failure to give adequate isolation distance results in contamination from foreign pollen that is carried by external agents of pollination. In order to ensure genetic purity, isolation distances need to be considered for the vegetable under consideration. Different

vegetable crops require different isolation distances depending on the mode and agent of pollination which may be either self- or cross-pollination (McCormack 2004). Failure to follow the required isolation distance requirement will result in the production of impure seeds which may not result in the production of the right type of vegetable crop for consumption or for sale. The performance of any crop depends on the genetic potential and good-quality seeds, combined with the provision of desired environmental conditions that will ensure judicious use of inputs such as water and nutrients (Singh et al. 2013). After obtaining a good seed with the right genetic make-up, it is necessary to grow the seeds on good soil for optimum performance. In seed production, the nutrient status of the soil has implications on the quality of seeds obtained and the overall vigour of the seedlings that would result from them. A good seed in a poor soil that lacks adequate nutrients will result in poor growth and productivity. The ideal practice is to analyze the environment and manipulate the planting dates to suit the specific crop variety to ensure that the crop is not negatively affected by the adverse environmental conditions that may affect the performance of the crop. This is because different crop varieties prefer specific environments and may respond differently in different growing environments (Osei et al. 2018). As a result, crop varieties are selected to match the specific environment using genotype × environment interaction analysis to ensure optimum and stable performance under different growing conditions (Singh et al. 2015).

5.7.2 Disease and Nematodes as Vegetable Problems

Vegetables like most crops are affected by several biotic and abiotic constraints that impact negatively on their productivity. Four types of organisms affect crops including vegetables. These include fungi, bacteria, viruses and nematodes (Little 2013). Most fungal diseases such as fruit rot, root rot and leaf spots occur under moist conditions, whereas the viral diseases are transmitted by insect vectors which feed on the sap of these vegetables. *Meloidogyne* sp. and reniform nematodes attack vegetables and cause severe economic losses in warm weather conditions and may hide in the soil causing severe damage on plant roots all year round.

5.7.3 Storage Facilities (Maintaining Viability in Long-Term Storage)

One major challenge with these species is the ability to maintain viability over the storage period. Seeds are living organisms and require ambient conditions which do not exist in most communities in Ghana (Elias et al. 2019). The length-inadequate seed storage facilities have been a major constraint to seed producers and even for farmers to their seeds. For smallholder farmers, locally improvised storage containers are sometimes used to preserve orthodox seeds though these may still be exposed to pests and disease pathogens. Unlike conventional cereals and pulses

whose seeds can be stored over fireplaces in most rural communities without losing their viability, vegetable seeds require special conditions in order to maintain their viability in long-term storage. Some vegetables like tomato, eggplant and the cucurbits do not require drying and extraction of seeds before storage. In these crops, the seeds are extracted and dried before storage. The fruits of some vegetables such as okra, roselle, pepper and jute mallow can be dried together with the seeds and stored till the seeds are needed before extraction.

5.7.4 Marketing (Demand vs. Supply)

Marketing of vegetable seeds is a major challenge in Ghana. This is because the seed industry has focused mainly on few cereal (rice, maize, sorghum and leguminous (cowpea, groundnut and soybean)). Most exotic vegetables such as cabbage, lettuce, carrot, cucumber, French beans and cauliflower are imported into the country with no local seed production outlets. Some of these seed dealers also sell imported hybrids of tomato, pepper, eggplant, etc. Due to the low scale of production of vegetables and absence of locally developed varieties, most farmers rely on the imported seeds to satisfy their seed requirement at the beginning of the season. Besides, there is little demand for seeds of most of these locally produced vegetables since few hardly meet the requirements of consumers. Importation of vegetables such as green pepper, tomatoes and eggplants from neighbouring countries has rendered the local vegetable industry unproductive, thereby reducing the number of farmers who cultivate these crops and hence the reduced demand. Additionally, the local vegetable seed system is not fully regulated and certified, so there are no certified vegetable seed growers as we have for other crops like maize, rice, soybean, groundnut and cowpea. The lack of certification and standardization has therefore limited the market opportunities for the producers of local vegetable seeds.

5.7.5 Technical Know-How

Vegetable seed production requires specialized techniques that help to retain genetic purity and viability of the resulting seeds. A good knowledge of the growth requirements, site selection (considering isolation distance), time of the year for optimum seed set, etc. and lack of proper knowledge on the biology of the crop and optimum conditions for both flowering and seed set will result in several losses incurred as a result of fruit drop or floral abortion when the flowering period coincides with hot weather conditions. It is important to take vegetable seed producers through adequate training from experts to ensure success in vegetable seed production. Another key constraint facing the vegetable seed sector is lack of skills and equipment for seed quality, variety testing and certification to evaluate the genetic quality of seed stock. Since seed quality depends on genetic components (defined by the variety), physical purity, viability and seed health, information on seed quality enables seed producers to determine the quality of seeds intended for

use (Elias et al. 2003). Variety testing is an important process that may target varietal identification and discrimination between different varieties and for checking genetic purity with the objective of varietal characterization of the variety (Zecchinelli 2009).

5.8 Conclusion

This paper sought to examine the vegetable seed system in Ghana and the prospects and challenges associated with vegetable seed production as a business. Quality seed and vibrant seed system are prerequisites for profitable agricultural production. Two types of systems operate in Ghana: formal and traditional systems, each with its own strengths and challenges. Vegetable seeds are obtained from two sources in Ghana: local source and imported seeds. Good-quality vegetable seeds are both genetically and physically pure. Vegetable seeds can be either recalcitrant or orthodox and have different preferences for storage conditions which must be strictly adhered to in order to prevent losses in production. Vegetable seed production requires sound agronomic and field production practices for optimum success. Post-production practices and operations such as cleaning, packaging and storing are very important in maintaining viability of seeds in storage. Vegetable seed production faces several challenges which range from genetic, environmental, pests and diseases, infrastructural, technical know-how and marketing challenges. These must be properly addressed to suit the crop, scale of production, ecology and the period for which the seeds are to be kept. Unlike other crops such as cereals, the vegetable seed system is not well developed. Most farmers save their own seeds or obtain from informal sources. This provides unique opportunity for its development. The initiation of government policies and programmes on vegetable production provides a huge potential for commercial certified vegetable seed production. Proper regulation of the system using appropriate approved standards will be very crucial in addressing these challenges.

References

Achaab A (2018) Principles and practices of seed production in Ghana. International Fertilize Development Center (IFDC), Muscle Shoals, AL. 33 p

Addo-Quaye A, Djokoto J (2013) Report on vegetable seed systems and policyintegrated agricultural research systems for the humid tropics: a CGIAR research program. December 2013

Ayana A, Afari-Sefa V, Emana B, Dinssa FF, Balemi T, Temesgen M (2014) Analysis of vegetable seed systems and implications for vegetable development in the humid tropics of Ethiopia. Int J Agric For 4(4):325–337

Badiane O, Nyanteng VK, Seini WA (1992) Food security, comparative advantages, and fertilizer use in Ghana. International Food Policy Research Institute, USA/Institute of Statistical, Social and Economic Research (ISSER), Legon

Bradford KJ (2006) Methods to maintain genetic purity of seed stocks. University of California Division of Agriculture and Natural Resources Publication 8189. University of California

Agriculture and Natural Resources, Davis, CA. ANR Communication Services Website: https://anrcatalog.ucanr.edu/pdf/8189.pdf

Chen NC (2001) Eggplant seed production. Asian training guide. Vegetable Research and Development Center, Shanhua, pp 1–4. https://afghanag.ucdavis.edu/fruits-nuts-vegetables/Vegetables/files/eggplant/Man_Veg_Eggplant_Seed_Prod_AVRDC.pdf

Diallo Y (2018) Identifying leading seed companies in Western and Central Africa. Landscaping study for the regional access to seed index for Western and Central Africa. Access to Seed Foundation, Amsterdam

Djokoto J, Afari-Sefa V, Addo-Quaye A (2015) Vegetable supply chains in Ghana: production constraints, opportunities and policy implications for enhancing food and nutrition security. Natl Acad Agric Sci 33(3):2113

Drechsel P, Keraita B (2014) Irrigated urban vegetable production in Ghana. Characteristics, benefits and risk mitigation, 2nd edn. International Water Management Institute, Colombo

ECOWAS (2008) Regulation C/REG.4/05/2008 on Harmonization of the rules governing quality control, certification, and marketing of plant seeds and seedlings in ECOWAS region. In: Sixteenth Ordinary Session of the Council of Ministers of the Economic Community of West African States (ECOWAS), 17–18 May, Abuja

Elias S, Garay A, Hanning S, Schweitzer L (2003) Testing the quality of seeds in cereals. Oregon State University Seed Laboratory Publication, Corvallis, OR. https://seedlab.oregonstate.edu/sites/seedlab.oregonstate.edu/files/cereal-quality-testing-2003.pdf

Elias S, Garay A, Young B, Chastain T (2019) Maintaining grass seed viability in storage: a brief review of management principles with emphasis on grass seeds stored in Oregon. Oregon State University Publication, Corvallis, OR. https://seedlab.oregonstate.edu/training-education/publications/maintaining-grass-seed-viability-storage

Etwire PM, Atople IDP, Buah SSJ, Abdulai AL, Karikari AS, Asungre P (2013) Analysis of the seed system in Ghana. Int J Adv Agric Res 1(2013):7–13

FAOSTAT (2019) Accessed 4 Jun 2019

George RA (2009) Vegetable seed production, 3rd edn. MPG Books Group, Bodmin. 329 p

Gerken A, Suglo J, Braun M (2001) Pesticides use and policies in Ghana. An economic and institutional analysis of current practice and factors influencing pesticide use. (10). Institute of Horticultural Economics, Hannover

Herpers S, Vodouhe R, Halewood M, De Jonge B (2017) The integrated seed sector development (ISSD) synthesis paper: the support for farmer-led seed systems in African seed laws. ISSD, Wageningen

IFPRI (2013) Ghana's commercial seed sector. Ghana strategy support programme. Working paper 32. IFPRI, Washington, DC

Koundinya AVV, Kumar PP (2014) Indian vegetable seeds industry: status and challenges. Int J Plants Anim Environ Sci 4(4):62–69

Kuhlmann K, Yuan Z (2016) Seed policy harmonization in ECOWAS: the case of Ghana. Working paper. Syngenta Foundation for Sustainable Agriculture, Basel. https://www.syngentafoundation.org/file/4481/download. Accessed 11 May 2019

Kumar S, Singh PK (2005) Mechanisms for hybrid development in vegetables. J New Seeds 6 (4):381–407

Little E (2013) Disease management in the home vegetable garden. University of Georgia Department of Agriculture Cooperative Extension Circular 862. University of Georgia Department of Agriculture, Athens, GA. https://secure.caes.uga.edu/extension/publications/files/pdf/C%20862_4.PDF

Mabe FN, Danso-Abbeam G, Ehiakpor S (2018) Assessment of implementation of planting for food and jobs programme: lessons and way forward. Feed Future Ghana Agric Pol Sup Proj. https://doi.org/10.13140/RG.2.2.34030.46402

McCormack JH (2004) Isolation distances: principles and practices of isolation distances for seed crops: an organic seed production manual for seed growers in the Mid-Atlantic and Southern

U.S. Version 1.5, pp 1–25. www.carolinafarmstewards.org/wp-content/uploads/2012/05/IsolationDistancesVer_1pt5.pdf
Mekbib F (2008) Farmers' seed system of Sorghum [Sorghum bicolor (L.) Moench] in the Center of Diversity: II. Seed quality, storage, protection and security. J New Seeds 9(3):191–211
Melomey LD, Danquah A, Offei SK, Ofori K, Danquah E, Osei M (2019) Review on tomato (Solanum lycopersicum, L.) improvement programmes in Ghana. In: Nyaku ST, Danquah A (eds) Recent advances in tomato breeding and production. IntechOpen Limited, London, pp 49–69. https://doi.org/10.5772/intechopen.75843
MoFA (2016) Agriculture in Ghana. Facts and figures. 25. Ministry of Food and Agriculture, Statistics, Research and Information Directorate (SRID), Accra, pp 3137–3146
Moharana DP, Singh V, Singh P, Singh AK (2016) Seed production in vegetable crops: the Indian prospects. Progress Res 11(Special-II):1436–1437. ISSN: 2454-6003
National Seed Plan (2015) Plan for the implementation of the National seed policy, pp 1–97
Nee G, Xiang Y, Soppe WJJ (2017) The release of dormancy, a wake-up call for seeds to germinate. Curr Opin Plant Biol 35:8–14
Netherlands-African Business Council (2014) Factsheet – Vegetables Ghana. African Business Council, The Hague. http://www.ondernemeninontwikkelingslanden.nl/sites/default/files/partnerkennis/Factsheet%20GhanaVeg.pdf
Osei MK, Annor B, Adjebeng-Danquah J, Danquah A, Danquah E, Blay E, Adu-Dapaah H (2018) Genotype x environment interaction: a prerequisite for tomato variety development. In: Nyaku ST, Danquah A (eds) Recent advances in tomato breeding and production. Intechopen Limited, London, pp 93–113
Poonia TC (2013) History of seed production and its key issues. Int J Food Agric Veterin Sci 3 (1):148–154
Pousseu L, Pegalepo E, Kouamé C, Bell JM, Kamga RT (2014) Diagnosis of seeds supply of leafy vegetables in Yaoundé, Cameroon. Afr J Food Agric Nutr Dev 14(4):9154–9166
Saavedra Y, Dijkxhoorn Y, Elings A, Glover-tay J, Koomen I, van der Maden E, Nkansah G, Obeng P (2014) GhanaVeg sector reports. Vegetables business opportunities in Ghana. Wageningen UR, Wageningen, pp 1–44
Sanwal SK, Patel KK, Yadav DS (2004) Vegetable production under protected conditions in the Neh Region: problems and prospects. Himalayan Ecol 12(2):1
Seini AW, Nyanteng VK (2003) Afrint macro study: Ghana report (revised). Institute of Statistical, Social and Economic Research (ISSER) University of Ghana, Legon
Singh B, Kaur P (2014) Prospects of hybrid seed production of vegetables in Punjab. IJLTEMAS 3 (10):95
Singh PM, Singh B, Pandey AK, Singh R (2010) Vegetable seed production - a ready reckoner. Technical Bulletin No. 37. IIVR, Varanasi
Singh RP, Prasad PVV, Reddy KR (2013) Impacts of changing climate and climate variability on seed production and seed industry. Adv Agron 118:49–110
Singh P, Singh RP, Singh MK, Mohapatra C (2014) Quality seed production, its testing and certification standard. In: Singh N, Kumar A (eds) Biologix, 3rd edn. Mewar Institute of Management, Sec-4C, Ghaziabad, pp 126–142
Singh RP, Prasad PVV, Reddy KR (2015) Climate change: implications for stakeholders in genetic resources and seed sector. Adv Agron 129:117–180
Sinnadurai S (1973) Vegetable production in Ghana. Acta Hortic 33:25. https://doi.org/10.17660/ActaHortic.1973.33.3
Sperling L, Cooper HD (2003) Understanding seed systems and seed security. In: Improving the effectiveness and sustainability of seed relief. Proceedings of a stakeholders' workshop, Rome, 26–28 May 2003. Food and Agriculture Organization, Rome
Zecchinelli R (2009) The influence of seed quality on crop productivity. In: Responding to the Challenges of a changing world: the role of new plant varieties and high-quality seed in agriculture. Proceedings of the Second World Seed Conference. FAO Headquarters, Rome, September 8–10, 2009, pp 150–159

Production of High-Quality Tropical Forage Legume Seeds

6

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Abstract

Production of quality seeds of tropical forage legumes is important to satisfy the growing market demand for planting of feed for livestock and for their environmental benefits. The overall quality of seeds is defined by genetic, physical, physiological, and health attributes. The entire production and storage process has a major effect on the quality of forage legume seeds. Therefore, attention needs to be given to all the stages of seed production and storage, to ensure the production and availability of high-quality forage seeds for use. This chapter covers the key attributes such as seed production, processing, maintenance/storage, and health testing, for the production of high-quality forage legume seeds.

Keywords

Forage · Tropical legumes · Quality seeds · Seed production · Seed processing

6.1 Introduction

Forage legumes are important for supplying animal feed and for their environmental benefits in extensive and intensive livestock production systems globally. Forage legumes are considered as the most important forage source due to the dual-purpose nature of a number of them as a protein-rich livestock feed and as grain for human consumption. The use of forage legumes for sustainable livestock production may be

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11,000 years old; however, some of the species were first used as grain for human consumption and more recently for fodder or pasture. An estimated more than 1500 out of a total of 17,000 legumes species reported worldwide are used as livestock feed. Of these, only a small number of species are widely used as cultivated forages and are incorporated into forage breeding programs with the aim of developing new varieties (Kulkarni et al. 2018). Not all legumes are suitable as forage because some species are woody and may have spines or thorns, while others have leaves with glands, glandular hairs or sticky exudates, and even compounds that are poisonous to livestock. The selection of which forage legume to grow also depends on climatic conditions, production system, and livestock needs.

6.2 Tropical Forage Legume Seed Systems

Consumption of livestock products (meat and milk) has grown notably around the world and is expected to grow further largely because of the population growth, rapid urbanization, rising incomes, and diversification of human diets. The productivity of livestock is constrained mainly due to low-quality fodder, and therefore, forage legume-based technologies are highly effective for intensifying meat and milk production on small farms by increasing the protein content in livestock feed (Ramesh et al. 2005; Guodao and Chakraborty 2005; Peters and Lascano 2003). While integrating more planted forage legumes in the farming system is a promising solution to meet the increasing demand for livestock products, the availability of high-quality tropical forage seeds/planting materials and lack of stable demand for forage seeds constrain forage legume production. In the absence of formal forage seed systems in many tropical countries, much of the production has been in the informal or traditional seed sector. Farmers undertake their own selection of promising plants from the forage crop and produce seeds to meet their own needs. In addition, they may also sell or exchange seeds within the local community. Therefore, there is a need to focus on quality seed production to increase forage productivity for sustainable livestock production.

6.3 Forage Legume Quality Seeds

Crop productivity is greatly influenced by the quality of seeds. Seed quality assurance has been reviewed at length for forages and is important where consumers recognize the link between high-quality seeds and crop performance (Beavis and Harty 1999). Quality assurance relates to seed customer's needs, reducing risk for producers, market acceptance, and use of best practices through the entire production and marketing process. In many countries, quality is controlled by seed legislation in the formal seed sector with seed certification for variety, purity, and germination. However, in many developing countries, forage seeds are produced and marketed by farmers, communities, cooperatives, and small-scale businesses where seed legislation may be inappropriate and difficult to apply. Government regulatory institutions

have limited resources and capacity to conduct quality certification across this wide range of forage producers: in these cases, the Quality Declared Seed (QDS) regulatory system, offers an alternative for tropical forages that do not easily fit within a conventional seed quality control scheme or where highly developed seed quality control activities are difficult to implement or make relatively little impact (FAO 2006). Producers working under a QDS system have the option to market forage seeds without full inspection, quality testing, and certification, which may be a more suitable regulatory system for forages. Usually, the seed producers are licensed and follow regulations to ensure quality seed is being produced with limited seed inspection to qualify seed for QDS status. Another system that links the value to the users with the seed quality is also used in marketing mainly in the USA called truth in labelling (Beavis and Harty 1999). This system has no minimum standards for quality and assumes that all sectors of the seed system can judge the value of the seed lot from the description of the quality and obligates seed vendors to label seed with accurate quality information in line with the truth in marketing requirements of trade practice laws.

The quality of the seeds is defined mainly based on four criteria, namely, genetic quality, physical quality, physiological quality, and health quality. The entire production and storage process has a major effect on the quality of forage legume seeds. The quality of seeds produced from the production process in forage legumes is largely determined by the climate (day length, temperature, and rainfall), nutrition of the soil and plant, and the harvest time of the seeds. However, forage legumes have a diverse range of attributes and requirements for seed production that is difficult to meet in one climate and environment (Andrade-de 1999), and forage seed production is best carried out in environments where the species are adapted. Several forage genera and species are very specific in their climate and soil requirements; for example, forages adapted to acid soils will not grow well when planted in areas with alkaline or neutral soils. In addition, the temperature and soil moisture during seed maturation are important to allow good seed development and filling and to avoid water or heat stress. Attention to quality in all stages of the process will result in the production of high-quality seeds for use in enhanced adoption of tropical forages.

6.4 Genetic Quality

Genetic quality in crops refers to variety purity and correctness. It contributes to more consistent and higher yield, better grain quality, and higher biotic and abiotic stress tolerance. Different varieties may have different morphologies, nutritional qualities, disease and pest resistance, and adaption to the environment. Selection of the best adapted variety for the production site and user needs will determine the performance. Quality forage seeds should be the correct variety to meet market standards.

Genetic purity, or trueness to variety, is established and maintained by production of outcrossing species in isolation from other varieties to avoid pollen

contamination, field and seed inspection during the growing season to rogue out any off types, and pedigree records and certification of variety labelling. Most tropical pasture legumes are predominantly self-pollinating but also show low levels of cross-pollination when grown within pollination distance of other varieties (Hacker and Hanson 1999). For example, *Stylosanthes* species and cowpea, which are primarily selfing, may show levels of outcrossing up to 22% (Cameron and Irwin 1986) and 10% (Kouam et al. 2012), respectively. Other species are cleistogamous, shedding the pollen inside the flower before it opens, including *Neonotonia wightii*, *Lotononis bainesii*, *Centrosema pubescens*, *Macroptilium atropurpureum* (Humphreys and Riveros 1986), *C. brasilianum* and *C. virginianum* (Grof 1970), *Desmodium sandwicense* (Rotar et al. 1967), *Lablab purpureus* (Kukade and Tidke 2014), and *Sesbania sesban* (Heering 1994). Although cleistogamous, a degree of outcrossing occurs if there is pollen flow and insect activity. This has been reported to be up to 18% in *D. sandwicense* (Rotar et al. 1967). This points to the need to produce seeds in isolation to retain variety purity when producing quality forage seeds. A distance of 100 m from other standing varieties is considered to be sufficient to reduce pollen flow, especially if other legume crops are planted in between the varieties.

Field inspection for variety correctness is an essential part of the process to produce quality seeds (HSU 1994). Inspection is usually done at flowering and/or seed harvest. Each registered variety has a detailed variety description that can be used to determine correctness, and any off types can be removed from the field. This will allow the seeds to be certified for variety and correctly labeled.

6.5 Physical Quality

This includes the physical properties such as seed purity, seed size, and seed weight and it covers the entire process of seed production and processing. Physical seed quality is without question the most important factor for marketing and requires careful attention throughout the seed production process to ensure production of seeds with a good germination rate which are free from weed seeds and diseases. Compared with sowing for general forage use, seed crops warrant more care and expense during the establishment phase. For example, seedbeds should be prepared more thoroughly and the seed sown more precisely, using higher seeding rates. Weeds need to be controlled from the outset. Essentially, the aim is to establish a strong, vigorous stand capable of producing profitable seed yields as soon as possible after sowing (Loch et al. 1999).

There are generally three groups of forage legumes: warm tropical, cool tropical, and subtropical legumes. Management of the seed crop for each group is based on their temperature adaptation (Andrade-de 1999).

6.5.1 Warm Tropical Legumes

Warm tropical legumes need a frost-free climate with reliable and well-defined wet and dry seasons and minimal rainfall of 800 mm to produce strong vegetative growth for a good seed crop. Some species, such as *M. atropurpureum* cv. Siratro and *C. pubescens*, require a long dry season with moisture stress to promote flowering.

6.5.2 Cool Tropical Legumes

Some perennial legumes, including *D. intortum*, *D. uncinatum*, *Macrotyloma axillare*, *N. wightii*, and *Vigna maranguensis*, need a cooler and longer wet season for good seed production.

6.5.3 Subtropical Legumes

The more subtropical species of legumes including *Trifolium semipilosum*, *Lotononis*, fine-stem stylo, and *Aeschynomene falcata* do not flower during winter avoiding frost. Moisture stress has little or no beneficial effect on flowering, although adequate moisture is important to support the vegetative growth required for a seed crop.

6.5.4 Production Environment

Production of quality forage legume seeds starts with selection of the most suitable environment for production. Sites must be accessible for good management and inspection and as much as possible located on land suitable for cultivation, irrigation, and fertilizer application. Day-length-sensitive legumes require a high density of shoots which are stimulated to flower by day length/temperature. This provides the necessary vegetative structure to maximize seed production. Other requirements for a suitable site include the following:

- Frost-free climate with adequate rainfall or irrigation over the growing season
- Soil pH suitable to most of the forage legumes species
- Area free of noxious weeds, pest, and diseases
- Adequate space to isolate cross-pollinated species
- Minimal rainfall during flowering, pollination, and seed harvesting

6.5.5 Land Preparation

For seed crops, thorough land preparation is essential to provide a clean and firm seedbed. Land leveling is preferred for irrigated systems or mechanized harvesting.

A clean and firm seedbed will ensure a dense plant population that will compete successfully with weeds, reduce contamination, and produce a high quantity of quality seed (Loch et al. 1999).

6.5.6 Seed Quality and Sowing of Seeds

Using good-quality seed with high purity and germination to establish forage legumes will usually enhance the production of certified seeds. Purity is expressed as percentages of seed of the sown variety, other crops and weeds, inert matter, and broken seed. Special attention must be given not to introduce new, potentially serious weeds into the forage legume seed production area (Loch et al. 1999). Seeds are usually sown in rows wide enough (50–100 cm apart) to reduce the quantity of seeds required and to make weeding and harvesting operations easy as soon as there is sufficient soil moisture to support germination and establishment. Seeding rates are often low because of the small seed size of many tropical legumes (3–8 kg/ha).

Seedling emergence depends on a complex interaction involving seed placement, size, hard seededness, genotype, and the microenvironment (the seedbed). Hard seededness prevents premature germination of seeds. There are two types of seed dormancy in forage legumes. Embryo dormancy occurs when the embryos are physiologically inactive due to intrinsic germination inhibitors or inactive enzyme systems. These need a period for dormancy to reduce after ripening before planting. Seed coat dormancy occurs due to a thick impermeable testa which prevents the entry of gases and moisture and the expansion and growth of the embryo or when chemicals in seed coats inhibit germination. It is important to reduce the hard seededness and improve germination for many forage legume crops. Seed scarification is used to physically damage the seed coat to allow imbibition. A number of seed scarification methods have been used by the researcher since the early twentieth century with variable results. Scarification may use mechanical, chemical, or environmental instruments to promote uniform germination. Heat, mechanical, and acid scarifications are among the most popular methods and found effective when performed carefully (Table 6.1). Hot water scarification is a high-risk method and open to operator error (cooked seed), and the process initiates germination so the seed cannot be stored for even short periods and, if sown and no rain falls, leads to mortality and poor emergence. If sowing is not possible (e.g., heavy rain falls during the scarifying period), the seed will ferment unless constantly aerated (Argel and Paton 1999).

6.5.7 Fertilizer Application

Adequate fertilizer applications are required to promote plant growth and subsequent high-quality seed production (HSU 1994). Mixing fertilizer with legume seed at the time of sowing can result in an interaction which reduces germination. Application

Table 6.1 Methods of breaking seed dormancy of forage legumes

Methods	Procedure	Species suitability
Mechanical scarification		
Sandpaper scarification	Abrade the seed coat with sandpaper manually	*Macroptilium atropurpureum*, *Pueraria phaseoloides*, *Neonotonia wightii*, *Macrotyloma axillare*, *Trifolium* sp.
Knife scarification	Nick the seed with a sharp knife or razor	*Lablab purpureus*, *Cajanus cajan*, *Medicago sativa*, *Stylosanthes* sp.
Drum scarification	Abrade the seeds using a machine with abrasive paper inside the drum	*Medicago* sp., *Stylosanthes* sp., *Lotononis* sp., *Trifolium* sp., *Desmodium intortum*, and *D. uncinatum*
Hot wire scarification	Abrade the seed coat using a pyrography tool	*M. atropurpureum*, *M. axillare*, *Stylosanthes* sp., *Centrosema* sp.
Chemical scarification	Dip seeds in strong acids such as sulfuric acid or use of organic solvents such as acetone, alcohol. Wash well with clean water after treatment	*Centrosema pubescens*, *Leucaena leucocephala*, *Desmanthus virgatus*, *Calopogonium mucunoides*, *M. atropurpureum*, *Clitoria ternatea*, *N. wightii*, *P. phaseoloides*, *Cratylia argentea*, *Stylosanthes* sp., *Medicago* sp., *Trifolium* sp.
Alternating and constant temperature scarification	Place imbibed seeds in high constant temperature or fluctuating temperature	*Stylosanthes scabra*, *T. subterraneum*, *T. hirtum*, *Phaseolus vulgaris*
Hot water scarification	Dip the seed in boiling water or hot water at a fixed temperature for few minutes	*Leucaena leucocephala*, *D. virgatus*, *C. pubescens*, *C. ternatea*

of phosphorus fertilizer is essential during establishment because legume seed crops are independent of soil N levels as long as they effectively fix atmospheric nitrogen.

6.5.8 Weeding

Weed control measures taken in the seedbed help ensure successful establishment of the seed crop. A pre-emergence herbicide may be used but is too expensive for many seed producers. The selection of planting time, thorough and repeated cultivation, crop rotation, and hand weeding (where cheap labor is available) are all alternative methods of weed control. Weeds can reduce seed yields and quality through competition and contamination. Legume seed crops are particularly vulnerable to weed invasion. Efficient weed control reduces contamination with weed seeds during harvesting. Weeding is comparatively easy in these crops, particularly if they also form a vigorous smothering canopy (HSU 1994).

6.5.9 Irrigation

Irrigation can be used to supplement rainfall and may also be used to induce flowering, improving synchronization of flowering and seed maturation. Some regions are naturally conducive to more synchronized flowering and uniform crop maturation because of their photoperiod and rainfall distribution. Defoliation (by cutting or grazing) and the deliberate use of water stress are management practices that can help synchronize maturation in legume seed crops.

6.5.10 Harvesting

Tropical forage legume species present a large diversity of morphological types. The positioning of pods and seeds also restricts the recovery of the seeds harvested; this may be formed inside a mat of vegetative growth (*Stylosanthes guianensis*) and below the soil surface (*Arachis pintoi*) or spread out on shrubs (*Leucaena leucocephala*). Efficient timing and harvesting techniques result in high quality and quantity of seed production (Andrade-de and English 1999). Even the most closely synchronized crops comprise inflorescences in various stages of maturity with further variation in flowering time within individual inflorescences, so some immature seeds will be present at harvest. Shedding of ripe seeds reduces yields, so the balance between maturity and shedding determines the standing (net) seed yield at any point of time and guides the optimum time to harvest.

Techniques for determining optimum harvest time vary with the species. Recognition of harvest ripeness is critical for forage legumes where the ripe seeds shatter readily; species such as *M. atropurpureum* and *S. guianensis* are the most difficult in this regard where there is premature shattering of seeds while still green (Loch et al. 1976). With shattering species, any delay in harvesting after the peak of standing seed yield will result in progressively lower yields because of seed shattering. Other legume species retain ripe seeds in the inflorescence for a time after maturity, and because there is less seed lost through shattering, the precise definition of optimum harvest time is also less important if seeds are to be sold to market in the coming season.

Indicators of harvest ripeness are usually based on a change in the color of pods or seeds, the degree of seed shattering, and the stickiness of the crop (Hopkinson and Loch 1977; Lambert 1982; English and Hopkinson 1985; Carmona et al. 1986). The seed or pods of some species, for example, siratro, tree lucerne, and *Leucaena*, change color as they ripen. The optimum harvest time usually occurs before maximum flower density occurs. Frost, drought, rainfall, and wind can also affect both the timetable of maturation in the crop and its morphological appearance, but with their accumulated experiences based on previous observation, growers usually cope with these limitations.

There are four broad approaches used when harvesting legume seed crops: a single destructive harvest of the standing crop, multiple harvesting of the standing crop, recovery of mature seeds shed from the standing crop, and the exhumation of

Table 6.2 Methods of seed harvesting

S. No.	Methods	Procedure	Species suitability
1	Hand picking	Picking mature pods of shattering species one by one	*Macroptilium atropurpureum*, *M. axillare*, *M. lathyroides*, *Chamaecrista rotundifolia*, *Leucaena leucocephala*, etc.
2	Stripping	Stripping the mature pods by hand/stripper	*Desmodium intortum*, *D. uncinatum*, *Melilotus albus*, *D. heterocarpon*
3	Sweeping	Sweeping the whole fallen seed from the ground using brooms/ sweepers	*Medicago scutellata*, *Stylosanthes hamata*, *S. guianensis*, *Centrosema pascuorum*, *M. truncatula*, etc.
4	Cutting	Cutting the top part or the whole plant using sickle/knife/ scythes and windrowing	*Lablab purpureus*, *S. scabra*, *N. wightii*, *Desmanthus virgatus*, *Aeschynomene americana*, *Vigna unguiculata*
5	Shaking	Shake the whole plant by hand inside paper bag or on canvas	*Stylosanthes scabra*, *S. guianensis*, *L. leucocephala*
6	Combine harvesting	Cutting the whole canopy using combined harvester	*Macroptilium atropurpureum*, *S. scabra*, *N. wightii*, *D. virgatus*, *A. americana*
7	Suction harvesting	Sucking the fallen seed from the floor	*Stylosanthes hamata*, *S. guianensis*, *M. atropurpureum*
8	Extraction from soil	Exhuming the seeds and soil and sieving the dry soil to separate seeds	*Arachis pintoi*, *V. subterranean*, *T. subterraneum*

buried seeds (Table 6.2). In general, the characteristics of the crop, its synchrony of development, the relative amounts of seed shed, and seed size and structure determine the efficiency of a particular harvesting method (Hill and Loch 1993). Harvesting can also be either mechanical or manual which depends on the costs and the availability of labor or machinery.

A single destructive harvest of the standing crop is the most widely used approach either using a conventional combine harvester or cutting the crop manually with a sickle. With hand harvesting, the top part of the canopy containing the inflorescences is cut using sickles and left in the field on canvas or bare soil to dry in the sun. The dried material is then generally beaten with wooden sticks and the threshed seed cleaned manually by traditional techniques. Sieves are used to remove material larger or smaller than the seed, followed by hand winnowing to remove the lighter material.

Hand picking is traditionally used to collect seed from twining species like *C. mucunoides*, *M. atropurpureum*, and *P. phaseoloides* or shrub/tree legumes like *L. leucocephala*. This process of using pickers who pluck or strip the ripe pods from the plant is repeated on a number of occasions as the crop progressively ripens. Hand picking is generally associated with casual seed production in plantation agriculture (from the cover crop of twining legumes) and with smallholder seed production systems. For twining legumes, the use of trellises or vegetative support (maize, elephant grass, sorghum, cassava) can increase the efficiency of hand picking. This

lifts the intertwined canopy of legume vines up from the soil, exposing the ripe pods and facilitating their identification and collection. Seeds obtained by hand picking can be of high quality. Depending upon the frequency and number of seeds collected per run, hand picking can be very efficient in recovering a greater proportion of the seed production from the crop.

Harvesting methods based on the recovery of fallen seeds have the potential to collect a greater proportion of the total seed yield than those which target the standing crop. The vigor of seed recovered from the ground is normally high, because seeds are mostly mature when shattering occurs (Hopkinson and Clifford 1993). Ground sweeping is used with *S. hamata* cv. Verano and *S. guianensis* cv. Graham. The first step is to expose the soil surface ready for sweeping by cutting the plants at the base (with a sickle or hoe) and removing them. Seed purity is invariably low in the materials swept up, so that seed cleaning can be quite a labor-demanding operation. Suction harvesting is useful for species that shed their seed as they ripen and/or cannot be collected efficiently (*S. hamata*, *S. guianensis*, *M. atropurpureum*).

Perennial *Arachis* species produce underground fruits, a characteristic unique among the tropical forage legumes and one that necessitates a different approach to seed harvesting through exhumation of the buried pods that can be undertaken manually or mechanically.

6.5.11 Seed Processing

The goal of seed processing is to obtain seed of the highest possible quality using the most economic method. The process refers to two distinct functions:

6.5.11.1 Cleaning

This refers to the process of removal of materials other than the desired seed such as other crop and weed seeds, sticks, straw, soil, and stones. The process starts with the drying of the plants and seeds. Some harvesting methods, including direct manual cutting, windrowing, and hand picking, already incorporate a sun-drying phase to facilitate threshing and so require little or no subsequent drying. Seeds recovered from the ground also require little or no drying, because they are shed at low moisture content and are exposed to sunshine after the vegetation is removed prior to suctioning or sweeping.

After drying, threshing can be performed manually, using animal power or mechanical threshing. The material can be threshed by trampling with the feet, lightly pounding with local wooden mortar, or beating with sticks. Threshed seed is then cleaned to remove seeds of contaminant species, soil, chaff, and poor-quality seeds. Seeds of subtropical and tropical legumes sometimes present considerable difficulties during the cleaning and processing of the harvested material. Problems are encountered because of their physical attributes of appendages and sticky pods. Winnowing and sieving are the normal means of cleaning seed. A range of standard

Table 6.3 Methods of seed processing/cleaning for forage legumes

S. No.	Methods	Procedure
1	Manual cleaning	Sorting out unwanted materials by winnowing and sieving manually
2	Pre-cleaning	Removal of materials other than seed that could impede seed flow and accurate cleaning
3	Mechanical cleaning with air screen unit (size separator)	Separation of seed according to size, shape, and diameter using flat air screen/cylindrical units which have perforations with round, square, triangular, or slotted
4	Mechanical cleaning with indented units (length separator)	Separation of seed based on difference in length by using pockets called indented cylinder/disc which sorts short seed from long seed or straw
5	Mechanical cleaning with gravity grader (density separators)	Separation of seeds based on differences in density using a deck which consists of a porous base of wire mesh or open-weave cloth

seed-processing systems can be used successfully with a number of tropical and subtropical forage species (Table 6.3).

6.5.11.2 Grading

This is the process of sizing seed to produce an even product suitable for use. Differences in the physical properties exhibited by seeds provide the basis for grading/separation (Linnett 1999). There are seven major principles on which most seed separations have been made:

1. Shape is the natural outline of seed. The perforated screen is the usual medium for separation based on difference in shape.
2. Size refers to differences in the individual bulk of seed with the same general outline. Separation on the basis of size is possible using screen or other density separator.
3. Width and thickness. Width refers to the narrowest dimension of the seed in cross section, while thickness is applied to the broader cross-sectional dimension. The screen utilizes this principle for separation.
4. Length is the longest dimension of seed. Indented cylinder and indented discs perform separation on this basis.
5. Density is measured by the specific gravity of seed. The gravity table is able to separate seed and contaminants of different densities.
6. Color is not routinely applied to forage seeds. Seeds may be separated electromechanically when a difference in color exists.
7. Flotation refers to the support of an object in a liquid or gas. When this occurs to differing degrees in a seed mixture, a flotation unit is able to make a separation.

6.6 Physiological Quality

This includes the viability and vigor of seed which are responsible for potential germination and subsequent seedling emergence and crop establishment in the field. The accepted hypothesis for many years was that seeds achieve maximum quality at the end of seed filling (termed physiological maturity) when seed moisture content is high and after that seed quality (viability and vigor) starts to decline because no further nutrients are accumulated in the seeds from the mother plant (Harrington 1972). Recent research findings have challenged this widely held assumption because many seeds continue to improve in quality for some time after physiological maturity (Ellis 2019). The meaning and definition of the term physiological maturity became confused, and the term mass maturity was introduced to designate the end of the seed-filling phase (Ellis and Pieta-Filho 1992). Harvest maturity is used to describe the seed developmental stage at the end of the seed maturation phase when seed moisture content has reduced further, approaching equilibrium with the ambient environment (Ellis 2019). This is the stage when most seed producers and farmers harvest their seeds. Yadav and Ellis (2016) demonstrated that seed improvement and deterioration processes cycle during maturation of wheat seeds subjected to rainfall during the maturation process, indicating that seed quality is not fixed at physiological maturity. Experiments with rice seed also demonstrated that longevity can be increased by high-temperature drying to reduce seed moisture content during late maturity after the seeds have been removed from the plant (Whitehouse et al. 2018). Phaseolus seeds also showed increased germination, desiccation tolerance, and longevity after air drying for up to 23 days after harvesting at mass maturity (Sanhewe and Ellis 1996a, b). More recent studies on *T. ambiguum* also indicated that germination and longevity were increased by a period of maturation drying of up to 10 days after mass maturity (Hay et al. 2010). These reports indicate that seed quality can be improved long after mass maturity and, in some cases, even after harvest maturity in cereal, legume, and forage seeds. The time of maximum quality during seed maturation varies with environment and crop, but maximum seed quality can be maintained or extended in some seed production environments beyond the previously accepted point of maximum seed quality (Ellis 2019).

6.6.1 Seed Quality During Storage

Seeds can be divided into those that can be dried and stored for long periods under cool conditions with little loss of longevity, termed orthodox seeds, and those that cannot withstand drying without loss of viability and therefore cannot be stored for long periods under cool temperatures, termed recalcitrant seeds. Most forage seeds fall into the orthodox category, and dry cool storage is used to ensure longevity and quality of seeds during the storage period (Chin and Hanson 1999). This is particularly important to retain seed quality during storage in tropical areas with high ambient temperatures and humidity.

Storage temperature and moisture content of the seeds are key to good longevity. Harrington (1963, 1970, 1972) suggested that the longevity of the seeds is doubled for each 1% reduction in seed moisture content down to about 4% seed moisture and for each 5 °C reduction in storage temperature between 50 and 0 °C. The effects of reductions in moisture content and temperature on seed longevity are independent, and different combinations of drying and cooling are used to ensure seed longevity and quality during storage for different periods. Cold stores are more frequently used for bulk seeds and commercial seed amounts because they can handle large quantities that need to be stored for relatively short periods, often only from one harvest to the next planting season. Longer-term seed storage, such as in genebanks, uses both seed drying and cold storage to ensure longevity of small seed samples with high genetic diversity for long periods.

The general relationship between moisture content, temperature, and seed longevity is consistent across species and has allowed modelling of seed longevity and determination of viability constants (Ellis and Roberts 1980, 1981). While these models have been applied to many crop species (Ellis and Hong 2007), it is only recently that sufficient information on longevity of forage species has been generated to apply the equations and determine longevity for forage genera of legumes (Ellis et al. 2017) and grasses (Ellis et al. 2019). Previously, some forage legume seeds were reported to have good longevity but without any estimates of possible storage periods (Chin and Hanson 1999).

6.6.2 Forage Seed Certification

Seed quality for germination and longevity are part of the seed certification system for quality assurance in the formal seed sector in many countries. The methods used to determine seed viability as well as purity may affect the final results declared for certification and should be standardized for seeds tested over time in different laboratories (Beavis and Harty 1999). For seed viability, this is usually the International Seed Testing Association (ISTA) which uses standard testing procedures in their accredited laboratories and issues international certificates. Many common tropical forage species are included in their standard testing protocols (ISTA 2019). However, in many countries in the tropics, the formal seed sector for forage seeds is not well developed, and forage seed certification is only now being introduced, or Quality Declared Seed regulatory systems or truth-in-labelling informal systems are preferred as part of forage seed system development to meet market demands for quality assurance.

6.7 Seed Health

This covers the production and maintenance of seeds, free from insect damage and diseases. Seed health is one of the important aspects to consider in producing high-quality forage seeds. Health of the seeds could be affected by several pathogens

including fungi, bacteria, viruses, and insect pests which could present either on the seed surface or internally in the seed coat and lead to low germination, reduced stand establishment, severe yield loss, or total crop failure in some circumstances. The list of the important pathogens and associated major diseases of forage legumes is available online (https://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=432&Itemid=614) and is reviewed by Trutmann (1994). Diseases are also reported to reduce the nutritive value and palatability of forages that ultimately affect animal health and productivity (Latch and Skipp 1987; Gardiner 1975; McKay 1993; Madin 1993). Seeds are also considered as an important means to carry over and disperse pathogens which has contributed to the local and international spread and survival of these pathogens (Kaiser et al. 2000), and therefore, the production of disease-free seeds is an important management option to control disease spread and support sustainable agricultural production. Pathogens could enter into the system at different stages, starting from the seedling stage in the glasshouse to the growth phase in field, followed by infection during harvest and storage. Therefore, appropriate control measures have to be taken to ensure the quality of seeds. Based on the types of pathogen, chemical, biological, cultural, and regulatory methods can be used to control the pathogen and associated loss. An appropriate fungicide or insecticide could be used to control the infestation by fungi and insects, respectively. Similarly, seed crop rotation and destroying of infected planting materials (plants and seeds) help in reduction of pathogen load in a particular place and environment.

6.7.1 Effects and Management of Pathogens

6.7.1.1 Bacteria

Bacteria infect the plants in the field, cause disease symptoms, and result in low productivity. Bacteria have a minimal role in seed deterioration during storage as they require free water and high temperature for their growth. Seeds that are stored at low temperature and moisture content are less susceptible to spoilage by bacteria during storage. If the moisture content of the seed increases by any means, then the seed deteriorates primarily due to fungi, respiration, heating, or premature sprouting, before any bacterial growth.

6.7.1.2 Fungi

Infection with fungi could happen at any stage of the seed production and storage process, and therefore, fungi are considered as major pathogens responsible for seed deterioration. Some fungal diseases such as anthracnose in cowpea (Cardona 1990) and *Stylosanthes* (Nan et al. 1998) are transmitted by seeds. Seeds must be free of these diseases to avoid risk of forage failure or transmission to other legume crops.

Storage fungi mainly belong to the genus *Aspergillus* and *Penicillium*, grow on dead cells of the seed surface, produce toxins, cause seed decay, and may kill the embryo leading to poor quality seeds. The presence of these fungi in seed lots leads to loss of seed viability and germination, discoloration, mustiness, and caking. These

storage fungi could easily be controlled by maintaining the low moisture in seed and low temperature and humidity in storage facilities, whereas fungi infecting plants in field could be controlled by using an appropriate fungicide treatment. Fungal infection in legumes is difficult to control, because the fungus is mostly located in the seed coat (Ellis and Paschal 1979; Tu 1988).

6.7.1.3 Viruses

A number of viruses are reported to infect a variety of forage legumes during seedling stages and under field conditions, but as the symptoms caused by viruses are not as striking as compared to other pathogens, it is difficult to diagnose without using an efficient detection tool. It has also been reported that about 50% of viruses affecting legumes are seed-borne and therefore have a significant role in seed deterioration during storage in addition to the risk of disease if clean seeds are not produced and marketed. Proper cultural practices such as varying sowing dates, rouging infected plants early in the season, and using nonhost plants as a border during planting are the only effective measures to control the losses caused by viral diseases.

6.7.1.4 Insects

The hot and humid climatic condition supports the growth of many insect pests, and therefore, insect pests are a serious problem for tropical and subtropical forage legumes. These insects are responsible for crop damage both in the field and in storage conditions. However, cool and dry seed storage conditions reduce the chances of insect infestation. Apart from this, appropriate chemicals such as insecticides or pesticides or a biological control method could be used to kill/restrict the growth of insects.

6.7.2 Seed Certification for Disease Status

Many of the pathogens infecting forage legumes are seed-borne; therefore, it is necessary to check the disease status of seed at harvest before its storage, distribution, selling to the open market, and international export. While most of the seed producers employ all the appropriate management options to produce disease-free seeds, there is a chance to have infection in the produced seeds. Therefore, a standard laboratory-based method is needed to be applied to check the infection of each suspected pathogen in the available seed lot. Most of the seed-producing companies/agencies operate a laboratory to test the disease-free status of seeds, but they are not permitted to issue certificates. Small-scale farmers or stakeholders don't have these facilities and may rely on truth in labelling to attest to the quality of the seeds. An independent seed certification authority has been established in each country to serve the seed producers and seed users and to perform this duty impartially. The procedures of seed certification used by these authorities are the same in all countries following the ISTA rules and issuing international certificates (ISTA 2019). These

processes assure the market on the disease quality of the tropical forage legume seeds for marketing and growers.

6.8 Conclusions

As the demand for tropical legume forages in the livestock value chain grows, the demand for high-quality seeds will also increase. Producers and seed merchants already need to address issues of quality seeds to meet the market demand, reduce risks for seed producers, and grow market confidence in seed quality in the tropical forage legume seed sector. Many of the tropical legumes in use by farmers were released in the 1960s (Hanson and Peters 2003). There are now better-adapted and disease-resistant varieties available for many of the widely grown forage species, and tropical forage seed production should focus on the production of quality seeds of these new varieties to provide farmers with a better choice of what to grow. This means that seed producers need to pay increased attention to processes that influence genetic and physical seed quality through the entire seed production chain to provide quality assurance for the market. This should be backed up by certification or labelling and use of a QDS regulatory system in the absence of a fully functional seed certification system for forage seeds. Greater attention to seed quality will support agribusiness and growth of the tropical forage legume seed sector in many tropical countries.

Developing a sustainable supply of planting material for tropical forage legumes should center on fostering private enterprises, supported by private and public sector investment, that avails best-quality forage seed to smallholder farmers conveniently and affordably. These enterprises can also provide opportunities for rural youth employment, which is becoming a major issue for governments in many tropical countries. Almost 88% of the world's youth live in developing countries where employment and entrepreneurial opportunities for them are limited (FAO, CTA and IFAD 2014). Employment opportunities in the production or marketing of forage legume seeds will grow as the market demand grows and should result in a well-functioning forage seed industry that provides quality forage seeds in response to the growth in demand in the end market.

References

Andrade-de RP (1999) Location of seed crops: legumes. In: Loch DS, Ferguson J (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, pp 129–140

Andrade-de RP, English BH (1999) Seed harvesting and drying. In: Loch DS, Ferguson JE (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, p 213

Argel PJ, Paton CJ (1999) Overcoming legume hardseededness. In: Loch DS, Ferguson JE (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, pp 247–265

Beavis CHS, Harty RL (1999) Seed quality assurance. In: Loch DS, Ferguson J (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, pp 283–301

Cameron DF, Irwin JAG (1986) Use of natural outcrossing to improve the anthracnose resistance of *Stylosanthes guianensis*. In: Proceedings of the DSIR Plant Breeding Symposium. Agronomy Society of New Zealand Special Publication No. 5. Agronomy Society of New Zealand, Christchurch, pp 224–227

Cardona JAO (1990) Seed transmission and control of cowpea anthracnose (*Colletotrichum dematium*). Revista Instituto Colombiano Agropecuario 25:265–274

Carmona R, Ferguson JE, Maia MS (1986) Maturacao para colheita de sementes de *Stylosanthes macrocephala* M.B.Fer.et S. Costa. Revista Brasileira de sementes 8(3):9–18

Chin HF, Hanson J (1999) Seed quality: seed storage. In: Loch DS, Ferguson J (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, pp 303–315

Ellis RH (2019) Temporal patterns of seed quality development, decline, and timing of maximum quality during seed development and maturation. Seed Sci Res 29:135–142

Ellis RH, Hong TD (2007) Quantitative response of the longevity of seed of twelve crops to temperature and moisture in hermetic storage. Seed Sci Technol 35:423–444

Ellis MA, Paschal EH (1979) Effect of fungicide seed treatment on internally seed-borne fungi, germination and field emergence of pigeon pea (*Cajanus cajan*). Seed Sci Technol 7:75–81

Ellis RH, Pieta-Filho C (1992) The development of seed quality in spring and winter cultivars of barley and wheat. Seed Sci Res 2:9–15

Ellis RH, Roberts EH (1980) Improved equations for the prediction of seed longevity. Ann Bot 45:13–30

Ellis RH, Roberts EH (1981) The quantification of ageing and survival in orthodox seeds. Seed Sci Technol 9:373–409

Ellis RH, Nasehzadeh M, Hanson J, Woldemariam Y (2017) Medium-term seed storage of 50 genera of forage legumes and evidence-based genebank monitoring intervals. Genet Resour Crop Evol 65:607–623

Ellis RH, Nasehzadeh M, Hanson J, Ndiwa N, Woldemariam Y (2019) Medium-term seed storage of diverse genera of forages grasses, evidence-based genebank monitoring intervals, and regeneration standards. Genet Resour Crop Evol 66:723–734

English BH, Hopkinson JM (1985) Verano stylo seed production. QLD Agric J 111:59–63

FAO (2006) Quality declared seed system. FAO Plant Production and Protection Paper. Food and Agriculture Organization of the United Nations, Rome, p 185

FAO, CTA, IFAD (2014) Youth and agriculture: key challenges and concrete solutions. Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Technical Centre for Agricultural and Rural Cooperation (CTA) and the International Fund for Agricultural Development (IFAD), Rome, p 128

Gardiner MR (1975) Lupinosis. J Agric West Aust 16:26–32

Grof B (1970) Interspecific hybridization in *Centrosema*: hybrids between *C. brasilianum*, *C. virginianum* and *C. pubescens*. QLD Dept Prim Indus Div Plant Indust Bull 564:385–389

Guodao L, Chakraborty S (2005) Stylo in China: a tropical forage legume success story. In: Proceedings of the XX International Grassland Congress: Offered papers, Dublin. Wageningen Academic, Wageningen, p 322

Hacker B, Hanson J (1999) Crop growth and development: reproduction. In: Loch DS, Ferguson J (eds) Forage seed production. Volume II. Tropical and subtropical species. CAB International, Wallingford, pp 93–111

Hanson J, Peters M (2003) Meeting the need for herbage seeds in developing countries. In: Loch DS (ed) Proceedings of the 5th International Herbage Seed Conference, Herbage seeds in the new millennium – new markets, new products, new opportunities. Queensland Department of Primary Industries, Cleveland, OH, pp 26–37

Harrington JF (1963) The value of moisture-resistant containers in vegetable seed packaging. Calif Agr Expt Stat Bull 792:1–23
Harrington JF (1970) Seed and pollen storage for conservation of plant gene resources. In: Frankel OH, Bennett E (eds) Genetic resources in plants; Their exploration and conservation. Blackwell Scientific Publications, Oxford, pp 501–521
Harrington JF (1972) Seed storage and longevity. In: Kozlowski TT (ed) Seed biology, vol III. Academic Press, New York, NY, pp 145–245
Hay FR, Smith RD, Ellis RH, Butler LH (2010) Developmental changes in the germinability, desiccation tolerance, hard seededness, and longevity of individual seeds of *Trifolium ambiguum*. Ann Bot 105:1035–1052
Heering JH (1994) The reproductive biology of three perennial *Sesbania* species (Leguminosae). Euphytica 74:143–148
Hill MJ, Loch DS (1993) Achieving potential herbage seed yields in tropical regions. In: Proceedings of the XVII International Grassland Congress, pp 1629–1635
Hopkinson JM, Clifford PTP (1993) Mechanical harvesting and processing of temperate zone and tropical pasture seed. In: Proceedings of the XVII International Grassland Congress, pp 1815–1822
Hopkinson JM, Loch DS (1977) Seed production of stylo in north Queensland. QLD Agric J 103:116–125
HSU (Herbage Seed Unit) (1994) Establishment and management of seed crop. ILCA training manual. Forage seed production. ILCA, Addis Ababa
Humphreys LR, Riveros F (1986) Tropical pasture seed production. FAO plant production and protection paper 8. FAO, Rome, p 203
ISTA (2019) International rules for seed testing 2019. International Seed Testing Association, Zurich
Kaiser WJ, Ramsey MD, Makkouk KM, Bretag TW, Açikgöz NA, Kumar J, Nutter FW Jr (2000) Foliar diseases of cool season food legumes and their control. In: Knight R (ed) Linking research and marketing opportunities for pulses in the 21st century. Proceedings of the Third International Food Legumes Research Conference, Adelaide, South Australia. Kluwer Academic Publishers, Dordrecht, pp 437–455
Kouam EB, Pasquet RS, Campagne P, Tignegre JB, Thoen K, Gaudin R, Ouedraogo JT, Salifu AB, Muluvi GM, Gepts P (2012) Genetic structure and mating system of wild cowpea populations in West Africa. BMC Plant Biol 12:113–126
Kukade SA, Tidke JA (2014) Reproductive biology of *Dolichos lablab* L. (Fabaceae). Indian J Plant Sci 3:22–25
Kulkarni KP, Tayade R, Asekova S, Song JT, Grover Shannon J, Lee JD (2018) Harnessing the potential of forage legumes, alfalfa, soybean, and cowpea for sustainable agriculture and global food security. Front Plant Sci 9:1314
Lambert GA (1982) Seed production of fine- stem stylo in the upper Burnett. QLD Agric J 108:57–58
Latch GCM, Skipp RA (1987) Disease. In: Baker MJ, Williams WM (eds) White clover. CABI, Wallingford, pp 441–460
Linnett B (1999) Seed cleaning and processing. In: Loch DS, Ferguson JE (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, pp 229–246
Loch DS, Hopkinson JM, English BH (1976) Seed production of *Stylosanthes guianensis*. 3. Effect of pre-harvest desiccation. Aust J Exp Agric Anim Husb 16:231–233
Loch DS, Cook BJ, Harvey GL (1999) Crop establishment. In: Loch DS, Ferguson JE (eds) Forage seed production. Volume 2: Tropical and subtropical species. CAB International, Wallingford, pp 141–157
Madin RW (1993) Weed, invertebrate and disease pests of Australian sheep pastures – an overview. In: Delfosse ES (ed) Pests of pastures, Weeds, invertebrate and disease pests of Australian sheep pastures. CSIRO, Canberra, ACT, pp 3–20

McKay A (1993) Development of annual ryegrass resistant to Anguina funesta, the vector in annual ryegrass toxicity. In: Delfosse ES (ed) Pests of pastures, Weeds, invertebrate and disease pests of Australian sheep pastures. CSIRO, Canberra, ACT, pp 80–84

Nan ZB, Hanson J, Woldemariam Y (1998) Effects of sulphuric acid and hot water treatments on seed borne fungi and germination of *Stylosanthes hamata*, *S. guianensis* and *S. scabra*. Seed Sci Technol 26:333–345

Peters M, Lascano CE (2003) Forage technology adoption: linking on-station research with participatory methods. Trop Grasslands 37:197–203

Ramesh CR, Chakraborty S, Pathak PS, Biradar N, Bhat P (2005) Stylo in India – much more than a plant for the revegetation of wasteland. Trop Grasslands 39:213

Rotar PP, Park SJ, Bromdep A, Urata U (1967) Crossing and flowering behavior in Spanish clover, *Desmodium sandwicense*. In: Mey E et al (eds) *Desmodium* species. Technical progress report 164. Hawaii Agricultural Experiment Station, Honolulu, HI, pp 3–13

Sanhewe AJ, Ellis RH (1996a) Seed development and maturation in *Phaseolus vulgaris.* I. Ability to germinate and to tolerate desiccation. J Exp Bot 47:949–958

Sanhewe AJ, Ellis RH (1996b) Seed development and maturation in *Phaseolus vulgaris.* II. Post-harvest longevity in air-dry storage. J Exp Bot 47:959–965

Trutmann P (1994) Management of diseases of tropical pasture plants. In: Lenne J, Trutmann P (eds) Diseases of tropical pasture plants. CAB International, Wallingford, pp 291–314

Tu JC (1988) The role of white mold-infected white bean (*Phaseolus vulgaris* L.) seeds in the dissemination of *Sclerotinia sclerotiorum* (Lib.) de Bary. Phytopathol Z 121:40–50

Whitehouse KJ, Hay FR, Ellis RH (2018) Improvement in rice seed storage longevity from high-temperature drying is a consistent positive function of harvest moisture content above a critical value. Seed Sci Res 28:332–339

Yadav G, Ellis RH (2016) Development of ability to germinate and of longevity in air-dry storage in wheat seed crops subjected to rain shelter or simulated supplementary rainfall. Seed Sci Res 26:332–341

Quality Seed Production of Sugar Beet in India

7

A. K. Mall, Varucha Misra, Santeshwari, B. D. Singh, and A. D. Pathak

Abstract

Sugar beet is another crop which possesses capability of producing sucrose, besides sugarcane. It is a flexible crop as it can be grown in number of soils including saline soils where crop like sugarcane fails to grow. It even has the capability to grow well under water-limiting conditions and in general requires less irrigation supply than sugarcane crop. Furthermore, it can be grown as an intercrop with autumn-planted sugarcane which will help in increasing the sugar productivity of our country and also lessens the burden of sugar production bestowed on sugarcane crop solely. Considering all the benefits of sugar beet cultivation, adaptability of superior variety to Indian agroclimatic condition is prerequisite for success of this crop for which intensive research is required for developing indigenous sugar beet varieties. In accordance, success has been achieved in standardization of quality seed production at various locations in our country at higher altitudes where low temperature prevails. These are Mukteshwar and Ranichauri (Kumaon Hills), Auli (Garhwal Hills), Shimla and Kalpa (Himachal Pradesh), Darjeeling (West Bengal), and Kashmir. Due to the variable climatic conditions in India at various places, seed production of this crop is more favorable, and in this respect, India may become independent producer of seed for this crop. With the increasing demand of sugar beet production, not only as sugar producer but also as bioethanol producer, multinational companies may be attracted toward our country for producing seeds at lower costs. In this respect, guarantee of seed availability of suitable varieties is needed. Considering all these aspects from this crop, there will be need of large quantity of high-quality sugar beet seeds. So, this chapter will be focused on seed biology of sugar beet, how sugar beet quality seeds are produced, as well as the seed enhancement technologies involved with this crop.

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Keywords

Seed · In situ · Transplantation · Pelleting · Multigerm · Monogerm · Sugar beet

7.1 Introduction

Seed is the main constituent of all the inputs required for good and proper production and productivity of any crop (Kanwar and Pawar 2017). Seed quality is an important aspect for good yield and production for any crop. Kanwar and Pawar (2017) had projected that 20–25% of productivity is affected by seed quality. Physical, physiological, and genetic attributes and storability are the various characters which determine seed quality. Production of quality seeds causes less disease and insect problem, increased rate of seedling, as well as uniform emergence, uniform plant population and maturity, and good response toward fertilizers and nutrients applied which in turn results in high return per unit area and profitability to producers.

Sugar beet (*Beta vulgaris* L.), Chenopodiaceae family (subtribe Beteae, tribe Cyclolobeae), is good source for sugar production and stands second as raw material after sugarcane. Sugar beet is a biennial crop and requires more than a year for producing seed. For a quality seed production, the first and foremost important aspect is the right selection of area where crop cultivation will take place. In accordance to seed, having high germination potential is also essential. Fauchere (2001) had revealed that in French varieties when population was 100,000 plants/ha, 4% higher root yields were reported compared to 70,000 plants/ha along with decrease in sugar by 0.36% for every 10% reduction in population (<100,000 plants/ha). Estimation reveals that for 200–250 g of beet roots, there is a requirement of 80–110 days of photothermal induction at 3–10 °C of temperature. In India, for good production of sugar beet seeds, sowing in month of June/July, thermal induction in mid-November to February (in hilly areas where low temperature prevails), emergence of flowering stalks in March/April, and seed harvest in July have been known. Sugar beet is gaining importance due to some of its in built capacities such as its good growth in drought and salt affected areas. Furthermore, the increasing use of green technology has made it an attractive crop due to its high ethanol content. To fulfill the needs of present times, requirement of large area covered by this crop is essential. This will require large quantity of improved and high-quality seeds. Therefore, in this chapter, seed biology of sugar beet, quality seed production, and seed enhancement technologies have been described.

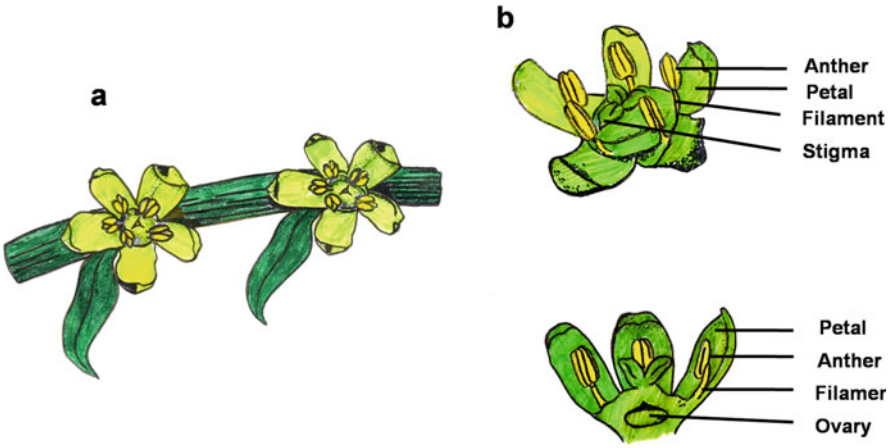

Fig. 7.1 Flower of sugar beet (*Beta vulgaris*). Flowers occur on the main axis of terminal portions. (**a**) The flower possesses three to four secure stigmas and tricarpellate pistil which is surrounded by five stamens. (**b**) Longitudinal section of flower showing different parts

7.2 Seed Biology

7.2.1 Morphology of Beet Flower

Flowers of *Beta vulgaris* are placed on the main axis of terminal portions and on the lateral branches subtended from the terminal (Fig. 7.1a). These are small in size and vary in color from green to white and arranged singly or in clusters of two to eight (Smith 1980) in sessile manner. Flowers are bisexual, pentamerous having either none or three bracts. They are rarely self-pollinating. Synchronous flowering is not seen in case of this plant. Flowering period of beet is approximately 4 weeks or longer. There is difference in flowers of monogerm genotypes and multigerms where monogerm flowers are borne single; however, in multigerms two to seven flowers in clusters are borne (Artschwager 1927; Smith 1980). The shoot which bear better developed glomerulus, on that shoot opening of flowers, occurs earlier in comparison to other shoot. The flowering starts from the top of the shoot, but the maturation of flowers occurs from base to top (Crane and Walker 1984). Secondary shoots rises later. For the purpose of fertilization, the stigmas remain receptive more than 2 weeks and provide better possibility to wind (Crane and Walker 1984). In each inflorescence, central flowers open earlier and produce seeds. These seeds yield plants and advance quality roots (OECD 2001). Favorable climatic condition supports proper blooming, flowering, and seed ripening. The flowering time between pollen parents and seed parents as well as pollen dispersal in seed is very significant for sugar beet seed production because it affects both quality and quantity of seed (Farzaneh et al. 2016).

7.2.2 Development of Reproductive Parts

Development of reproductive parts of sugar beet crop occurs during second growing season. At the stage of generative growth, when vernalization process phase initiates, the internode increases and forms shoots. Leaves of these shoots are ellipsoid in shape. For growth of reproductive phase, temperature of 4–13 °C is favorable, whereas vegetative growth phase needs favorable temperature above 21 °C. The leaf axils emerge out flowers bearing shoots and panicles. Generally a single, strong, and unbranched shoots of flowers develop. The flowering stems grow erect up to 2 m in height. It is rough, wrinkled, and green in color. Majorly the secondary shoots grow in upright direction, but there are certain which hangs down. About 90–110 days of exposure of temperature is required to cultivate sugar beet commercially. The seed which grows easily requires short period of exposure of temperature in comparison to the seed which do not grows easily for vernalization process (OECD 2001).

7.2.3 Female Reproductive Organ

Formation of ovary occurs by fusion of three leaves into a single gynoecium. The gynoecium is partially inferior. It is relatively three-cornered in cross section and flattened on the top. Gynaeceum wall and ovule are jointed together with a short funicle. The ovary of the flower is raised in position with three to four secure stigmas. It is almost sessile and mainly consists of tricarpellate pistil which is surrounded by five stamens and a perianth of five narrow sepals (Smith 1980) (Fig. 7.1a, b). The growth of gynoecium and perianth (calycine and pentamerous) occurs together. Elliott and Weston (1993) and Esau (1977) had revealed that ovary is inferior as it is located at the base of sepals and stamens whereas flower is epigynous (having ovary enclosed in receptacle and floral parts as well as stamens are located above). A gland-like structure present in each flower of ovary secretes appropriate amount of honey and also joints flowers together. Each middle flower of the cluster blossoms prior to the surrounded flowers. Maturation of fruits occurs when perianth gets hardened. The arrangement of seeds is in campylotropous manner. On the basis of environmental condition and quality of seed, the opening of the flowering bud takes 5–6 weeks after initiation of reproductive growth, and this phase continues up to several weeks (OECD 2001).

7.2.3.1 Compatibility

At the time when flower opens, there is immature stigma, and this shows strongly self-incompatible tendency of beet flower. Sometimes the crop has few or no seeds. Every beet population has self-fertilizing ability. Diploid shows more frequency of fertilization than the tetraploids. Diploid characteristic of flower has higher compatibility for self-fertilization (OECD 2001).

7.2.4 Male Reproductive Organ

Gynoecium of the flower is surrounded by five stamens. Anthers of flowers are either equal in size of perianth lobe or shorter than it. Approximately 17,000 pollen grains per anther have been counted (Marlander et al. 2011). Opening of anthers occurs instinctively as soon as formation of longitudinal break occurs in each anther. Initially pollen grains when released are adhered to each other which later on get dry up. During anther dehiscence, lobes of stigma are closed and thereafter open up very slowly. In the beginning it takes 5–7 h after the opening up of flowers, and this process is continuous after next 30 h or so (Artschwager and Starrett 1933). Large variations have been observed in rate of opening and expanding of stigma lobes among its flowers (Bincardi et al. 2005).

7.2.5 Pollen

The pollen grains are rounded in shape with wart like exines. Artschwager and Starrett (1933) had found the time of pollen germination after it reaches to stigma (in particular receptive surface) is less than equal to 2 h when anthers dehiscence. Total 85,000 grains per flower and 10,000 flowers per bush had been counted (OECD 2001). Hecker (1988) had observed that diploid monogerm produce nearly 1.5×10^9 pollen grains plant^{-1} while tetraploid monogerm generate more than 21 per cent pollen grain against diploid. Depending on the environmental condition specially moisture, the pollen grains survive up to 24 h (OECD 2001). Artschwager and Starrett (1933) had defined the pollen of sugar beet as the one possessing three nuclei (of which two are generative nuclei and one is tube nucleus) surrounded by cytoplasm and its inclusions and covered by plasma membrane. In short it can be expressed as tricellular (Hecker 1988). Artschwager (1940) had revealed that life of pollen of sugar beet is short. An important aspect during flowering is the humidity in air should not be too low (Marlander et al. 2011) and relative humidity for release of pollen grains at a maximum rate is 60–70%, but when this parameter goes down to 42%, pollen production also decreases irrespective of ideal weather conditions (Scott 1970).

7.2.6 Pollen Dispersal

Darmency and Klein (2009) stated that the studies of pollen dispersal of sugar beet were conceded during twenty-first century for higher seed production. Dispersal of pollen generally occurs through wind. Studies on 129 visiting insect species have been revealed that cross-pollination of sugar beet seed crop done mostly through carriage of pollen by insects (Free et al. 1975). The tetraploid crop produces larger and few amounts of pollen, so insect pollination plays an important role to cross-pollination for hybrid. Harding and Harris (1994) stated that the airborne pollen distributed horizontally up to 8 km from the originating field. In another study,

pollen was found to be carried out up to 1200 m (approx. 0.75 miles) (Bodnar 2010). When environmental conditions are not favorable or pollen tube growth is disturbed, production of empty fruit occurs. Such type of fruits should be eliminated during subsequent processing of seed lot (Scott 1970; Alcaraz et al. 1998). Scott (1966) reported decrease in humidity as the sun rises and demonstrate increase in emission at diurnal periodicity of release of pollen. Peak is observed at 900–1000 GMT, but emission maintains its increase till early phase of afternoon, and there is a decrease in emission afterward. However, there has been variation observed in its pattern when rainfall occurs as some amount of pollens is being emitted due to high humidity (Scott 1966). Viability of pollen remains only for 24 h, but when stored in cold and dry refrigerator, it may be viable for little longer after being shed from its flower (Bodnar 2010).

7.2.7 Pollination

The tetraploid plants produce pollen grains later in the day. In the morning, the stigma of tetraploid plants becomes more receptive but during this period pollen grains not produced through male sterile plant. In this situation, contamination from wild and weedy forms of diploid occurs. This cross results production of weedy forms of beet. This type of contamination is very serious problem in beet crop (OECD 2001) (Fig. 7.2).

7.2.8 Sugar Beet Fruit

The gynoecium of the flower is covered with shriveled and wrinkled remaining part of perianth. Due to concescent gynoecium, fruit shows fleshy pericarp with a cap. The pericarps of the fruits are flattened structures, formed by ovary walls which are important character for the formation of embryo. Pericarp becomes harden progressively. The embryo placed horizontally, rounded and irregular in shape and glabrous with the rostellum (OECD 2001). Mature embryo sac consists of one egg cell and one persistent and one degenerated synergid. One central cell occurs with two fused polar nuclei and five to six antipodals. The degeneration of one of the synergids appears before pollination in the maturing process. Secretary activities may be possessed by antipodals of central cell. Maturing of embryo sac of this plant is suggestive to be pollination independent (Bruun 1987). The fruits are collected in to seed balls. For better seed production air, temperature and relative humidity are the two important factors. It affects from pollination to seed ripening stages of growth and yield of seed (Farzaneh et al. 2016).

Fig. 7.2 Seed production of different germplasm at IISR Sugarbeet Breeding Outpost, Mukteshwar (Nainital)

7.2.9 Seed and Seed Structure

Seeds are flattened, placed horizontal, and glabrous with rostellum. Weight of 1000 seed balls varies from 18–30 to 32–50 g, whereas 1000 seeds' weight is of 1.5–2.0 or 5–6 g (OECD 2001). The sugar beet seed is light in weight and light to dark brown in color. The shape of seed is circular and 1–2 mm in diameter (Fig. 7.4). A small beak-shaped root causes kidney-shaped indentation. Flowering of sugar beet in 1 ha generally produces about 20 crores of seeds (Benjamin and Bell 1985). The shape of sugar beet fruit is like a capsule; seed is imbedded in hollow area with a small lid-like structure and attached to pericarp of fruit (Fig. 7.3). Seeds generally consist seed ball which is generated by two to four true seeds (OECD 1993). Smith (1980) stated that the receptacle of the flower cluster encloses the ovary and these receptacles form an irregular dry body which is called seed ball. Each seed ball contains two to eight and sometimes three to four seeds. Every seed ball contains single variety in each seed. Most of the seed balls produce multigerm seeds. There are very few varieties which produce only monogerm seed. Ly (2017) reported that intensive research which had been done in 1948 among 300,000 plants in 4-acre seed production field of North Salem, Oregon, results only five sugar beet plants having monogerm seeds.

Fig. 7.3 Sugar beet plant bearing seed and flowers: Receptacle of flower encloses the ovary and forms seed ball which contains seeds

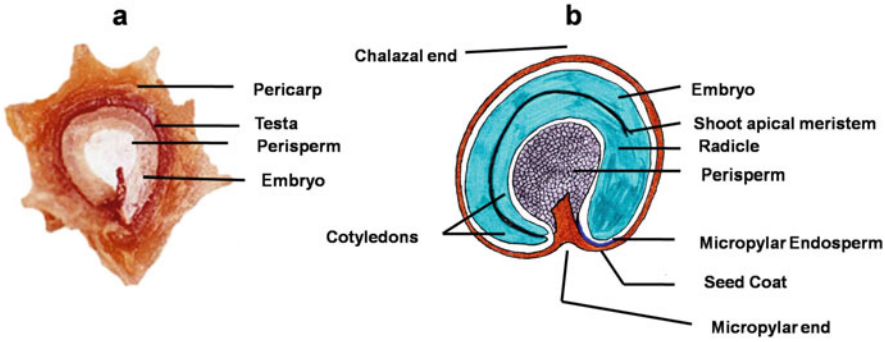

Fig. 7.4 Structure of sugar beet seed (**a**) Longitudinal section of seed showing embryo and perisperm of seed. (**b**) Structure of ovule

7.2.9.1 Monogerm Seed

Monogerm seed refers to the ones having a single embryo and whose fruit in certain cases is used in propagation (Fig. 7.5). Fruit of monogerm seeds is either genetically modified or fragmented by mechanical means so as to contain one single embryo. The main benefit of producing such seeds is to avoid the process of thinning in sugar beet so as to reduce the cost of labor (Redei 2008). McFarlane (1971) had showed that availability of first monogerm germplasm was in 1951 named SLC 101 after which years later commercialization of such seeds began.

Fig. 7.5 Monogerm seeds: Single embryo is the characteristic feature of such seeds

Fig. 7.6 Multigerm seeds: Many embryo is the characteristic feature of such seeds and gives rise to multiple plants

7.2.9.2 Polygerm/Multigerm Seed

Multigerm seeds are defined as the ones which are formed by aggregation of several fruits (Klotz 2005) (Fig. 7.6). In other words when flowers grow in clusters and fused together by their petals, multigerm seeds formation occur. Such seed balls germinate into two to five seedlings sprout all at once. When such seeds undergo emergence, manual thinning is important for seeds developed by this process so as to ignore the competition between plantlets (Biancardi et al. 2010).

7.3 Seed Dispersal

At the end of the flowering season, large amount of wild beet mature seed produced. These seeds mainly shed instantaneous proximity of maternal plant. Long-distance dispersal of seed quantity is very low. After dispersal the seeds of sugar beet which are falling on the ground do not germinate as easily as wild *Beta* species. It may be because of the presence of some germination inhibitor or because of poor soil and

seed contact (OECD 1993). Perisperm is very important for germination purpose found in very low quantity. This cause also inhibits the seedling and early growth.

7.3.1 Spacing and Plant Population

For high yield early planting usually decreases the risk of frost. For maximum germination seed should be planted 1–1.25 in. deep in soil. Shallow depth for early planting is beneficial. 4.5–5 in. distance between seeds and 22 in. row for planting is suitable for quality germination (Khan 2019). Intra-row spacing must be kept between 5 and 8 in.. However, high yields are obtained at 22 × 8 in. spacing of row to row and plant to plant. This spacing provides about 100,000 plants or roots ha^{-1}.

7.3.2 Germination and Its Process

Multigerm seeds generally used for sowing at the rate of 10 kg/ha. Polished and unpolished both types of seeds are used for sowing. Polishing of seeds not only affects the quicker germination but also reduces the inocula of seed-borne pathogen. The drawback of polishing is only the breakage of seed, and this adversely affects the germination (Motiwale et al. 1991). Sugar beet seed requires fairly cool climate, good rain fall or irrigation, and bright sunshine during its growth period. The optimum temperature for seed germination is about 15 °C and for growth and sugar accumulation 21 °C. Germination of seed (Fig. 7.7) becomes improved when soaked in water for 4–6 hrs and aerated overnight before sowing (Mall et al. 2018). Sugar beet germinates in to bud form, just above or below the soil surface; hence it is known as hemicryptophytes crop. Stem elongation occurs in first year in very few plans, i.e., bolting, but in most of the plants, it grows in second growing season. Bolting process and elongation of stem have been observed at 1–4 °C temperature, the very specific environmental condition. Length of day, quality of light, and duration of low temperature also affect the growth.

7.3.3 Seed Production Technology

Production of high-grade seed is possible when roots produced are of suitable size, shape, and quality. However, root size at times be of less important parameter in respect to high-grade seed production as size depends upon the environmental conditions in which the crop will grow. Crown part also plays a significant role for development of the size of sugar beet. The main root carries high sugar content. The leaves of crown should be normal in size not very small or very large. Abnormally large sugar beet roots contain very low amount of sugar (OECD 2001). Most of the factories recommend from 6 to 9 kg of sugar beet seed to the acre, since a good stand is of prime importance in producing a satisfactory crop of sugar beets. Sugar beet

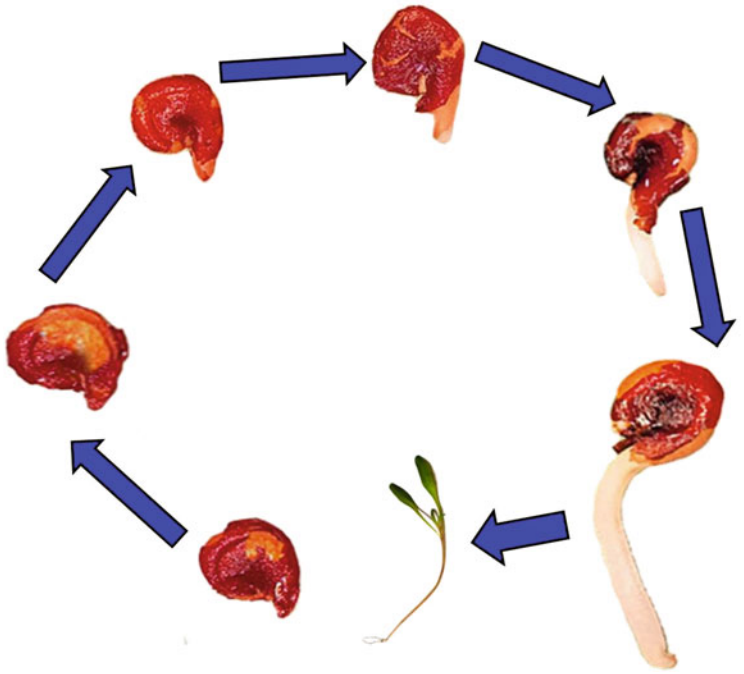

Fig. 7.7 Different stages of seed germination in sugar beet. (**a**) and (**b**) are dry seeds. (**c**) Imbibed seed showing rupture of outer testa and sprout of inner testa. (**d**–**f**) Protrusion of radicle through all the seed covering layers

seed industry is very important for the future development of sugar beet production and also for production of sugar from sugar beet (OECD 2001).

For production of high-quality seeds, there are two process/methods involved in sugar beet. These are:

1. In situ or direct sowing method
2. Transplantation method or indirect method

In direct sowing method, genotypes which are needed to be reproduced are sown at places where harvesting of seeds will take place. The main benefit of seed production by this method lies in the fact that deep and broader roots are developed in same place without being disturbed (Srivastava et al. 1986). Smith (1987) had revealed that seeds were sowed at a distance of 6–14 cm within row whereas row to row distance was maintained at 60–75 cm. Furthermore, single row of pollinators, depending on environment and pollen-producing pollinators, is being sown after every three to four rows of cytoplasmic male sterility (CMS) lines. Sowing of seeds (combination of monogerm and multigerm) in ratio of 10:1 is another way by which seeds can be produced by this method. Produced multigerm and monogerm seeds are separated by grading (Hecker and Helmerich 1985). It is important to keep in mind

Fig. 7.8 Root stecklings ready for transplantation

that sowing of monogerm and multigerm seeds (parent) should be done in separate marked rows so that their growth and development can be observed carefully along with timely trimming of both sorts of plants and timely removal of any fertile and analogous/off type plants if seen. Frost plays an important role in seed production process of sugar beet by this method. Campbell (1968) had showed that temperatures <-12 °C causes severe damage and higher loss to seeds production of monogerm seeds. In case of providing protection from frost, beets are placed at far-off distance so as to survive under low temperatures. This way of seed production has been adopted by various countries such as North America, Hungary, Yugoslavia, and France for monogerms and United Kingdom and Denmark for multigerms. However, in India for sugar beet seed production, favorable low temperatures are seen at higher altitudes such as Kashmir valley and Himachal Pradesh. In this method, favorable temperature should be of 1.66–7.22 °C for a period of 2–3 months, but if favorable temperatures do not occur, it causes mortality of roots (Campbell 1968). It is interesting to know that no incidence of any insect, pest, or disease has been observed either on root or seed crop. This is why the sugar beet crop is preferred to seed crops of temperature vegetables by the seed growers.

In transplantation method, sugar beets are firstly sown in a nursery. Bornscheuer et al. (1993) had revealed that generally after the process of vernalization at a specific time, the roots are transplanted to places where the seeds are to be produced. These uprooted roots are known as stecklings (Fig. 7.8). Prior to uprooting, leaves are trimmed and have petioles of size of 4–5 cm. The roots are also trimmed to develop lateral roots where they will be planted for flowering. Furthermore, these stecklings are dipped in fungicide solution so as to prevent from fungal infection (Biancardi et al. 2010). Row to row distance for transplanted stecklings plantation should be 70–80 and 40–50 cm within row. When stecklings are planted, petioles should only be above the soil. Furthermore, soil surrounding stecklings should be completely be compressed. After stecklings are being planted, care should be taken for weed control (by hoeing) and aphid (Black and green aphids) control prior and later to bolting for prevention of virus infection in plants.

In producing seeds from this method, field history (at least of 10 years) where these transplants will be planted as well of the nursery should be known as it may cause contamination (Bornscheuer et al. 1993). Another important aspect which

should be taken care of is the seed germinating ability of the genotypes which are to be planted prior to sowing should be known so as to attain uniform emergence and germination. The range between 1,000,000 and 1,200,000 plants/ha is considered as ideal stand for seedlings grown by this method (CAC 1996). Seed to seed distance in monogerm seed should range between 2 and 3 cm, while in multigerm ones, this distance is dependent on mean number of embryos in a single seed of multigerm possess. In India it is planted in October end and transplanted in December end. In this method, root stand, root uniformity, and weather conditions play an important role for quality seed production. In general, it is known that roots (size 3–4 cm) have good survival capability when transplanted to other place for quality seed production (Biancardi et al. 2010). Though small roots of sugar beet are better in terms of less transportation costs and mechanical operations, they are more prone to drought conditions. During flowering, no chemical spray or application should be done on to plants. It is recommended that hybrid variety parents should be planted in separate rows so as to avoid any mixing of varieties. Though seed production by this method is more costly in comparison to direct sowing method as it requires more labor, seeds produced in this way are much more of quality (Biancardi et al. 2010).

There are many differences in seed production of sugar beet in India and other countries which may be due to the occurrence of favorable conditions for its production. Table 7.1 clearly gives a clear-cut glimpse of the seed production of sugar beet by both methods in India and other countries.

7.3.4 Difference Between Direct and Indirect Seed Production of Sugar Beet

There are various differences between direct and indirect method of seed production which have been briefly mentioned in Table 7.2:

7.3.5 Sugar Beet Seed Storage and Seed Enhancement Technologies

Sugar beet seed storage is another important criteria for maintain quality seeds. It is known that sugar beet seeds do not deteriorate for 1 year, if stored properly; however, when sugar beet seeds are stored for >3 years even following good storage practices, seeds deteriorate and show reduced rate of germination. The following are some of the precautions to be practiced for maintaining quality seeds:

1. Temperatures >90 °F for longer periods should be avoided as it may cause reduction in seed quality.
2. Avoid storing seed in buildings having high temperature, trucks/cabs exposed to direct sunlight.
3. Seed storage should be done in dry, low humidity, and cool environments having temperature of 35 and 70 °F.

Table 7.1 Comparison of seed production methods in India and in other countries

Characteristics		Transplantation method		Direct method	
		Other countries	India	Other countries	India
Seed requirement (kg ha^{-1})	Monogerm	10		5–6	
	Multigerm	10–20		10	
Seed to seed distance (cm)	Monogerm	2–3	10	7 (Chile), 13/10 (France), 5 (Oregon, USA)	15–20
	Multigerm	Mean of embryos $seed^{-1}$	15–20	4 (England)	15–20
Row to row distance (cm)	Monogerm	50–75 (France) 70 (Turkey)	45	75 (Chile), 60/ 75 (France), 61 (Oregon, USA)	60
	Multigerm	50–75 (France) 70 (Turkey)	50	50 (England)	60
Nursery plantation (month)		August	October	–	
Steckling harvesting (month)		February–March (France and Italy) May (UK)	December	–	
Bolting (month)		End April to end May	March	August (US)	February–March
Flowering (month)		November–December (Egypt)	April–May	August (US)	March–April
Seed harvest (month)		July and August of following year	September	July and August of following year	August–September
Stecklings transplantation details					
Seed to seed distance (cm)	Monogerm	40–50	45	–	
	Multigerm	40–50	25–30		
Row to row distance (cm)	Monogerm	70–80	60		
	Multigerm	70–80	50		
Foliage removal of steckling above crown (cm)		2–3 (Italy) 7–8 (France)	3–4		
Fertilizer dosage (kg ha^{-1})	Nitrogen	60–70 (France) 50–60 (Italy)	240 (spilt in two doses)	41.37 (Lithuania)	240 (spilt in two doses)
	Potassium (K_2O)	60–70 (France) 100–120 (Italy)	80	103.44 (Lithuania)	80
	Phosphorus (P_2O_5)	40–50 (France) 120–140 (Italy)	80	155.17 (Lithuania)	80

– Not applicable

4. Sugar beet seed is known to be fragile so it should be handled carefully while storing.
5. Long drops into a planter hopper or dropping seed boxes can reduce seed quality.

Seed enhancement technologies in sugar beet include priming, pelleting, and coating process. These processes have been extensively used for improving sugar yield and to minimize or reduce the losses related with pests and disease infestation.

1. **Seed priming:** In general, it is a hydration process in controlled way. Hussain et al. (2016) had showed that during early stages of germination prior to radical protrusion, seed priming activates metabolic processes. Chomontowski et al. (2019) had revealed that this process causes uniform emergence at a faster pace after planting. Management of temperature and soil moisture content in seed priming causes regulation of germination of sugar beet seeds. This process involves number of steps: Phase I, known as imbibitions where suitable temperature and moisture seeds take up water; Phase II, involves activation of biochemical processes; germination initiates; and Phase III, emergence of roots and hypocotyls from sugar beet seed. Furthermore, seed priming causes many benefits such as increase in emergence rate, facilitates seed germination particularly under adverse environmental conditions, enhances yield, etc. It is a cheap and simple tool which helps sugar beet to produce good yield in less time (Jalali and Salehi 2013; Jisha et al. 2013; Mukasa et al. 2003; Orzeszko-Rywka and Podlaski 2003). The seed process in this technology involves exposure of seeds to water in controlled environment. This is basically triggering seed for germination process. Seed priming technology has further three types of sub-methods, viz., hydro-priming, solid matrix priming, and osmo-priming. In hydro-priming process, exposure of seeds occurs either in control environment in fixed time or in limited water uptake (Fujikura et al. 1993). In solid matrix priming process, Jisha et al. (2013) and Finch-Savage and Bassel (2015) revealed that the seeds are placed in aqueous solution with absorbent and solid particles where the ratio between them decides the hydration in seeds. In osmo-priming process, the seeds are exposed to an aerated solution of inorganic salts and mannitol having low potential of water (Bourgne et al. 2000; Heydecker et al. 1973; Wright et al. 2003). Furthermore, inert osmoticum like polyethylene glycol may also be used as aerated solution in this process (Capron et al. 2007). As stated earlier though all process ensures good germination yet there are variations in its processing. To evade overhydration hydro-priming process necessitates firm control in time, while in osmo-priming and solid matrix priming, water availability is limited by using liquid/solid particles for creating chemical environment (Bezhin et al. 2018). Adel et al. (2017) had showed that seed priming technologies used prior to seedling help in initiating metabolic processes. Bezhin et al. (2018) had illustrated that for speeding the rate of germination and emergence in sugar beet, priming of seeds is an important useful technology, but favorable conditions in field are also necessary for it. Chomontowski et al. (2019) had revealed that seed priming

Table 7.2 Comparison of direct and indirect seed production

Category	Direct method	Indirect method
Selection of stecklings for phenotypic characters or diseases	No prior selection	Prior selection
Life cycle	Plants complete their life cycle in one place	Plants complete their life cycle in two places, viz., the seeds were sown at other place while stecklings in favorable location where flowering occurs
Roots morphology	Deeper and broader root system	Less deeper and thin root system
Cost of cultivation	No transplanting is necessary, thereby eliminating the costs involved	Extra cost involved as transplanting is required
Countries involved	Direct seeding is used in the USA (Oregon), southwest France, England, Yugoslavia, Hungary, India, and Greece	India, Italy, and southwest France
Lodging	Increase	Less
Irrigation required	Less	More
Vegetative development	Increase	Less
Flowering	Slightly earlier	Later
Susceptibility to frost	Yes	No
Contamination by weed beet seeds	Yes	No
Agro-technological and breeder's requirements	Low adoptability	High adoptability
Rotational risk	High	Low
Risk from adverse winter weather conditions	High	Reduce
Seeds distribution in beds	More sparsely	Less sparsely
Winter survival	Lower	Higher
Labor involved	Less	More
Seed interval between crops (minimum)	10 years	5 years
Propagation ratio	1:300–1:800	1:150–1:550
Off type selection	Feasible	High efforts
Breeders flexibility for adapting to area for cultivation according to varietal importance	Flexible	Limited
Sugar yield	Less	Increase (at rate of 1.32 t ha^{-1})

Fig. 7.9 Pelleted Seed with uniform appearance

causes reduction in adverse effects of pelleting in germination. Furthermore, QB 1 technology of seed priming had improved rate of germination, its speed, and its ability along with enhancement in leaf area, plant growth, and root yield (Chomontowski et al. 2019).

Benefits of seed priming

(a) Growth and development at fast pace of plants
(b) Higher levelling in the field
(c) Control weed growth
(d) Uniform emergence and roots formation (in respect to shape and size) (Heyes et al. 1997; Michalska-Klimaczak et al. 2018)

2. **Pelleting:** Seed pelleting is another process by which seeds are prevented from infections. Sugar beet pellets were first made by Germans. This process technology produces big, rounded and smooth seed pellets which are uniform in size. These seed pellets are ideal carrier for treatments of protection of crop such as pesticides and fungicides. In this process, seeds are pelleted with pelleting material (major ingredient is of wood fiber) so as to give uniform shape (Fig. 7.9). This in turn causes higher suitability for drilling equipment (Blunk et al. 2017). Antonio and Antunes (2007) had showed that use of polymer as a pelleting material on seeds enhances the potassium content in sugar beet as well as act as antifungal agent.

   Benefits of seed pelleting

   (a) Prevention of clogging by singling seeds
   (b) Absorption of moisture
   (c) Nutrients supply
   (d) Birds/animals protection

3. **Coating:** Coating is a process in which seeds are basically protected from pathogens, pests, diseases, etc., with the help of application of chemicals applied on to the seeds. The chemicals applied are in general synthetic polymer by which a solid thin coating is created over seeds (Hoseini et al. 2013) (Fig. 7.10). Besides, some new methods are also being developed for improving germination. This technique helps in controlling seedling emergence and germination of seeds (Hoseini et al. 2013).

   Benefits of seed coating

   (a) Tight adherence on seeds

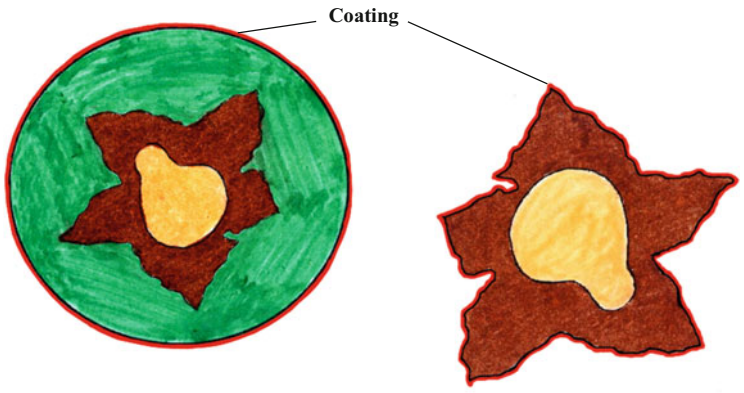

Fig. 7.10 Coated seed showing solid thin coating of synthetic polymer

(b) Prevention in loss of active materials such as nutrients, fungicides, etc.
(c) Bestow temperature-sensitive water permeability to seeds

7.4 Conclusion

For any crop to be flourishing, seeds are the first and foremost foundation. With the advancement of science and technology, agriculture is modernizing, but even then the use of steady quality seed for good production is necessary. Quality seed production possesses some important characteristics such as variety, germination percentage, vigor, appearance, disease occurrence, etc. Production of high-quality seeds is the result of high precision breeding and research and management strategies. Genetic characteristics, yield, quality, and storability are the factors which govern seed quality.

Sugar beet is an important crop not only for sugar production, but it has multifarious other uses, one important, being high potential for ethanol production. For production of good roots, quality seed production is the most important factor. Good and high yielding seeds will give benefits to farmers. High-quality seed production is difficult in this crop as it requires lot of precision and favorable weather conditions. India is one among such countries where high-quality seeds of this crop are being produced. In this respect, right selection of area is first and foremost important aspect. In India, high-altitude places possess the capability of producing high-quality sugar beet seeds. IISR Lucknow has been producing its seeds at its Outpost Mukteshwar. Sugar beet is a biennial crop whose reproductive parts (favorable temperature of 4–13 °C) develop during second growing season. It forms two types of seeds, viz., multigerm and monogerm. Multigerm seed production is generally easier than monogerms. Production of seeds involves two methods; one

is direct method, while other is transplantation method. Of both methods, transplantation method is beneficial as it has higher sugar yield. Production of high-grade seed is possible when roots produced are of suitable size, shape, and quality. Though on basis of research findings, root steckling having positive correlation with quality and high seed production, however, it is mainly governed by Genotype x Environment interaction. Crown part may also play a significant role for the development of the size of sugar beet. Another important aspect is storage of seeds by maintaining their high quality. There are the three seed enhancement technologies, i.e., priming, pelleting, and coating process that will help not only in improving sugar yield but also in reducing the pest and disease losses responsible for seed deterioration.

References

Adel P, Tajabakhsh M, Fathollah D, Ghiyasi M (2017) Sugar beet quantitative and qualitative yield related traits as affected by different seed priming treatments. Am J Sustain Agric 11:13–18

Alcaraz G, Genter T, Laillet G, Rageot D (1998) Sugar beet pollen biology. In: Proceedings of the 61st Congress of the International Institute for Beet Research Brussels, pp 393–399

Antonio Z, Antunes T (2007) Evaluation of powder properties from the seed coat of tingui (*Magonia pubescens*) as an excipient for seed pelleting. Estud Biol 29(67):171–178

Artschwager E (1927) Development of flowers and seed in the sugar beet. J Agric Res 34:1–25

Artschwager E (1940) Sugar beet pollen germination in relation to environmental conditions. Proc Am Soc Sugar Beet Technol 2:133–140

Artschwager E, Starrett RC (1933) The time factor in fertilization and embryo development in the sugar beet. J Agric Res 47:823–843

Benjamin LR, Bell N (1985) The influence of seed and plant density on variation in plant size of red beet (*Beta vulgaris* L.) crops. J Agric Sci Camb 108:221–230

Bezhin K, Santel HJ, Gerhards R (2018) The effect of sugar beet seed priming on sugar beet yield and weed suppressive ability. J Plant Sci 6(4):149–156

Biancardi E, McGrath JM, Panella LW, Lewellen RT, Stevanato P (2010) Sugar beet. In: Bradshaw JE (ed) Root and tuber crops (Handbook of plant breeding). Springer, New York, NY; Dordrecht; Heidelberg; London, pp 173–219

Bincardi E, Campbell LG, Skaracis GN, Biaggi MD (2005) Genetics and breeding of sugar beet. Science Publishers Inc., Enfield, NH, p 391

Blunk S, Malik AH, Heer MID, Ekblad T, Bussell J, Sparkes D, Fredlund K, Sturrock CJ, Mooney SJ (2017) Quantification of seed–soil contact of sugar beet *(Beta vulgaris*) using X ray computed tomography. Plant Methods 13:71–85

Bodnar A (2010) Sugarbeet biology. Biology Fortified. https://biofortified.org/2010/08/sugar-beet-biology/. Accessed on 8 Nov 2019

Bornscheuer E, Meierholz K, Wunderlich KH (1993) Seed production and quality. In: Cooke DA, Scott RK (eds) The sugar beet crop. Chapman & Hall, London, pp 121–153

Bourgne S, Job C, Job D (2000) Sugarbeet seed priming: solubilization of the basic subunit of 11-S globulin in individual seeds. Seed Sci Res 10:153–161

Bruun L (1987) The mature embryo sac of the sugar beet, Beta vulgaris: a structural investigation. Nord J Bot 7(5):543–551

CAC (Cooperativa Agricola Cesenate) (1996) Tech Publ, Cesena, Italy

Campbell SC (1968) Sugar beet seed production in Oregon. Report of the 31st Winter Congress of the International Institute for Sugar Beet Research, pp 165–174

Capron I, Corbineau F, Dacher F, Job C, Come D, Job D (2007) Sugarbeet seed priming: effects of priming conditions on germination, solubilization of 11-S globulin and accumulation of LEA proteins. Seed Sci Res 10(3):243–254

Chomontowski C, Wzorek H, Podlaski S (2019) Impact of sugar beet seed priming on seed quality and performance under diversified environmental conditions of germination, emergence and growth. J Plant Growth Regul. https://doi.org/10.1007/s00344-019-09973-2

Crane E, Walker P (1984) *Beta vulgaris* L. In: Pollination directory for world crop. IBRA, Hill House, pp 28–29

Darmency H, Klein EK (2009) Pollen dispersal in sugar beet production fields. Theor Appl Genet 118:1083–1092

Elliott MC, Weston GD (1993) Biology and physiology of the sugar-beet plant. In: Cooke DA, Scott RK (eds) The sugar beet crop. Chapman & Hall, London, pp 34–66

Esau K (1977) Anatomy of seed plants, 2nd edn. John Wiley & Sons, New York, NY

Farzaneh S, Kamkar B, Ghaderi-Far F, Chegini MA (2016) Effects of pollinator line characteristics on quantity and quality of monogerm hybrid seed production in sugarbeet (Beta vulgaris L). Int J Plant Prod 10(1):13–28

Fauchere J (2001) Influence de la qualite des graines sur les varieties experimentees. Proc IIRB 64:257–268

Finch-Savage W, Bassel G (2015) Seed vigour and crop establishment: extending performance beyond adaptation. J Exp Bot 67:567–591

Free JB, Williams IH, Longden PC, Johnson MG (1975) Insect pollination of sugar beet (*Beta vulgaris*) seed crops. Ann Appl Biol 81:127–134

Fujikura Y, Karssen C, Kraak H, Basra A (1993) Hydropriming, a simple and inexpensive priming method. Seed Sci Technol 21:639–642

Harding K, Harris PS (1994) Risk assessment of the release modified plants: a review. Ministry of Agriculture Fisheries and Food, London, p 54

Hecker RJ (1988) Pollen characteristics of diploid and tetraploid sugarbeet. J Sugar Beet Res 25 (1):55–62

Hecker RJ, Helmerich RM (1985) Sugar beet breeding in the United States. In: Russell GE (ed) Progress in plant breeding. Butterworths, London, pp 37–61

Heydecker W, Higgins J, Gulliver R (1973) Accelerated germination by osmotic seed treatment. Nature 246:42–44

Heyes V, Osborne B, Halmer P, Hughes M (1997) Advanced sugar beet seed goes international. Br Sugar Beet Rev 67:39–41

Hoseini M, Feqenabi F, Tajbakhsh F, Babazadeh-Igdir H (2013) Introduction of seed treatment techniques (seed priming). Int J Bioscie 3(5):1–12. https://germains.com/us/product-category/seed-pelleting-us/

Hussain S, Khan F, Hussain HA, Nie L (2016) Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. Front Plant Sci 7(116):116

Jalali AH, Salehi F (2013) Sugar beet yield as affected by seed priming and weed control. Arch Agron Soil Sci 59:281–288

Jisha K, Vijayakumari K, Puthur J (2013) Seed priming for abiotic stress tolerance: an overview. Acta Physiol Plant 35:1381–1396

Kanwar HS, Pawar R (2017) Current status of vegetable seed industry in India and future prospects. In: Advances in quality seed production of vegetable crops. Centre of Advanced Faculty Training in Horticulture (Vegetables), Solan, pp 1–6

Khan M (2019) Sugar beet production guide. NDSU University of Minnesota, pp 1–52. http://www.ag.ndsu.edu/Extension

Klotz KL (2005) Anatomy and Physiology. In: Biancardi E, Campbell LG, Skarcis GN, de Biaggi M (eds) Genetics and breeding of sugar beet. Science Publishers Inc., Enfield, NH, pp 9–19

Ly L (2017) Why do multiple seedlings sprout from a beet seed? https://www.gardenbetty.com/why-do-multiple-seedlings-sprout-from-a-beet-seed/#hC5qQfjqicVIzhHK.99. Accessed on 7 Nov 2019

Mall AK, Misra V, Kumar M, Shahabuddin SD, Pathak AD (2018) Sugarbeet: crop management in India. ICAR-Indian Institute of Sugarcane Research, Lucknow, p 50

Marlander B, Lange T, Wulkow A (2011) Dispersal principles of sugar beet from seed to sugar with particular relation to genetically modified varieties. J Fur Kulturpflanzen 63:349–373
McFarlane JS (1971) Variety development. In: Johnson RT, Alexander JT, Rush GE, Hawkes GR (eds) Advances in sugar beet production: principles and practices. Iowa State Univ. Press, Ames, IA, pp 401–435
Michalska-Klimaczak B, Wysznski Z, Pacuta V, Rasovsky M, Rozanska A (2018) The effect of seed priming on field emergence and root yield of sugar beet. Plant Soil Environ 64(5):227–232
Motiwale CRS, Agnihotri VP (1991) The sugarbeet cultivation. Indian Institute of Sugarcane Research, Lucknow, p 14
Mukasa Y, Takahashi H, Taguchi K (2003) Accumulation of soluble sugar in true seeds by priming of sugar beet seeds and the effects of priming on growth and yield of drilled plants. Plant Prod Sci 6:74–82
OECD (1993) Traditional crop breeding practices: an historical review to serve as a baseline for assessing the role of modern biotechnology. OECD, Paris. ISBN 92-64-14047-6
OECD (2001) Series on harmonization of regulatory oversight in biotechnology no. 18, Consensus document on the biology of sugar beet (*Beta vulgaris* L.), OECD environment, health and safety publications ENV/JM/MONO11. OECD, Paris
Orzeszko-Rywka A, Podlaski S (2003) The effect of sugar beet seed treatments on their vigour. Plant Soil Environ 49:249–254
Redei GP (2008) Monogerm seed. In: Encyclopedia of genetics, proteomics and informatics. Springer, Dordecht, p 1252
Scott RK (1966) Pollen release. Annual Report of Rothamsted Experimental Station. Rothamsted Experimental Station, Harpenden
Scott RK (1970) The effect of weather on the concentration of pollen in sugar beet seed crops. Ann Appl Biol 66:119–127
Smith GA (1980) Sugar beet. In: Hybridization of crop plants. American Society for Agronomy – Crop Science Society of America, Madison, WI, pp 601–616
Smith GA (1987) Sugar beet. In: Fehr WR (ed) Principles of cultivar development, vol 2. Macmillan, New York, NY, pp 577–625
Srivastava SN, Misra SR, Singh K (1986) Evaluation of fungitoxicants against *Rhizoctonia bataticola* causing charcoal root rot of sugarbeet. Ind J Sugarcane Technol 3:89–90
Wright B, Rowse H, Whipps JM (2003) Microbial population dynamics on seeds during drum and steeping priming. Plant Soil 255:631–640

Seed-Infesting Pests and Its Control Strategies

8

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Abstract

Seeds are the major sources of food (cereals, pulses, edible oils, nuts) globally. They are nutritionally rich source of oils, proteins, carbohydrates and many vitamins and minerals. However, seeds are prone to pest attacks. They are the shelter for many pests, which feed on them and affect their germination. Therefore, pest control is an important aspect in order to get healthy seeds. There are different classes of insects and they are specific to a particular seed type. So, precise management strategies are required to control them. Currently, many methods of seed protection are available, and further study is going on to deal with many aspects of environmentally sound pest management approaches. This chapter reviews the seed-infesting pests, which poses a serious risk for seeds, their detection and management.

Keywords

Seed protection · Food security · Seed-infesting pests · Detection of pests · Physical control · Chemical control · Biocontrol of stored seed pests

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8.1 Introduction

In order to achieve 'food for all', it is mandatory to increase seed production and develop advance methods to store and preserve seeds for future utility. Cereals, pulses, oilseeds and nuts have been an unprecedented source of food, and they are produced and stored in bulk since time immemorial. Seeds of the food crop, being rich source of minerals and nutrients, are prone to be attacked by insects during crop cultivation as well as during storage. Insect attack causes huge crop losses. Therefore, to meet food security, it is important to develop mechanisms to protect crops, seeds and stored seeds and food products from attack by insects to avoid economic losses due to harmful effect on seed quantity and quality, particularly during long-term storage (Pimentel and Zepp 1991). If pest control will not be adequate, contaminated seeds and seed products can cause health, economic, legal and aesthetic problems.

According to a recent estimate, one quarter or one third of the world grain crop is lost each year during storage of surplus dried seeds. Much of this damage is caused by insect infestation leading to huge economic loss, which is caused because of physical damage, inferior quality of seeds/grains and rejection by consumers and loss of market value. Moreover, infested seeds also affect the health and can cause safety issues. Allergic reactions to stored food arthropods have been stated many times (Van Lynden-van Nes et al. 1996), which is caused due to contamination of seeds with faeces, remnants of skins and dead bodies of pests (Scott 1991). In established countries, occurrence of a few insects can cause a severe loss in its market value since the consumers take it as downgraded or rejected completely (Pinniger et al. 1984). Any trace of live or dead insects, their odours, webbing and frass in stored seeds will lead to loss of faith of the consumers, which will ultimately cause financial loss. Therefore, measures need to be taken to avoid pests in stored seeds in order to inhibit food contamination. It is the moral responsibility of the professionals (involved in seed storage and processing) to prevent seed contamination; otherwise, it may affect human health as well as monetary position.

There are several sources of seed infestation, and it can start either from the field or during storage. Seeds can get exposed to pests through carried-over commodities, waste and rejects, agricultural machineries used for seed processing, etc. Seeds may get exposure to the pests during processing plants farm grain stores and their storage in re-used sacks. Seeds/grains can also get infested during transportation from one place to another and also from alternative hibernation sites and hosts. Protection of stored seeds of crop plants is highly needed since pest attack not only causes loss in its amount but also deteriorates its superiority.

There is an immediate need to increase the food production by 60% to feed an ever-growing population (Alexandratos and Bruinsma 2012). In order to achieve this goal and to safeguard food and nutritional security, in addition to proliferation in production and distribution, scientist should also pay attention to improve techniques to reduce food losses due to seed-infesting pests. During ancient days, approaches, such as mingling seeds with dry soil and wood ash leading to lethal dehydration of insects and fumigation with certain plant extracts, packing grains in

pits with a layer of straw and use of granaries made up of mud for storage, were adopted (Levinson and Levinson 1989; Lee 1960; Mellart 1961). Since then, advanced methods of storage have come up. However, ancient mud-brick granaries are used even now at certain places like Africa, and now they have been improvised and are made with steel and concrete in most of the developed countries (Reed 1992). Other old methods for pest control that are still in use include use of admixture and fumigation. However, nowadays, scientists have started exploring new approaches for pest control because of the harmful effects of pesticides and fumigants. It is the demand of the hour that pest control strategies should apply amalgamations of new and old techniques to emphasize the environment-friendly and non-chemical aspects of pest control, i.e. biocontrol along with the judicious use of pesticides.

8.2 Seed-Infesting Pests

Stored seeds of all kinds are subject to attack by insects. There are different varieties of pests that attack on stored seeds, and they are prevalent in a particular geographical region. Pests of grains are catalogued into primary and secondary pests. Primary pests are also called as whole grain pests since they can enter into an intact seed and pod to feed on the embryo, endosperm or cotyledons. Seed-infesting pests of the orders Coleoptera, Lepidoptera and Psocoptera are classified as the primary pests. Rice weevil, the granary weevil, the lesser grain borer and the Angoumois grain moth are good examples of primary pests. On the other hand, secondary pests feed on damaged seeds. Confused and red flour beetles, Indian meal moth, Mediterranean flour moth and the saw-toothed grain beetles are most commonly found secondary pests of stored seeds and their products such as cheese and flour (Athanassiou and Arthur 2018). A list of different types of pests of cereals, pulses and nuts are given in Table 8.1.

8.3 Detection of Pests in the Stored Food

The detection of pest infestation is essential for quality assurance and to ensure prolonged shelf life of the stored food. Detection of pests can be done by both direct and indirect methods. In direct methods, pests are detected by visual inspection approach, acoustic detection approach and chemical detection. These methods lead to accurate identification of the insect. However, these methods are unreliable for measuring physical and chemical parameters, labour-intensive, time-consuming and only practical for areas of limited size. On the other hand, indirect methods are used to identify the pest's infection through various remote sensing techniques which include spectrophotometry, spectral line imaging, spectrometry, imaging of fluorescence, thermo-radiometry and thermography (Barber et al. 2007). The most commonly used conventional as well as modern methods are described in Table 8.2.

Table 8.1 List of seed-infesting pests and the crop plants

Scientific name	Common name	Affected seeds
Sitophilus oryzae (L.)	Rice weevil	Rice
S. zeamais Motschulsky	Maize weevil	Maize
S. granarius (L.)	Granary weevil	Wheat
Rhyzopertha dominica (F.)	Lesser grain borer	Peanuts
Prostephanus truncatus (Horn)	Larger grain borer	Maize, cassava
Sitotroga cerealella	Angoumois grain moth	Rice, pearl millet, maize, sorghum, wheat, barley
Acanthoscelides obtectus (Say)	Bean weevil	Vetches, beans
Callosobruchus maculatus (F.) *C. chinensis* *C. analis* *C. phaseoli*	Cowpea weevils	Cowpea, green gram, lentils Chickpea, lentils, green gram, broad beans, soybean, adzuki beans, cowpeas Bean and other legumes Pigeon pea, mung bean, cowpea, hyacinth bean, chickpea
Caryedon serratus	Groundnut bruchids	Peanuts
Zabrotes subfasciatus	Mexican bean weevil	Lima bean, common bean
Tribolium castaneum *T. confusum*	Rust red flour beetle Confused flour beetle	Maize, groundnut, oats, Brazil nut, barley, walnuts, lentil, rice, beans, pea, almond, rye, sorghum and wheat Grain products
Cryptolestes ferrugineus	Rusty grain beetle	Rice, sorghum, wheat, maize, barley
C. pusillus *C. pussilloides*	Flat grain beetles	Groundnut, barley, wheat, rice
Trogoderma granarium Everts	Khapra beetle	Wheat, barley, oats, sorghum, maize, rice, alfalfa, beans, tomato, cotton, groundnut
T. variabile	Warehouse beetle	Wheat, sunflower, carrot, tomato
O. surinamensis	Saw-toothed grain beetle	Maize, wheat
Cadra cautella	Tropical warehouse moth or dried currant moth or almond moth	Almonds, fig, soybean, groundnut, date palm
Corcyra cephalonica	Rice meal moth	Rice, cassava, nutmeg, maize, millet, pearl millet, sorghum, wheat
Plodia interpunctella	Indian meal moth	Groundnut, maize
L. bostrychophila *L. entomophila*	*Liposcelis* psocid	Wheat
Carpophilus hemipterus (L.)	Dried fruit beetle	Common fig, prunes, apricot, plum, maize
Ephestia elutella	Tobacco moth or chocolate moth	Tobacco, coco beans

(continued)

Table 8.1 (continued)

Scientific name	Common name	Affected seeds
Cadra figulilella	Raisin moth	Date palm, raisin, figs
Lasioderma serricorne (F.)	Cigarette beetle	Tobacco, oilseeds
Stegobium paniceum (L.)	Drugstore beetle	Almond, peanuts
Trogoderma glabrum and *Trogoderma ornatum*	Carpet beetles	Cottonseed, barley, rice
Alphitobius diaperinus	Lesser mealworm	Grains

8.4 Measures Taken to Reduce the Crop Losses

Microorganisms, insects and other pests are the major impediments for safety of the stored grains, and therefore, some precautionary measures must be taken to safeguard the stored products. Various methods have been adopted to get rid of pest infection in stored seeds, and they are specific to geographical distribution, grains or food commodities, etc. Drying, aeration, dry heating and hermetic storage ($O_2 < 5\%$; $CO_2 > 10\%$) are some of the traditional practices being used very commonly all over the world. Some recent methods such as microwave heating (at energy levels 2.45–9.7 GHz), application of gaseous ozone, corona discharge (application time 5–20 min), ionizing radiation, pulsed light, supercritical carbon dioxide co-solvent system and ultra-superheated steam are being developed for controlling insect growth in stored grains. In addition, plant essential oils (EO), plant derivatives and vegetable oils are also being explored for their potential to prevent growth of invaders in stored food (Mohapatra et al. 2017). Some of them are practised sporadically, while some are still in the laboratory testing stage. There are various physical, mechanical, chemical and biological methods that are practised to control seed-infesting pests.

8.5 Physical and Mechanical Methods

Pest infection in stored food grain can be controlled by manipulating physical environment (e.g. temperature, relative humidity, moisture content in the grains and percentage of atmospheric gases in the intergranular air) or by giving physical treatment. Physical treatments include mechanical impact, physical removal of pests, use of physical barrier to prevent the entry of insects, inert dusts, ionization irradiation and light and sound.

Table 8.2 Different methods of detection of seed-infesting pests

Methods of detection	Definition/description	Pests	References
Visual inspection	Stored seeds are observed visually to find pests	*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*, *Tribolium* sp., *Oryzaephilus surinamensis*, *Cryptolestes* spp., mites	Stejskal et al. (2015)
Probe sampling and sieving	Sieving method is used for the recovery of pest from seed sample	*Tribolium castaneum*	Stejskal et al. (2015)
Trap method	Trap devices based on insects wander towards air are used for detection and monitoring of insect infestation	*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*, *Tribolium* sp., *Oryzaephilus surinamensis*	
Visual lures	Lights of wavelength 280–600 nm are used for allurance of insects towards light	*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*, *Tribolium* sp., *Oryzaephilus surinamensis*	Stejskal et al. (2015)
Pheromones	Sex and aggregated pheromone chemicals are used in traps on adhesive-coated surface to catch insects	*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*, *Tribolium* sp., *Oryzaephilus surinamensis*	Stejskal et al. (2015)
Berlese funnel method	Dry heated grain samples are kept below screened Berlese funnel apparatus, so that insects move in a funnel and captured in above kept alcohol-containing jar	*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*, *Tribolium* sp., *Oryzaephilus surinamensis*	Stejskal et al. (2015)
Uric acid method	Insect's excreta are used as tracing element for infestation	*Sitophilus granarius*, *S. oryzae*, *Rhyzopertha dominica*	Stejskal et al. (2015)
Hidden infestation detector	Three circular base plates are placed over one another and are covered with ninhydrin-treated filter paper, which moisturize the grain. Grains infested with pests are stained and can be detected	*Sitophilus oryzae* *Sitotroga cerealella* *Callosobruchus maculatus*	Dakshinmurthy and Ali (1984)
Electrically conductive roller mill	When seeds are infested with pests, the moisture content of the seed increases that can be detected by increse in electrical conductance	*Sitophilus zeamais* *Sitotroga cerealella*	Pearson and Brabec (2007)

(continued)

Table 8.2 (continued)

Methods of detection	Definition/description	Pests	References
Solid phase micro-extraction (SPME)	Volatiles/pheromones released by either larvae or adult insects can be evaluated by gas chromatography-mass spectrometry	*Sitophilus* sp., *Rhyzopertha* sp., *Acanthoscelides* sp., *Sitotroga* sp.	Laopongsit et al. (2014)
Electronic nose (e-nose)	E-nose instrument's sensors work on electronic aroma detection principle to detect the volatile compounds from the stored food grains and hence thereby insets	*Sitophilus* sp., *Rhyzopertha* sp., *Acanthoscelides* sp., *Sitotroga* sp.	Wilson (2012)
Machine vision with visible domain	Multispectral wavelength analysis with pattern recognition is used to detect pests	*R. dominica*	Aviara et al. (2016) Zayas and Flinn (1998)
X-ray imaging	X-rays with 0.12–12 KeV energy are used for image formation and detection of invisible insects in stored grains	*Cryptolestes ferrugineus* *T. castaneum* *Plodia interpunctella* *S. oryzae (L.)* *R. dominica (F.)* *C. maculatus*	Kotwaliwale et al. (2014) Karunakaran et al. (2003) Chelladurai et al. (2014)
Thermal imaging	Infrared energy uses heat signature to produce thermogram, which detects infestation of grains	*Sitophilus* sp., *Rhyzopertha* sp., *Acanthoscelides* sp., *Sitotroga* sp.	Nanje Gowda and Alagusundaram (2013)
Electronic grain probe insect counter (EGPIC)	EGPIC sensors having infrared beam passes through the fallen insects on sensor head acquires all the information and transmitted for data analysis	*Sitophilus* sp., *Rhyzopertha* sp., *Acanthoscelides* sp., *Sitotroga* sp.	Shuman and Epsky (2001)
NIR spectroscopy	NIR hyperspectral imaging system collects the changes in chemical imaging and detects infection	*Sitophilus granaries* (L.)	Elizabeth et al. (2002) Kim et al. (2003) Xing and Guyer (2008) Ridgway and Chambers (1996)
Acoustic detection	Acoustic sensors detect the mechanical waves produced by the movement of insects	*S. zeamais*	Eliopoulos et al. (2015) Pearson and Brabec (2007) Kiobia et al. (2015) Leblanc et al. (2011)

1. **Drying/aeration:** The safe storage moisture levels for cereal grains, legumes and oil seeds have been reported to about 12%, 10% and 8%, respectively. To make it possible, grains must be dried to the moisture content of designated safe limit before storage, physical damage during harvesting and storage of the grains must be minimum, and dry and clean insect-proof storage conditions should be ensured. Pest and pathogen growth conditions are harboured by bins which are non-aerated and low air flow drying process (Langseth et al. 1993). Hence, adequate aeration should be provided to the grains to prevent damage from invaders.
2. **Inert dusts:** These are dusts which are unreactive chemically having insecticidal capability. Insects get killed physically by inert dusts due to dehydration. These inert dusts are more effective at low relative humidity. There is renewed interest in technology associated with use of inert dusts in grain storages (Hagstrum and Subramanyam 2000; Mahdi and Khalequzzaman 2006). Since long time, inert dusts have been used in North America and Africa to control insects in the stored grains. Inert dusts may be of different types:
   (a) **Sands and other soil components:** These insecticides are traditionally used as a protective layer on the top of stored seed (Golob and Webley 1980).
   (b) **Diatomaceous earth (DE):** These are the fossilized remains of diatoms. They have a fine opaline silica shell. DE is the most effective modified form of DE which shows insecticidal, repellent and ovicidal effects against *Callosobruchus maculatus*. It is generally used in integrated pest management programme for stored seed grains.
   (c) **Silica aerogel:** It is light non-hygroscopic powder produced by drying an aqueous solution of sodium silicate (Quarles 1992).
   (d) **Non-silica dusts:** These dusts include rock phosphate and lime (calcium oxide) and can be used to control pests in the stored food (Fam et al. 1974; Golob and Webley 1980).
   (e) **Particle films (Kaolin and bentonite clays):** The kaolinite-based particle films may be used as a dry dust material in stored product environments. These are more commonly used in organic markets in place of diatomaceous earth. The particle film M-96-018 has been reported to be effective against *Tribolium castaneum* (Herbst) and *Tribolium confusum* (du Val) and beetles (Arthur and Puterka 2002).

   Inert dusts kill insects by removing the moisture content present in their body. These dusts act by either scratching (diatomaceous earths) or absorbing (silica aerogel) the insect's waxy coating. In the absence of waxy coating, insects get dehydrated due to excessive loss of moisture content to the dry grain and air present in intergranular spaces. Because of their non-toxic nature to humans as well as animals, inert dusts are more advantageous. They may be used as continuous protectant in stored grains and also do not affect the baking quality of grains (e.g. wheat). In the USA, diatomaceous earths are registered as food additive.

3. **Hermetic storage:** Hermetic storage means storage of food grains in an airtight storage structure, which may be either flexible or rigid and may be under-ground or over-ground. The systems used for hermetic storage can be of organic or vacuum or modified atmospheric storage type (Navarro 2012). In the organic hermetic storage, oxygen depletes, and carbon dioxide content rises gradually due to respiration. In this condition, the growth of insect, other pests and toxin-producing microorganisms is retarded. In vacuum hermetic storage, partial air pressure is maintained by sucking out most of the air. This results in an oxygen-deficit environment that prevents the growth of insects and other pests. In the third type, usually carbon dioxide or nitrogen is flushed to generate controlled atmosphere that retards the growth of insects and microorganisms (Villers et al. 2006). Thus, in all types of hermetic storage, oxygen level is depleted very rapidly, which creates an unfavourable environment for pest growth and survival.
4. **Thermal control:** Insect population can be controlled in stored food by keeping it at either low temperature or high temperature.
   (a) **Control of insects by low temperatures:** The optimal temperature for insect growth is in the range of 25–33 °C. Low temperature retards the process of development in insects and thus lengthens the time before their population reaches to a certain level where they can damage the stored seeds significantly. Although no development occurs at these low temperatures, still insects and mites remain alive for long periods, and they start damaging grain, as and when they get optimum temperature for their growth. Thus, this method may be used as a preventative rather than a cure. Insects, after their exposure to low temperatures, become acclimatized, and their cold hardiness also becomes enhanced by two to ten times. Low temperature has been reported to prevent *Sitophilus oryzae* (L.) but shows no effect on *Anisopteromalus calandrae* (Burks et al. 1999).
   (b) **High-temperature disinfestation:** All insects can be killed in stored grains by raising their temperatures up to 60–65 °C for a few seconds, and it can be achieved by heating the grains by various methods, e.g. hot-air convection, infrared or microwave radiation. There are various methods, e.g. counter-flow heat exchanger (Lapp et al. 1986), fluidized beds, spouted beds, pneumatic conveyors, high-frequency waves (Nelson and Kantack 1966), infrared waves, microwaves (Locatelli and Traversa 1989) and solar radiation (Kitch et al. 1992), that have been used to disinfest seeds with good satisfaction. To achieve disinfection with minimal damage, maximum kernel temperatures and its residence time must be controlled very carefully (Evans et al. 1983). Since high temperatures can adversely affect the seed quality such as baking quality of wheat, malting quality of barley, and the germination of almost all seeds, it is therefore mandatory to decipher alternative methods (Evans 1987). For better results, a thin layer of grains in passed under an infrared or microwave radiation source. In a radiant heating system, a separate equipment for cooling of grains is also included.

Other methods have been demonstrated at laboratory or pilot plant stage only.

5. **Microwave heating treatment:** It works on the principle of dielectric heating with radio waves and microwaves in which volumetric heat is produced due to the vibration and collision of various polar molecules such as water. Microwaves may be used to disinfest insects (Mohapatra et al. 2014, 2015; Nyzam and Rahim 2019). It causes differential heating due to difference in composition of the materials. The microwave heating enhances the electrolyte, Ca^{2+}, protein and DNA leakage from the pest and pathogen.
6. **Ionizing irradiation:** Ionizing radiation is a successful method for the decontamination of stored seeds as it damages causal organisms by producing highly reactive ions or free radical-charged molecules (Hallman 2013). It may extend the shelf life of grains over 6 months (Lima et al. 2011). The efficiency of radiation depends on various factors like method of radiation, exposure time and genera, since each genus responds differently (Banks and Fields 1995; Nemțanu et al. 2014). After irradiation with gamma rays at 0.5 kGy, insects may show complete mortality in 14 days for rusty grain beetles, 70 days for saw-toothed grain beetles, 28 days for red flour beetles and 200 days for grain mites. This method is advantageous as it can be used for all life stages of the insects, no chemical residue is left and nutritional value also remained unaffected if used at low doses. However, irradiation may affect seed germination.
7. **Light and sound:** Light may be used to allure flying insects into traps (Banks and Fields 1995). A short time exposure to 1 MHz sound at 14.5 W cm^{-2} has been reported to kill all stages of *S. granarius* at 26 °C in wheat but was not successful at commercial level (Banks and Fields 1995).
8. **Pulse light application:** Pulsed light (PL) is an emerging non-thermal technology that requires high-intensity light for a very few seconds (microseconds), to decontaminate food. The high-intensity light produces photothermal and/or photochemical reactions in the contaminating microbes and then eliminates them (Gomez-Lopez et al. 2007). Its efficiency depends on voltage input, the time of exposure and distance between electrodes (Maftei et al. 2014).
9. **Ultra-superheated steam technology:** In this technology, saturated steam is converted into superheated steam by heating under constant pressure. Superheated steam is then used to dry food materials. Superheated steam (160–170 °C) decontaminates naked oats (*Avena sativa* L.) from pest and pathogens without affecting seed quality (Chang et al. 2015). Recently, a new technology known as ultra-superheated steam technology (USST) has been developed by FBI Co. Ltd. (Tokyo, Japan) which incorporates high-frequency induction heating (IH) technology. The eddy current-based induction heating can produce very high temperature (300–500 °C) at which highly energized radicals are produced.
10. **Supercritical CO_2 co-solvent system:** Under high pressure and temperature (above a critical point), CO_2 behaves like a liquid having greater solubility. This property is used in SC-CO_2 co-solvent system to extract bio-compounds from microbial cells which causes their elimination from the stored grains.

Inactivation of microorganisms with the application of supercritical carbon dioxide has been evaluated for different microorganisms, and it was observed that only wet cells (not dry cells) of microorganisms can be sterilized (Kamihira et al. 1987). This method can be extended to get protection from pests as well.

8.6 Chemical Control

In chemical control, chemical compounds are used to prevent, destroy or repel pests. These methods are advantageous as they act fast, their availability is high and they are reliable too. But these chemical pest control agents have various serious drawbacks and risks such as low selective toxicity, low biodegradability and development of pesticide resistance in target organisms, which is associated with the excessive and improper use of these compounds. In chemical control, ozone application, fumigation, contact insecticides, residual surface treatment and use of aerosols and semiochemicals are the commonly used strategies to control seed-infesting pests.

1. **Ozone application:** Ozone is a well-known sterilant that can be used against insects present in stored seeds at certain levels (<45 ppm). Ozone can be easily generated from the oxygen present in atmosphere, and during disintegration, it gets converted to oxygen leaving no deposits, making it environment-friendly. However, it is extremely unstable and gets converted into oxygen rapidly. Ozonation can retard the growth of pathogens (Zotti et al. 2008). Its major disadvantage is that it is highly corrosive for many of the metals (Mason and Strait 1998). It has been reported that treatment of corn with ozone neither affects the popping volume of popcorn nor causes any harmful effect on fatty acid and amino acid composition of corn. Moreover, no disadvantageous concern was observed on milling characteristics of corn (Méndez et al. 2002). This indicates that even repeated ozone treatments may be given without getting any adverse effect on the quality of grain for end-users. Thus, ozonation may be a potential alternative to conventional methods implemented for pest control.
2. **Fumigation:** In fumigation an area is completely filled with fumigants, which are pesticides in gaseous form to suffocate and poison the pests within the fumigated area (Vijayanna 2006). Afterwards these chemical compounds enter into the body of the insect and get circulated all over the body through trachea and tracheoles. Fumigation is an important method for the elimination of seed-infesting pests. This method is very effective if storage is well sealed and the grain temperature is above 50 °F.

   Phosphine, methyl bromide and sulphuryl fluoride are most commonly used fumigants that were being used for the protection of seed and its products throughout the world (Rajendran and Sriranjini 2008). Methyl bromide and phosphine are used for fumigation purpose to protect legumes and cereals. However, sometimes these additives can cause health issues, and it can also contribute to depletion to the stratosphere ozone layer and hence has been

declared an ozone-depleting substance and has been phased out completely. Phosphine (PH_3) fumigation has been used as the main procedure to protect stored seeds from pests. It was being used widely and very frequently, but due to its continuous use, resistance against PH_3 was developed in many pests such as *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens) (Opit et al. 2012; Nayak et al. 2013; Kaur et al. 2015; Malekpour et al. 2018).

Few other fumigants such as sulphuryl fluoride, ethyl formate and carbonyl sulphite and ethanedinitrile are also used to kill termites, cockroaches and mites. A mixture of ethylene dichloride and carbon tetrachloride is used for fumigation of empty godowns to kill the eggs, larvae and adults of stored insect pests. Vapours of mixture while used in ordinary concentrations are usually non-toxic to man, but massive fumes may show anaesthetic effects. Similarly, other fumigants Grain-O-Cide (mixture of carbon bisulphide and carbon tetrachloride) and HCN gas (generated by metal phosphide preparations) are used to fumigate godowns and public store houses. Besides this, some of the most common fumigants used to control stored grain insects are carbon disulphide, carbon tetrachloride, ethylene dichloride, ethylene oxide, methyl bromide, chloropicrin, trichloroethylene, sulphur dioxide, methyl format and trichloroacetonitrate (Upadhyay and Ahmad 2011). However, synthetic chemical compounds are quite poisonous to people, and many of the seed-infesting pests have established resistance against these compounds. Sulphuryl fluoride fumigation has been reported for the control of insects infesting stored grain (wheat) that are resistant to phosphine (Opit et al. 2016). But now the use of sulphuryl fluoride is also prohibited (Zhu et al. 2018). Recently, a study was conducted, and it was concluded that the biological extract methyl ketone undecanone might be a good alternative to currently being used fumigants for various pest applications (Zhu et al. 2018).

3. **Contact insecticides:** Despite considering various bio-rational approaches, which intend to avoid the use of chemical insecticides in stored products, contact insecticides are even now the most successful insecticide and a worthy option to methyl bromide (Phillips and Throne 2010; Zettler and Arthur 2000; Fields and White 2002; Arthur 2012). Organophosphates (OP) and pyrethroids (PY) are amongst the most frequently practised contact insecticides. Amongst PY, deltamethrin and bifenthrin are the two compounds used very often (Whitehead 2007; MacBean 2012; Golic et al. 2016). Recurrent use of OP- and PY-based insecticides can also lead to development of resistance in stored product pests (Champ and Dyte 1976; Boyer et al. 2012). The effectiveness of contact insecticides can be further increased by giving various temperature treatments along with OP- and PY-based insecticides (Golic et al. 2016). Contact insecticides pirimiphos-methyl and spinetoram have been reported to be effective for phosphine-resistant *Sitophilus oryzae* and *Oryzaephilus surinamensis* (Agrafioti and Athanassiou 2018).
4. **Residual surface treatment:** Residual surface treatments are given to a wide area, where seed processing is executed and food products are manufactured

(Arthur 2012). Residual surface insecticides may control different developmental stages of pests and their growth regulators, showing detrimental effect on immature insects. In the USA, cyfluthrin (Tempot) is labelled as a residual surface treatment for interior surfaces. There are several biological and physical factors that affect the residual effectiveness of cyfluthrin (Arthur 1999).

5. **Aerosols:** Aerosols are liquid insecticide formulations made up of small size particles (5–50 μm) that cannot penetrate through the skin, and it is mechanically atomized and then dispensed by an equipment that can be fixed as well portable and is applied within an interior structure on the volume basis (Peckman and Arthur 2005). Nowadays, application systems incorporate formulations comprised of an oil-based carrier and a propellant such as CO_2 that helps in aerosol dispersion (Arthur 2012). Methoprene acts as an aerosol with excellent residual efficacy, and it is highly effective on *T. castaneum*. In field studies where different packaging surfaces were treated with methoprene, complete suppression of the larvae of *T. castaneum* has been reported, while residual testing period was 16 weeks (Sutton et al. 2011). Insecticide-incorporated bags may also be used for the control stored product beetles (Kavallieratos et al. 2017).
6. **Semiochemicals for pest control in stored products:** Semiochemicals are used to control insect pests by manipulating their behaviour. Insects use these signalling chemicals to attack intruders, identify members of same colony and find mates, food and oviposition sites (Phillips 1997; Burkholder 1990; Foster and Harris 1997). The females pick their oviposition site very judiciously on a host plant to secure feeding and survival for their offspring (Rees 2004; IITA 1988). Therefore, female insects lay their eggs selectively on the top host available (Awmack and Leather 2002; Scheirs et al. 2003). This role of chemical cues makes them vital agents for selective insect pest control (Burkholder 1990). On the basis of characterization of the chemical cues in insects, semiochemical-based management strategies have been developed for pest control in stored grains (Phillips et al. 1993, 1996; Sharma and Fadamiro 2013).

There are many plant species, which produce chemicals with potential as repellents or antifeedants for the control of stored grain pests (Mondal and Khalequzzaman 2010). After thorough assessment, these compounds are extracted from plants and can be used for pest management in stored foods (Allotey and Azalekor 2000). For example, *C. maculatus* gets attracted to legume seeds, which is mediated by 2-ethylhexanol (Ajayi et al. 2015). This attractant can be used in managing *C. maculatus* in stored legume seeds. Similarly, chemicals such as quinines could be used as practical control agents.

These semiochemicals are more advantageous compared to other chemical control methods, as they are natural plant products, e.g. essential oils and their components. Thus, they show very less toxicity to mammals (in majority of cases), can degrade very rapidly and are available locally (Rajendran and Sriranjini 2008). Therefore, a semiochemical-based management strategy could provide an alternative to the use of insecticides, by exploiting natural volatile signalling processes to manipulate insect behaviour and their control in stored seeds. However,

there are some difficulties in the practical applications of semiochemicals in pest management, and that's why not being used worldwide.

8.7 Biological Control of Pests with Microbial Biopesticides

Since chemicals used for controlling stored seed pests are not environment-friendly and also cause development of insecticide resistance amongst the pests, microbial biological control for pests involving pathogens (viruses, bacteria and fungi) that affect growth and survival of pests together with other biological control methods involving predators, parasitoids and pheromones of pests can be the potential alternative to chemical control (Cox and Wilkin 1996; Moore et al. 2000). Microbial biological control agents act slowly in comparison to chemical insecticides, but are directly from nature, and they do not cause any risk neither to the consumers nor to the environment.

8.7.1 Bacillus thuringiensis

Bacillus thuringiensis (B.t.) has been widely used for pest control. B.t. produces protein crystals; when insects ingest these crystals, they are fragmented into endotoxins at alkaline pH, which increases the permeability of epithelial cells and causes gradual lysis of the cells, paralysis or septicaemia and death of insects. Taking its extensive insecticidal spectrum, transgenics containing the gene encoding for Cry protein have been generated, and they have shown adequate resistance against insect attack. Different strains of B.t. have been reported that works against stored seed pests (Blanc et al. 2002; Bozlağan et al. 2010; Yılmaz et al. 2012; Contreras et al. 2013) such as modified as well as original, or the recombinant Cry3A protoxin was tested against *Tribolium castaneum* (Mostafa et al. 2013), and it was found that modified as well as original Cry3A were equally effective. Additionally, B.t. endotoxin has also been tested against mites affecting stored seeds (Erban et al. 2009) such as study of Cry1Ab in transgenic *Zea mays* affected the emergence and survival of *Sitotroga cerealella* (Sedlacek et al. 2001). Similar activity of Cry proteins was also reported in a transgenic variety of maize, MON810 and TC 1507, which works efficiently against a few species of seed-infesting pests: *Ephestia elutella*. *E. cautella* and *P. interpunctella* and *E. kuehniella*, *Sitophilus oryzae* and *Liposcelis bostrychophila* (Hubert et al. 2008a). These results clearly suggest that transgenic plants harbouring Cry gene can provide effective range of resistance against seed-infesting pests. However, efficiency of genetically modified plants depends on the expression of Cry genes, and scientists need to perform field study to ascertain that these plants are able to provide resistance even under field conditions. Moreover, development of resistance against B.t. has also been observed in a few lepidopteran pests such as *P. interpunctella* (McGaughey 1985) and *E. cautella* (McGaughey and Beeman 1988). The solution to this problem has also been taken up, and Cry proteins have been used along with trypsin inhibitor and

chitinases from soybean and diflubenzuron, and it was found to be more effective (Hubert et al. 2008b; Oppert et al. 2011).

8.7.2 (EPF) Entomopathogenic Fungi

Out of 1000 species of entomopathogenic fungi, only few isolates of EPF (*Beauveria bassiana*, *Paecilomyces fumosoroseus*, *Metarhizium anisopliae* and *Lecanicillium muscarium*) have been well studied and commercialized as pest control agents since they are amenable to mass production and formulation and they are also listed in EU database of insecticides. EPF infects the seed-infesting pests via spores, which germinates on the insect cuticle and pierces into the inner layers and proliferates within the insect body causing extremes of malnourishment, change in physiological conditions and difference in the levels of biochemical compounds and synthesis of toxic metabolites finally causing disease and death of the insects. The mycelium may produce spores under suitable environmental conditions that can infect other hosts. Most of the investigations done till now focus on laboratory studies mainly, with only few exceptions (Cherry et al. 2007; Meikle et al. 2001; Sabbour 2012). Further study is going on to check the potential of EPF as a biological control agent.

8.7.3 Baculoviruses

Baculoviruses are double-stranded DNA containing viruses, which are pathogenic to lepidopteran species. Once pests ingest baculoviruses, occlusion bodies are formed that are released into more of infective virus particles at alkaline pH of the midgut and are free to enter gut cells and fat body cells, causing gut expulsion and mortality of insects. Baculoviruses are transmitted to the progenies of next generation via infected females. Baculoviruses can serve as important biocontrol agents, but they are host specific. Anagnou-Veroniki et al. 2005 reported the effectiveness of a baculovirus isolated from *Spodoptera exigua*, *Mamestra brassicae*, *Cydia pomonella* or *Helicoverpa zea*, against *E. kuehniella*. Another granulovirus isolated from *P. interpunctella* and *E. kuehniella* had shown potential for control of the same species (Moore et al. 2000; McVean et al. 2002a, b). An approach combining granulovirus infection together with malnourishment was also found to be effective (Burden et al. 2002; McVean et al. 2002a, b). A commercial product based on granulovirus isolated from *E. kuehniella* has been registered for the control of this species in stored nuts (Vail et al. 1993).

In addition, protozoa and nematodes have also shown competences for being used as potent biocontrol agent for pest control in laboratory conditions; the potential of these organisms to suppress naturally occurring populations under field conditions remains to be established.

8.7.4 Natural Enemies of Pests as Biocontrol: Predators

A predator, *Xylocoris flavipes* (Reuter), belonging to Anthocoridae (Hemiptera) has been widely studied as a biocontrol agent (Yamada et al. 2013), and they can eat small arthropods (Lattin 2000). Members of this genus were observed in stored seeds, and amongst all, *X. flavipes* is the most explored species, and it has been described as a well-known predator for eggs and immature beetles and moths. Hence, it is a good option to be used as a biocontrol agent (Jay et al. 1968; Brower and Mullen 1990). These are effective against *Tribolium confusum*, *T. castaneum*, *Sitophilus zeamais*, *S. granarius* (L.) and *Lasioderma serricorne* (F.) (Press et al. 1975; Schöller and Prozell 2011a, b). They were also reported to be operative against a few moths like *Plodia interpunctella* (Hübner) and *Sitotroga cerealella* (Reichmuth 2000; Reichmuth et al. 2007; Rabinder and Virk 2011). Berger et al. 2017 reported recently its potential activity against seed beetles (Coleoptera: Bruchinae). Recently, it was reported that if *X. flavipes* is used together with other predators or some insect growth regulators, it becomes more effective (Murata et al. 2007; Rabinder and Virk 2011; Ferdous et al. 2010).

8.7.5 Natural Enemies of Pests as Biocontrol: Parasitoids

There are four most appearing parasitoids in stored seeds: *Anisopteromalus calandrae*, *Theocolax elegans*, *Lariophagus distinguendus* and *Habrobracon hebetor*. There are many hosts for *A. calandrae* (Williams and Floyd 1971; Reichmuth et al. 2007; Ngamo et al. 2007), and this parasitoid was reported to suppress the population of *Rhyzopertha dominica* found in stored wheat grains (Mahal et al. 2005). Similarly, *Theocolax elegans* was found to reduce the population of *S. zeamais* (Williams and Floyd 1971). Similarly, *Lariophagus distinguendus* can also be useful as a means of biocontrol. This parasitoid has the capacity to find its hosts inside the bulk-stored seeds and their products (Steidle and Schöller 2002; Adarkwah and Sch 2012). *Habrobracon hebetor* is an ectoparasitoid, and its main hosts belong to the family Pyralidae (Schöller 1998) and are useful as biocontrol agents (Kovalenkov and Meshcheriakova 1983; Balevski 1984).

8.8 Conclusion and Future Developments

Since synthetic pesticides are harmful for the environment and it also affects the human health, it is mandatory to look for its alternative options. Recent study in this direction has been pursued, and a couple of alternatives such as semiochemicals, radiation, essential oils, different ways of biological control and use of inert materials and nanoparticles have come up. Use of semiochemicals as attracticide, deterrent or repellent for mass trapping and mating disruption is very exciting. Practising radiations (such as microwaves, X-rays, gamma rays and electron beams) is also an advantageous method to control pests. Use of biopesticides of

microbial origin such as ɔ endotoxin of B.t. and abamectins from *Streptomyces avermitilis* is preferred over synthetic chemicals. Similarly, biocontrol methods also involve use of EMF, baculoviruses, protozoa and nematodes to control grain pests. In addition, diatomaceous earths (fossils of diatoms), zeolites and kaolin have also been evaluated for their activity against seed-infesting pests. A unique approach, use of nanoparticles, for seed protection has also been investigated, which can be useful for the delivery of pheromones, attracticide, repellent or deterrents of natural origin. Further research to figure out more substitutes to synthetic pesticides is highly recommended.

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References

Adarkwah C, Sch M (2012) Biological control of Plodia interpunctella (Lepidoptera: Pyralidae) by single and double releases of two larval parasitoids in bulk stored wheat. J Stored Prod Res 51:1–5

Agrafioti P, Athanassiou CG (2018) Insecticidal effect of contact insecticides against stored product beetle populations with different susceptibility to phosphine. J Stored Prod Res 79:9–15

Ajayi OE, Balusu R, Morawo TO, Zebelo S, Fadamiro H (2015) Semiochemical modulation of host preference of Callosobruchus maculatus on legume seeds. J Stored Prod Res 63:31–37

Alexandratos N, Bruinsima J (2012) World agriculture towards 2030/2050: the 2012 revision. In: ESA Working paper No. 12-03, Rome, FAO

Allotey J, Azalekor W (2000) Some aspects of the biology and control using botanicals of the rice moth, Corcyra cephalonica (Stainton), on some pulses. J Stored Prod Res 36(3):235–243

Anagnou-Veroniki M, Kontodimas DC, Chaleplidi S, Georgiadou AG, Menti H (2005) Laboratory evaluation of microbial control products on the Mediterranean flour moth Ephestia kuehniella (Zeller) (Lepidoptera: Pyralidae). Bull OILB/SROP 28:169–172

Arthur FH (1999) Efficacy of cyfluthrin as a residual surface treatment on concrete against Tribolium castaneum and T. confusum. In: Zuxun J, Quan L, Yongsheng L, Xianchang T, Linaghua G (eds) Proceedings of the Seventh International Working Conference on Stored Product Protection, Beijing, China, October 1998. Sichuan Publishing House of Science and Technology, Chengdu, pp 891–895

Arthur FH (2012) Aerosols and contact insecticides as alternatives to methyl bromide in flour mills, food production facilities, and food warehouses. J Pest Sci 85(3):323–329

Arthur FH, Puterka GJ (2002) Evaluation of kaolinite-based particle films to control Tribolium species (Coleoptera: Tenebrionidae). J Stored Prod Res 38(4):341–348

Athanassiou CG, Arthur FH (eds) (2018) Recent advances in stored product protection. Springer, New York, NY

Aviara NA, Fabiyi OE, Ojediran JO, Ogunniyi OI, Onatola IT (2016) Application of computer vision in food grain quality inspection, evaluation and control during bulk storage. In: 37th Annual Conference and Annual General Meeting–"MINNA 2016"

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Annu Rev Entomol 47(1):817–844

Balevski N (1984) Use of parasite Habrobracon hebetor Say for biological control. Rastitelna zashtita 32:28

Banks J, Fields PG (1995) Physical methods for insect control in stored-grain ecosystems, vol 353. Marcel Dekker, New York, NY

Barber DT, Black D, Dow AgroSciences LLC (2007) Detection and control of pests. US Patent 7,212,112

Berger A, Degenkolb T, Vilcinskas A, Schöller M (2017) Evaluating the combination of a parasitoid and a predator for biological control of seed beetles (Chrysomelidae: Bruchinae) in stored beans. J Stored Prod Res 74:22–26

Blanc M, Kaelin P, Gadani F (2002) Bacillus thuringiensis (Bt) for the control of insect pests in stored tobacco: a review. Beitr Tabakforschung Int Contrib Tob Res 20(1):15–22

Boyer S, Zhang H, Lempérière G (2012) A review of control methods and resistance mechanisms in stored-product insects. Bull Entomol Res 102(2):213–229

Bozlağan I, Ayvaz A, Öztürk F, Acik L, Akbulut M, Yilmaz S (2010) Detection of the cry1 gene in Bacillus thuringiensis isolates from agricultural fields and their bioactivity against two stored product moth larvae. Turk J Agric For 34(2):145–154

Brower JH, Mullen MA (1990) Effects of Xylocoris flavipes (Hemiptera: Anthocoridae) releases on moth populations in experimental peanut storages. J Entomol Sci 25(2):268–276

Burden JP, Griffiths CM, Cory JS, Smith P, Sait SM (2002) Vertical transmission of sublethal granulovirus infection in the Indian meal moth, Plodia interpunctella. Mol Ecol 11(3):547–555

Burkholder WE (1990) Practical use of pheromones and other attractants for stored-product insects. In: Behavior-modifying chemicals for insect management: applications of pheromones and other attractants. Marcel Dekker, New York, NY, pp 497–516

Burks CS, Hagstrum DW, Baker JE (1999) Selection of cold injury treatments to facilitate release of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) reared on the rice weevil (Coleoptera: Curculionidae). J Econ Entomol 92(2):473–479

Champ BR, Dyte CE (1976) Report of the FAO global survey of pesticide susceptibility of stored grain pests. FAO, Rome

Chang Y, Li XP, Liu L, Ma Z, Hu XZ, Zhao WQ, Gao GT (2015) Effect of processing in superheated steam on surface microbes and enzyme activity of naked oats. J Food Process Preserv 39(6):2753–2761

Chelladurai V, Karuppiah K, Jayas DS, Fields PG, White NDG (2014) Detection of Callosobruchus maculatus (F.) infestation in soybean using soft X-ray and NIR hyperspectral imaging techniques. J Stored Prod Res 57:43–48

Cherry AJ, Abalo P, Hell K, Korie S (2007) Farm-scale trials to compare the entomopathogenic fungus Beauveria bassiana with pirimiphos methyl+ deltamethrin and essential oil of lemon grass for protection of stored cowpea against Callosobruchus maculatus (Coleoptera: Bruchidae). Ann Appl Biol 151(1):1–10

Contreras E, Rausell C, Real MD (2013) Proteome response of Tribolium castaneum larvae to Bacillus thuringiensis toxin producing strains. PLoS One 8(1):e55330

Cox PD, Wilkin DR (1996) The potential use of biological control of pests in stored grain. HGCA research review. HGCA, London

Dakshinmurthy A, Ali N (1984) A simple device for determining hidden infestation in grains. In: Proc. Of the XXI Annual Convention of ISAE held at New Delhi during April, 1984

Eliopoulos PA, Potamitis I, Kontodimas DC, Givropoulou EG (2015) Detection of adult beetles inside the stored wheat mass based on their acoustic emissions. J Econ Entomol 108 (6):2808–2814

Elizabeth BM, Dowell FE, Baker JE, Throne JE (2002) Detecting single wheat kernels containing live or dead insects using near infrared reflectance spectroscopy. In: ASAE Meeting Paper No 23067. ASAE, Washington, DC, pp 1–10

Erban T, Nesvorna M, Erbanova M, Hubert J (2009) Bacillus thuringiensis var. tenebrionis control of synanthropic mites (Acari: Acaridida) under laboratory conditions. Exp Appl Acarol 49 (4):339–346

Evans DE (1987) The survival of immature grain beetles at low temperatures. J Stored Prod Res 23 (2):79–83

Evans DE, Thorpe GR, Dermott T (1983) The disinfestation of wheat in a continuous-flow fluidized bed. J Stored Prod Res 19(3):125–137
Fam EZ, El-Nahal AKM, Fahmy HSM (1974) Influence of grain moisture on the efficiency of silica aerogel and katelsous used as grain protectants. Bull Ent Soc Egypt Econ Ser 8:105–114
Ferdous A, Imam MZ, Ahmed T (2010) Antioxidant, antimicrobial and cytotoxic activities of Samanea saman (Jacq.) Merr. Stamf J Pharmaceut Sci 3(1):11–17
Fields PG, White ND (2002) Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu Rev Entomol 47(1):331–359
Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annu Rev Entomol 42(1):123–146
Golić MP, Andrić G, Kljajić P (2016) Combined effects of contact insecticides and 50° C temperature on Sitophilus oryzae (L.)(Coleoptera: Curculionidae) in wheat grain. J Stored Prod Res 69:245–251
Golob PAV, Webley DJ (1980) The use of plants and minerals as traditional protectants of stored products. FAO, Rome
Gomez-Lopez VM, Ragaert P, Debevere J, Devlieghere F (2007) Pulsed light for food decontamination: a review. Trends Food Sci Technol 18(9):464–473
Hagstrum DW, Subramanyam B (2000) Monitoring and decision tools. In: Alternatives to pesticides in stored-product IPM. Springer, Boston, MA, pp 1–28
Hallman GJ (2013) Control of stored product pests by ionizing radiation. J Stored Prod Res 52:36–41
Hubert J, Nesvorna M, Zemek R, Stara J, Stejskal V (2008a) Effects of metabolic inhibitors on activity of Cry1Ab toxin to inhibit growth of Ephestia kuehniella larvae. Pest Manag Sci 64:1063–1068
Hubert J, Kudlíková-Křížková I, Stejskal V (2008b) a. Effect of MON 810 Bt transgenic maize diet on stored-product moths (Lepidoptera: Pyralidae). Crop Prot 27(3-5):489–496
IITA (International Institute of Tropical Agriculture) (1988) Annual report 1988/89. IITA, Ibadan
Jay E, Davis R, Brown S (1968) Studies on the predacious habits of Xylocoris flavipes (Reuter) (Hemiptera: Anthocoridae). J Ga Entomol Soc 3:126–130
Kamihira M, Taniguchi M, Kobayashi T (1987) Sterilization of microorganisms with supercritical carbon dioxide. Agric Biol Chem 51(2):407–412
Karunakaran C, Jayas DS, White NDG (2003) X-ray image analysis to detect infestations caused by insects in grain. Cereal Chem 80(5):553–557
Kaur R, Subbarayalu M, Jagadeesan R, Daglish GJ, Nayak MK, Naik HR, Ramasamy S, Subramanian C, Ebert PR, Schlipalius DI (2015) Phosphine resistance in India is characterised by a dihydrolipoamide dehydrogenase variant that is otherwise unobserved in eukaryotes. Heredity 115(3):188–194
Kavallieratos NG, Athanassiou CG, Arthur FH (2017) Effectiveness of insecticide-incorporated bags to control stored-product beetles. J Stored Prod Res 70:18–24
Kim SS, Rhyu MR, Kim JM, Lee SH (2003) Authentication of rice using near-infrared reflectance spectroscopy. Cereal Chem 80(3):346–349
Kiobia DO, Tumbo SD, Cantillo J, Rohde BB, Mallikarjunan PK, Mankin RW (2015) Characterization of sounds in maize produced by internally feeding insects: investigations to develop inexpensive devices for detection of Prostephanus truncatus (Coleoptera: Bostrichidae) and Sitophilus zeamais (Coleoptera: Curculionidae) in small-scale storage facilities in sub-Saharan Africa. Fla Entomol 98(2):405–410
Kitch LW, Ntoukam G, Shade RE, Wolfson JL, Murdock LL (1992) A solar heater for disinfesting stored cowpeas on subsistence farms. J Stored Prod Res 28(4):261–267
Kotwaliwale N, Singh K, Kalne A, Jha SN, Seth N, Kar A (2014) X-ray imaging methods for internal quality evaluation of agricultural produce. J Food Sci Technol 51(1):1–15
Kovalenkov VG, Meshcheriakova TV (1983) Reserve plantations for Trichogramma and Habrobracon. Zashchita rastenii 12:16

Langseth W, Stenwig H, Sogn L, Mo E (1993) Growth of moulds and production of mycotoxins in wheat during drying and storage. Acta Agric Scand B Plant Soil Sci 43(1):32–37
Laopongsit W, Srzednicki G, Craske J (2014) Preliminary study of solid phase micro-extraction (SPME) as a method for detecting insect infestation in wheat grain. J Stored Prod Res 59:88–95
Lapp HM, Madrid FJ, Smith LB (1986) A continuous thermal treatment to eradicate insects from stored wheat. American Society of Agricultural Engineers, St Joseph, MI. Microfiche collection
Lattin JD (2000) Minute pirate bugs (Anthocoridae). In: Schaefer CW, Panizzi AR (eds) Heteroptera of economic importance. CRC Press, Boca Raton, FL, pp 607–637
Leblanc MP, Gaunt D, Fleurat-Lessard F (2011) Experimental study of acoustic equipment for real-time insect detection in grain bins—assessment of their potential for infestation risk prediction during long term storage periods. IOBC WPRS Bull 69:79–88
Lee N (1960) Harvests and harvesting through the ages. Cambridge University Press, New York, NY
Levinson HZ, Levinson AR (1989) Food storage and storage protection in ancient Egypt. Boletin De Sanidad Vegetal 17:475–482
Lima KDSC, e Souza LB, de Oliveira Godoy RL, França TCC, dos Santos Lima AL (2011) Effect of gamma irradiation and cooking on cowpea bean grains (Vigna unguiculata L. Walp). Radiat Phys Chem 80(9):983–989
Locatelli DP, Traversa S (1989) Microwaves in the control of rice infestations. Ital J Food Sci 2:53–62
Macbean C (2012) The pesticide manual, 16th edn. BCPC, Alton
Maftei NA, Ramos-Villarroel AY, Nicolau AI, Martín-Belloso O, Soliva-Fortuny R (2014) Pulsed light inactivation of naturally occurring moulds on wheat grain. J Sci Food Agric 94:721–726
Mahal N, Islam W, Parween S, Mondal KAMSH (2005) Effect of Anisopteromalus calandrae (Hymenoptera: Pteromalidae) in controlling residual populations of Rhyzopertha dominica (Coleoptera: Bostrichidae) in wheat stores. Int J Trop Insect Sci 25(4):245–250
Mahdi SHA, Khalequzzaman M (2006) Toxicity studies of some inert dusts with the cowpea beetle, Callosobruchus maculatus (Fabricius) (Coleoptera: Bruchidae). J Biol Sci 6(2):402–407
Malekpour R, Rafter MA, Daglish GJ et al (2018) The movement abilities and resource location behaviour of *Tribolium castaneum*: phosphine resistance and its genetic influences. J Pest Sci 91:739. https://doi.org/10.1007/s10340-017-0935-z
Mason LJ, Strait CA (1998) Stored product integrated pest management with extreme temperatures. In: Temperature sensitivity in insects and application in integrated pest management, pp 141–177
McGaughey WH (1985) Insect resistance to the biological insecticide Bacillus thuringiensis. Science 229(4709):193–195
McGaughey WH, Beeman RW (1988) Resistance to Bacillus thuringiensis in colonies of Indian meal moth and almond moth (Lepidoptera: Pyralidae). J Econ Entomol 81(1):28–33
McVean RIK, Sait SM, Thompson DJ, Begon M (2002a) Dietary stress reduces the susceptibility of Plodia interpunctella to infection by a granulovirus. Biol Control 25(1):81–84
McVean RI, Sait SM, Thompson DJ, Begon M (2002b) Effects of resource quality on the population dynamics of the Indian meal moth Plodia interpunctella and its granulovirus. Oecologia 131(1):71–78
Meikle WG, Cherry AJ, Holst N, Hounna B, Markham RH (2001) The effects of an entomopathogenic fungus, Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes), on Prostephanus truncatus (Horn) (Col.: Bostrichidae), Sitophilus zeamais Motschulsky (Col.: Curculionidae), and grain losses in stored maize in the Benin Republic. J Invertebr Pathol 77 (3):198–205
Mellart J (1961) Hacilar: a Neolithic village site. Sci Am 205(2):86–98
Méndez WA, Valle J, Ibarra JE, Cisneros J, Penagos DI, Williams T (2002) Spinosad and nucleopolyhedrovirus mixtures for control of Spodoptera frugiperda (Lepidoptera: Noctuidae) in maize. Biol Control 25(2):195–206

Mohapatra D, Giri SK, Kar A (2014) Effect of microwave aided disinfestation of Callosobruchus maculatus on green gram quality. Int J Agric Food Sci Technol 5(2):55–62
Mohapatra D, Kar A, Giri SK (2015) Insect pest management in stored pulses: an overview. Food Bioprocess Technol 8(2):239–265
Mohapatra D, Kumar S, Kotwaliwale N, Singh KK (2017) Critical factors responsible for fungi growth in stored food grains and non-Chemical approaches for their control. Ind Crop Prod 108:162–182
Mondal M, Khalequzzaman M (2010) Toxicity of naturally occurring compounds of plant essential oil against Tribolium castaneum (Herbst). J Biol Sci 10(1):10–17
Moore D, Smith SM, Lord JC (2000) Pathogens. In: Alternatives to pesticides in stored-product IPM. Kluwer Academic Publishers, Boston, MA
Mostafa MAM, Vlasák J, Sehnal F (2013) Activities of modified Cry3A-type toxins on the red flour beetle, Tribolium castaneum (Herbst). J Appl Entomol 137(9):684–692
Murata M, Imamura T, Miyanoshita A (2007) Suppression of the stored-product insect Tribolium confusum by Xylocoris flavipes and Amphibolus venator. J Appl Entomol 131(8):559–563
Nanje Gowda NA, Alagusundaram K (2013) Use of thermal imaging to improve the food grains quality during storage. Int J Curr Agric Res 1(7):34–41
Navarro S (2012) The use of modified and controlled atmospheres for the disinfestation of stored products. J Pest Sci 85(3):301–322
Nayak MK, Holloway JC, Emery RN, Pavic H, Bartlet J, Collins PJ (2013) Strong resistance to phosphine in the rusty grain beetle, Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in Australia. Pest Manag Sci 69(1):48–53
Nelson SO, Kantack BH (1966) Stored-grain insect control studies with radio-frequency energy. J Econ Entomol 59(3):588–594
Nemțanu MR, Braşoveanu M, Karaca G, Erper İ (2014) Inactivation effect of electron beam irradiation on fungal load of naturally contaminated maize seeds. J Sci Food Agric 94 (13):2668–2673
Ngamo TSL, Kouninki H, Ladang YD, Ngassoum MB, Mapongmestsem PM, Hance T (2007) Potential of Anisopteromalus calandrae (Hymenoptera: Pteromalidae) as biocontrol agent of Callosobruchus maculatus (F.) (Coleoptera: Bruchidae). Afr J Agric Res 2(4):168–172
Nyzam SZA, Rahim RA (2019) Heat distribution under microwave heating treatment. Bull Elect Eng Informatics 8(1):136–141
Opit GP, Phillips TW, Aikins MJ, Hasan MM (2012) Phosphine resistance in Tribolium castaneum and Rhyzopertha dominica from stored wheat in Oklahoma. J Econ Entomol 105(4):1107–1114
Opit GP, Thoms E, Phillips TW, Payton ME (2016) Effectiveness of sulfuryl fluoride fumigation for the control of phosphine-resistant grain insects infesting stored wheat. J Econ Entomol 109(2):930–941
Oppert B, Morgan TD, Kramer KJ (2011) Efficacy of Bacillus thuringiensis Cry3Aa protoxin and protease inhibitors against coleopteran storage pests. Pest Manag Sci 67(5):568–573
Pearson T, Brabec DL (2007) Detection of wheat kernels with hidden insect infestations with an electrically conductive roller mill. Appl Eng Agric 23(5):639–645
Peckman PS, Arthur FH (2005) Insecticide space treatments in food plants. In: Heaps JW (ed) Insect management for food storage and processing, 2nd edn. AACC International, Minneapolis, MN, pp 175–182
Phillips TW (1997) Semiochemicals of stored-product insects: research and applications. J Stored Prod Res 33(1):17–30
Phillips TW, Throne JE (2010) Biorational approaches to managing stored-product insects. Annu Rev Entomol 55:375
Phillips TW, Jiang XL, Burkholder WE, Phillips JK, Tran HQ (1993) Behavioral responses to food volatiles by two species of stored-product coleoptera, Sitophilus oryzae (curculionidae) and Tribolium castaneum (tenebrionidae). J Chem Ecol 19(4):723–734

Phillips TW, Phillips JK, Webster FX, Tang R, Burkholder WE (1996) Identification of sex pheromones from cowpea weevil, Callosobruchus maculatus, and related studies with C. analis (coleoptera: Bruchidae). J Chem Ecol 22(12):2233–2249

Pimentel D, Zepp A (1991) Environmental and economic effects of reducing pesticide use. Bioscience 41(6):402

Pinniger DB, Stubbs MR, Chambers J (1984) The evaluation of some food attractants for the detection of Oryzaephilus surinamensis (L.) and other storage pests. Kansas State University, Manhattan, KS

Press JW, Flaherty BR, Arbogast RT (1975) Control of the red flour beetle, Tribolium castaneum, in a warehouse by a predaceous bug, Xylocoris flavipes. J Georgia Entomol Soc 10(1):76–78

Quarles W (1992) Diatomaceous earth for pest control. IPM Pract 14(5/6):1–11

Rabinder K, Virk JS (2011) Role of Blaptostethus pallescens Poppius and Xylocoris flavipes (Reuter) in the suppression of Corcyra cephalonica Stainton in stored rice grain. J Biol Control 25(4):329–332

Rajendran S, Sriranjini V (2008) Plant products as fumigants for stored-product insect control. J Stored Prod Res 44(2):126–135

Reed CARL (1992) Development of storage techniques: a historical perspective. In: Storage of cereal grains and their products. American Association of Cereal Chemists, St Paul, MN, pp 143–156

Rees DP (2004) Insects of stored products. Csiro Publishing, Clayton, VIC

Reichmuth C (2000) Biological control in stored product protection. IOBC WPRS Bull 23 (10):11–24

Reichmuth C, Schöller M, Ulrichs C (2007) Stored product pets in grain: morphology-biology-damage-control. AgroConcept Verlagsgesellschaft, Bonn

Ridgway C, Chambers J (1996) Detection of external and internal insect infestation in wheat by near-infrared reflectance spectroscopy. J Sci Food Agric 71(2):251–264

Sabbour M (2012) Evaluations of some bioagents against Sitophilus oryzae under laboratory and store conditions. IOBC/WPRS Bull 81:135–142

Scheirs J, De Bruyn L, Verhagen R (2003) Host nutritive quality and host plant choice in two grass miners: primary roles for primary compounds? J Chem Ecol 29(6):1373–1389

Schöller M (1998) Biologische Bekämpfung vorratsschädlicher Arthropoden mit Räubern und Parasitoiden–Sammelbericht und Bibliographie. In: Reichmuth C (ed) 100 Jahre Pflanzenschutzforschung. Wichtige Arbeitsschwerpunkte im Vorratsschutz. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Heft, vol 342. Parey, Berlin, pp 85–189

Schöller M, Prozell S (2011a) Biological control of cultural heritage pest Coleoptera and Lepidoptera with the help of parasitoid Hymenoptera. J Entomol Acarol Res 43(2):157–168

Schöller M, Prozell S (2011b) Potential of Xylocoris flavipes (Hemiptera: Anthocoridae) to control Tribolium confusum (Coleoptera: Tenebrionidae) in Central Europe. IOBC/WPRS Bull 69:163–168

Scott HG (1991) Nutrition changes caused by pests in food. In: Gorham JR (ed) Ecology and management of food-industry pests. Association of official analytical chemists. Food and drug administration technical bulletin 4, Virginia, USA, pp 463–467

Sedlacek JD, Komaravalli SR, Hanley AM, Price BD, Davis PM (2001) Life history attributes of Indian meal moth (Lepidoptera: Pyralidae) and Angoumois grain moth (Lepidoptera: Gelechiidae) reared on transgenic corn kernels. J Econ Entomol 94(2):586–592

Sharma KR, Fadamiro HY (2013) Fire ant alarm pheromone and venom alkaloids act in concert to attract parasitic phorid flies, Pseudacteon spp. J Insect Physiol 59(11):1119–1124

Shuman D, EPSKY ND (2001) Commercialization of the electronic grain probe insect counter. In: Proceeding of an International Conference on Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA, vol 29, pp 665–677

Steidle JL, Schöller M (2002) Fecundity and ability of the parasitoid Lariophagus distinguendus (Hymenoptera: Pteromalidae) to find larvae of the granary weevil Sitophilus granarius (Coleoptera: Curculionidae) in bulk grain. J Stored Prod Res 38(1):43–53

Stejskal V, Hubert J, Aulicky R, Kucerova Z (2015) Overview of present and past and pest-associated risks in stored food and feed products: European perspective. J Stored Prod Res 64:122–132

Sutton AE, Arthur FH, Zhu KY, Campbell JF, Murray LW (2011) Residual efficacy of synergized pyrethrin+ methoprene aerosol against larvae of Tribolium castaneum and Tribolium confusum (Coleoptera: Tenebrionidae). J Stored Prod Res 47(4):399–406

Upadhyay RK, Ahmad S (2011) Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World J Agric Sci 7(5):527–549

Vail PV, Tebbets JS, Mackey BE, Curtis CE (1993) Quarantine treatments: a biological approach to decision-making for selected hosts of codling moth (Lepidoptera: Tortricidae). J Econ Entomol 86(1):70–75

Van Lynden-van Nes AMT, Koren LGH, Snijders MCL, Van Bronswijk JEMH (1996) Medical impact of arthropod allergens. In: Proceedings of the Second International Conference on Urban Pests, Edinburgh, Scotland, pp 17–30

Vijayanna SV (2006) Effect of fumigation on seed quality during storage of ground nut. Dissertation, Univ. Agric. Sci., Dharwad, Karnataka, India

Villers P, Bruin TD, Navarro S (2006) Development and applications of the hermetic storage technology. In: Proceedings of the 9th International Working Conference on Stored-Product Protection, ABRAPOS, Passo Fundo, RS, Brazil, 15–18 October 2006. Brazilian Post-Harvest Association (ABRAPOS), Passo Fundo, pp 719–729

Whitehead BR (2007) The pesticide guide 2007. CAB International, Wallingford

Williams RN, Floyd EH (1971) Effect of two parasites, Anisopteromalus calandrae and Choetospila elegans upon populations of the maize weevil under laboratory and natural conditions. J Econ Entomol 64(6):1407–1408

Wilson AD (2012) Review of electronic-nose technologies and algorithms to detect hazardous chemicals in the environment. Proc Technol 1:453–463

Xing J, Guyer D (2008) Comparison of transmittance and reflectance to detect insect infestation in Montmorency tart cherry. Comput Electron Agric 64(2):194–201

Yamada K, Yasunaga T, Artchawakom T (2013) The genus Xylocoris found from plant debris in Thailand, with description of a new species of the subgenus Arrostelus (Hemiptera: Heteroptera: Anthocoridae). Acta Entomol Musei Nationalis Pragae 53(2):493–504

Yılmaz S, Ayvaz A, Akbulut M, Azizoglu U, Karabörklü S (2012) A novel Bacillus thuringiensis strain and its pathogenicity against three important pest insects. J Stored Prod Res 51:33–40

Zayas IY, Flinn PW (1998) Detection of insects in bulk wheat samples with machine vision. Trans ASAE 41(3):883–888

Zettler JL, Arthur FH (2000) Chemical control of stored product insects with fumigants and residual treatments. Crop Prot 19(8–10):577–582

Zhu J, Dhammi A, van Kretschmar JB, Vargo EL, Apperson CS, Michael Roe R (2018) Novel use of aliphatic n-methyl ketones as a fumigant and alternative to methyl bromide for insect control. Pest Manag Sci 74(3):648–657

Zotti M, Porro R, Vizzini A, Mariotti MG (2008) Inactivation of Aspergillus spp. by ozone treatment. Ozone Sci Eng 30(6):423–430

9 Disease-Causing Seed Pathogenic Microorganisms and Their Management Practices

Avinash Marwal and R. K. Gaur

Abstract

Seed diseases are a universal problem, and it creates pressure on limited food supplies, and therefore, employment of effective and sustainable disease management practices is quite important/necessary. There are several beneficial microorganisms which are used for better growth, survival of seed and protection against soilborne diseases and pests. The present article summarizes the currently available information on disease-causing seed pathogenic microorganisms and their management practices.

Keywords

Crop seeds · Fungi · Bacteria · Viruses · Viroids · Management practices · Antimicrobial compounds

9.1 Background

Seed pathogen causes severe damages reducing germination, seedling vigour, and affecting photosynthetic ability; overall it affects the sale due to the use of infected seeds. This drop in seed quality is considered to cause a major economic impact (Nowicki et al. 2012; Manhas and Kaur 2016). These pathogenic microbes include numerous species of plant-infecting fungi, bacteria, viruses and viroids (Mahmood et al. 2017; Prajapat et al. 2013a, b; Singh and Dilworth 2009; Nehra et al. 2019a). It

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is well known that plant microbiota are principally accumulated from external source/inoculum, which can be in harmful pathogenic form (Marwal et al. 2014a; Sahu et al. 2013), or beneficial endophytes (evade in pathogen transmission) having a significant impact on plant health (Sudheep et al. 2017; Rybakova et al. 2017), whereas plant seeds are acknowledged/involved in the vertical transmission of microbes from parent plants to the next generation (Johnston-Monje and Raizada 2011; Lebeis et al. 2015; Shade et al. 2017).

Prominently, many of these plant pathogenic microbes are easily seedborne in nature, which means they can endure in the plant seeds and are conveyed via infected seeds to new fields and locations (Adam et al. 2018; Agler et al. 2016). With globalization, a large quantity of contaminated commercial seeds are being exported and imported, which may be the foremost reason for the spread of pathogenic microbes throughout the world (Gitaitis and Walcott 2007; Constable et al. 2019). Therefore, new strategies to combat microbial plant diseases are exceedingly required and are needed to stop and reduce seed transmission of microbial pathogens (Darrasse et al. 2010; Sahu et al. 2014a; Makarovsky et al. 2018). The latest technology which helps in the management of pathogenic microbes is the genome editing tool. RNAi (RNA interference)-mediated gene silencing approach (Das et al. 2011; Marwal et al. 2012a, b) is still in practice, whereas CRISPR (clustered regularly interspaced short palindromic repeats)/Cas system is the emerging technology to combat against plant diseases (Cody and Scholthof 2019). Several crops have been developed which showed resistance to fungal, bacterial and viral disease using these genome editing tools (Chen et al. 2019; Ortigosa et al. 2019; Collemare et al. 2019). In this chapter, we address the various diseases related to seeds and their causal agents. It also includes their management practices for seed protection, thus possessing sturdy inhibitory activity against plant pathogens but least impact on crop health and the environment.

9.2 Fungal Microbes Infecting Crop Seeds

Pathogenic fungi are considered to be generalist (infecting wide range of host) in nature and also have the characteristics for demonstrating diverse degrees of host specialization (Zhang et al. 2019; Marwal et al. 2012a, b). But these pathogenic groups of fungi are often genetically different, with each species causing maximum disease occurrence on its host of origin (Viswanathan et al. 2019; Marwal et al. 2014b). One of the studies reveals the generalist nature of pathogenic ascomycete fungi *Pyrenophora semeniperda* in the seeds of *Bromus tectorum* (known as cheat grass or downy brome) and other winter annual grasses like *Bromus arvensis*, *Bromus diandrus*, *Bromus rubens* and *Taeniatherum caput-medusae* clearly suggesting its wide host range (Meyer et al. 2014; Beckstead et al. 2014, 2016).

The country Nicaragua where the latest technology in agriculture sector is yet to be accomplished does not have any access to certified healthy seeds for their small in-land grown pulses (especially *Phaseolus vulgaris* L: the common bean), resulting in huge yield loss of this legume due to pathogenic fungi-infected seeds. ‘INTA

Rojo' is the major local bean cultivar of common bean legume and was recorded to be infected by 11 pathogenic fungi, namely, *Macrophomina phaseolina*, *Fusarium incarnatum*, *Diaporthe* sp. *(Phomopsis)*, *Colletotrichum capsici*, *Fusarium equiseti*, *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Corynespora cassiicola*, *Fusarium chlamydosporum*, *Penicillium citrinum* and *Aspergillus flavus*. These harmful microorganisms are easily transmitted by legume seeds and restrict seed germination and even destroy the seedlings, affecting the movement of nutrients by infecting the roots and vascular system, thereby diminishing plant growth (Jimeânez et al. 2012; Mancini et al. 2016; Schwartz et al. 2005; Marcenaro and Valkonen 2016).

Over time, phytopathologists have believed in the influence of surrounding environment in their experiments of plant diseases, where interaction among the hosts, pathogens and environment is a driving force in the onset of disease (Hou et al. 2018; Marwal et al. 2018, 2019a; Long et al. 2015). Talking about climate change, as described by Boddy et al. (2014), the changing environment can have both positive (increasing infection) and negative (decreasing infection) effects on fungal disease occurrence/incidence in seeds, which strongly correlates to the crop yield loss of economically important plants by even a slight change in their environmental conditions (Paterson et al. 2013). The environmental condition comprises changes in precipitation, light intensity, relative humidity and wind speed, which directly assists in the transmission of seedborne fungal microbes (Popovski and Celar 2013).

Sclerotinia homoeocarpa belongs to *Rutstroemiaceae* family which does not produce any spores and is responsible for dollar spot diseases of turfgrasses infecting only on the leaves. Turfgrasses are the species of grasses which are widely used in golf courses, and its seeds are transmitted over long distances. *Sclerotinia homoeocarpa* pathogenic fungi were detected in a number of seed lots of turfgrasses in the United States using basic molecular technique, i.e. nested PCR with specific primers (Rioux et al. 2014).

All the reported pathogenic fungi are either externally/internally seedborne or externally/internally embryal in nature. On the one hand, *Fusarium oxysporum* is the major seed-infecting fungus of *Solanum lycopersicum* and causes root rot and wilt diseases in tomato crops. On the other hand, *Alternaria solani* is the second most pathogenic species and causes early blight of tomato (Mehrotra and Agarwal 2003). Up to date identified seedborne pathogenic fungi of *Solanum lycopersicum* are *Sclerotinia* sp., *Rhizoctonia* sp., *Pythium* sp., *Verticillium* sp., *Cladosporium* sp., *Rhizopus stolonifer*, *Aspergillus flavus*, *Alternaria alternata*, *Rhizopus arrhizus*, *Fusarium equiseti*, *Colletotrichum gloeosporioides*, *Aspergillus clavatus*, *Penicillium digitatum*, *Bipolaris maydis*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium moniliforme* and *Fusarium solani* (Nishikawa et al. 2006; Al-Kassim and Monawar 2000).

For the same crop plant, in an another study in Saudi Arabia in the course of the year 2011 and 2012, over hundreds of samples of *Solanum lycopersicum* were examined to identify the seedborne pathogenic fungi via deep freezing blotter and agar plate methods. In continuation to this, their distribution across the country was also explored using the canonical correspondence analysis. In the results, 30 genera

and 57 species of fungi were identified, and the maximum incidence was recorded from the Al-Madena province. From all the diagnosed fungi from tomato seeds, eight of them were known to be pathogenic to seeds in nature, and they were *Botrytis cinerea*, *Rhizoctonia solani*, *Alternaria alternata*, *Verticillium albo-atrum*, *Pythium aphanidermatum*, *Drechslera* sp., *Fusarium oxysporum* and *Cladosporium herbarum* (Al-Askar et al. 2014).

Management/control of disease-causing pathogenic fungi is a must for the enhancement of seed production, lessening the yield losses and basically curtailing the health risks employed by toxins and allergen substances released by these harmful microbes. The well-organized propagation of fungal conidia by either wind dispersal or rain splash establishes another task for the control of these pathogens (Bamisile et al. 2018). Use of resistant cultivars and integrated pest management is quite important in averting seedborne fungal diseases of various economically important crop species. In an attempt to control the spread of *Macrophomina phaseolina* in common bean in Nicaragua which causes the famous charcoal rot disease, various crop management approaches were taken into consideration that include the usage of biological control agents, crop rotation practices, testing resistant cultivars in the fields, decreasing the crop density/canopy in the field and even managing the soil humidity favourable for a healthy plant growth (Diniz Cavalcante et al. 2014; Sharma et al. 2015).

Mycosphaerella graminicola is a well-known filamentous fungus pathogen spread by both sexual ascospores and asexual pycnidiospores. Simple polymerase chain reaction (PCR) technique is sufficient to identify the pathogenic fungus in contaminated seeds. Despite the environmental risk, cost, etc., fungicides are widely used to control the spread of *Mycosphaerella graminicola*. In the management of *Mycosphaerella graminicola*, strobilurin (under the trade name QoI) fungicide is majorly used which shows a strong effect against the mitochondrial protein cytochrome b of this fungus. Another well-known fungicide is the triazole, which is a sterol demethylation inhibitor that shows its action on CYP51, the cytochrome P450 eburicol 14-demethylase enzyme of the ergosterol biosynthesis pathway of the fungus (Stergiopoulos et al. 2010; Orton et al. 2011).

Across the world *Alternaria brassicicola* fungus reasons for enormous economic crop losses of crucifers by causing black spot disease and damping off of seedlings (Reis and Boiteux 2010). To avoid the use of toxic fungicides, biocontrol agents and their bioactive metabolites which are environmental friendly can be used instead. *Streptomyces hydrogenans* strain DH16 is a wonderful biocontrol agent whose culture supernatant (10%) was used to treat the *Alternaria brassicicola*-infected seeds. Employment of this management technique significantly improved the seed germination (75–80%) and the vigour index (1167–1538) of crucifers (Manhas and Kaur 2016). *Didymella pinodes* causes a very common blight disease in *Pisum sativum* (field peas). Rhizobium is recognized as a plant growth-promoting endosymbiont, and when its inoculum was treated on *Pisum sativum* seeds, it reduces the *Didymella pinodes* load in the seed. This management technique enhanced the crop yield, and the rhizobium inoculation also persuaded changes in the seed proteome

and metabolome making *Pisum sativum* resistant to pathogenic fungus *Didymella pinodes* (Sistani et al. 2017; Simonsen et al. 2017).

From the above-mentioned dicot crop plants, pathogenic fungi were also identified to harbour monocot species. One such is the fungal pathogen *Fusarium fujikuroi* responsible for bakanae disease in rice, and the infected seeds upon germination display chlorotic symptoms and have extended growth of stem parts and no edible grains. For the management of *Fusarium fujikuroi*, seed sterilization was done with the application of ozone gas and arc discharge plasma. Here rice seeds were completely sterilized which is quite effective in this case. (Kwack et al. 2014; Marwal et al. 2014c; Kitazaki et al. 2014; Carter et al. 2008; Kang et al. 2015).

9.3 Bacterial Pathogens Harbouring Seeds

Bacterial infection in seeds seemingly had a damaging effect on the seedlings and overall plant development. Spreading of contaminated seeds from one region to another easily provides an effective way of introducing bacterial pathogens into crops at an early stage (Roberts et al. 2019). This movement of seedborne pathogenic bacteria is also responsible for crossing international/intercontinental boundaries, thereby disease in new area (Syed-Ab-Rahman et al. 2019). Both slow- and fast-growing pathogenic bacterial strains are sufficient to kill both dormant and nondormant seed lots regardless of their inoculum loads eventually harming the yield. Fast growers affect seed at early stage, whereas the slow growers release toxins which affect the seeds' growth at later stage (Darrasse et al. 2007; Darsonval et al. 2008; Wightwick et al. 2010).

The chief route for the bacteria in reaching the seeds is the floral organs, and the major ones in the list causing disease in seeds are *Xanthomonas euvesicatoria* (bacterial spot of pepper), *Pseudomonas syringae* pv. tomato (bacterial speck of tomato), *Acidovorax citrulli* (fruit blotch of watermelon), *Pseudomonas syringae* pv. glycinea (bacterial blight of soybean), *Xanthomonas campestris* pv. campestris (cauliflower) and *Clavibacter michiganensis* subsp. michiganensis (bacterial canker of tomato) (Burdman and Walcott 2012; Dutta et al. 2014; Yan et al. 2017). *Clavibacter michiganensis* subsp. michiganensis (Cmm) is a gram-positive bacterial species responsible for the devastating disease, i.e. bacterial wilt (symptoms wilting) and bacterial canker in *Solanum lycopersicum* plant affecting the crop production (Frenkel et al. 2016; Sen et al. 2015; Han et al. 2017).

Pseudomonas and *Bacillus* strains produce various antimicrobial metabolites like phenazines, pyrrolnitrin and hydrogen cyanide (HCN) and even degradative enzymes. Both were found to be effective in disease control. Even the extracts and essential oils of plants have a probable way in controlling the seedborne pathogens. Few examples include the extract fragarin from strawberry leaves and essential oils of marjoram, oregano, thyme and dictamnus (Daferera et al. 2003; Boudyach et al. 2010). Initial methods of bacterial management were the treatment of seeds with low concentration of acids and even sanitizing the soil itself with formaldehyde; some success was achieved in this case. Likewise subjecting the seeds to high temperature

of 48–52 °C was effective for some seed cultivars. Certain chemicals have also been tested chiefly with the copper-based compounds, i.e. copper hydroxide and copper sulphate. Bactericides such as streptomycin, mancozeb and their combinations were too fruitful (Kasselaki et al. 2011; Hausbeck et al. 2000; Werner et al. 2002; Divsalar et al. 2014; Nandi et al. 2018).

In the race of employing better management practices, antimicrobial peptides (AMPs) can also solve the infections caused by pathogenic bacterial strains. In humans AMPs work in immune responses against pathogens, and the same holds true in the case for plants. An example is the BP178 peptide, for which a synthetic gene was constructed beneath the governance of endosperm-specific promoter and was then introduced in the rice plants. After showing good expression, the BP178 peptide was purified, and its antibacterial activity was positively tested against bacterial plant pathogen *Dickeya* sp., accountable for the dark brown sheath rot of rice. Further the transgenic rice developed from this experiment also revealed strong resistance to the fungal pathogen *Fusarium verticillioides* which causes bakanae disease in rice (Montesinos et al. 2017; Takaiwa et al. 2015).

Taking a step forward to regulate the transmission of bacterial pathogens via seed, it seems better option to sanitize them from seed-producing crops. Control techniques were aimed, including thermotherapy and the use of a biocontrol agent to halt seed transmission of plant pathogenic agents. Under the trade name EndoSeedTM, a plant growth-promoting strain *Paraburkholderia phytofirmans* PsJN was taken into consideration and tested its effect on seeds of many plant species via spraying the inoculum on the parent plant at the flowering stage. The *Paraburkholderia phytofirmans* PsJN easily compete with plant pathogens for nutrients, and this limits seed transmission of phytopathogenic bacteria (Vázquez-de-Aldana et al. 2013; Valeria and Gianfranco 2013; Goggin et al. 2015; Torres-Cortes et al. 2019).

In another study of employing the use of biocontrol agents, bacteriophages have also been tried in bacterial management practices as it can be easily prepared with low cost of production. One such example is the virus bacteriophage CMP1 belonging to the *Siphoviridae* family. Bacteriophage CMP1 was firstly isolated from *Clavibacter michiganensis* subsp. michiganensis (Cmm), a seedborne pathogenic bacterium found infecting the tomato stems. Bacteriophage CMP1 produces one major macromolecule endolysin which possesses peptidase activity and can easily lyse Cmm only specifically. This bacteriophage CMP1 endolysin gene was identified and cloned in a suitable manner and later used to produce transgenic tomato plants, showing complete resistance to Cmm (Jones et al. 2007; Wittmann et al. 2016).

Nanoparticles have a vast application in various fields ranging from medical to agriculture sector (Marwal and Gaur 2019; Das et al. 2014, 2016). A nanoconjugate was developed called bovine submaxillary mucin-silver nanoparticles (BSM-Ag NPs). Silver nanoparticles are known for their strong antibacterial activity, whereas mucins which are large extracellular glycoproteins are found in mucus of all animals; both of them were combined and tested against gram-positive (*Clavibacter*) and gram-negative (*Acidovorax* and *Xanthomonas*) bacteria. Results obtained were

significantly remarkable where the nanoconjugate at low concentration successfully inhibits the growth of these bacterial strains mentioned above. Similarly the conjugate was treated on melon seeds which effectively prevents seed to seedling transmission of *Acidovorax citrulli*, the chief pathogen of Cucurbitaceae across the globe (Bansil and Turner 2006; Lamichhane et al. 2016; Franci et al. 2015).

There is a serious need to diagnose and characterize the mechanisms and even the ways that permit pathogenic bacteria to move across the continents between countries and to understand their epidemiology for better management and solving regulatory issues. Moreover to make the management effective against these harmful pathogens, understanding of the factors responsible for their transmission must be on priority as to identify the places where they were not previously present, and countries must devise their effective regulations. It might be possible that the pathogenic bacteria can be transmitted to non-host seeds leading to serious seedborne diseases. Therefore, testing of seed health, seed certification and quarantine should be compulsory along with their treatment of bactericides and if necessary eliminate seedborne bacterial inoculum to stop the spread in the importer country. Despite using these precautions and management practices, a better knowledge of seedborne bacteria is required (Gitaitis and Walcott 2007; Johnson et al. 2011).

9.4 Virus/Viroid Diseases in Seeds

There are a number of ways which are responsible for the transmission of plant viruses (Prajapat et al. 2014). Trading (export/import) of plant materials, mainly seeds, is the easy source of distribution of plant viruses to geographic regions sufficient to cause epidemics at new places. Numerous plant viruses are known to be seedborne, for example, *Tobamovirus, Apple mosaic virus, Prunus necrotic ringspot virus, Sweet potato leaf curl virus, Tomato yellow leaf curl virus, Maize dwarf mosaic virus*, etc. (Ludmerszki et al. 2017; Kannan et al. 2018). Geminiviruses are the major ones which are transmitted by seed, viruliferous whitefly, leafhoppers, treehoppers, aphids, etc. (Gaur et al. 2011; Marwal et al. 2019b; Nehra et al. 2019b). Seed transmission may be externally (seed coat) or internally (embryo), and the virus makes through to the seed from the infected mother plant tissue or through the infected pollen grains (Reingold et al. 2015).

Apart from viruses, there are also viroids which are seedborne, and among them are the pospiviroids which infect solanaceous crops. Tomato, capsicum and chilli seed lots were found infected with pospiviroids in Australia. The major ones detected were *Tomato apical stunt viroid, Pepper chat fruit viroid, Citrus exocortis viroid, Tomato chlorotic dwarf viroid, Columnea latent viroid* and *Potato spindle tuber viroid* (Aviña-Padilla et al. 2018). Around 231 types of plant viruses and viroids have already been documented for seedborne transmission route. Tomato is consumed all across the world, and its seeds are a ground of numerous viruses and viroids like *Tomato streak virus* (TSV), *Pepino mosaic virus*, *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato mosaic virus*, *Tomato apical stunt viroid* (TASVd), *Arabis mosaic virus*, *Tomato black ring virus* and TYLCV-Israel

(TYLCV-IL) (Hadas et al. 2004; Antignus et al. 2007; Córdoba-Sellés et al. 2007; Kil et al. 2016).

Ilarviruses are also on the list of infecting various tree species. *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) are from the genus *Ilarvirus* (*Bromoviridae* family), contain tripartite genomic elements and are commonly found in mixed infections on stone fruit hosts. Both PNRSV and PDV are easily transmitted by seeds. There are also other means of their spreading, i.e. vegetative propagation, grafting and pollen grains (Kozieł et al. 2018; Marwal and Gaur 2017; Rubio et al. 2017; Umer et al. 2019). *Maize streak virus* from the genus *Mastrevirus* reaches to the maize seed through vascular puncture of the seeds. Further there are more viruses transmitted through seeds of Solanaceae crops like *Sweet potato leaf curl virus* (SPLCV) infecting sweet potato (*Ipomoea batatas*) and tomato (*Solanum lycopersicum*) and Fabaceae crops like *Mung bean yellow mosaic virus* (MBYMV) infecting black gram (*Vigna mungo*) (Redinbaugh 2003; Kim et al. 2015; Kothandaraman et al. 2016).

Circulifer species of arthropods are responsible for transmission of two famous viruses in Iran, i.e. *Beet curly top virus* (BCTV) and *Beet curly top Iran virus* (BCTIV). BCTV and BCTIV were identified in the seed extract of petunia through PCR and IC-PCR (Anabestani et al. 2017). Similar approaches were considered for *Pea seedborne mosaic virus* (PSbMV) which generally colonizes the leaves of pea plants (*Pisum sativum* L.) and its seeds (Fabre et al. 2014). Another in the list is the *Tobacco ringspot virus* (TRSV) which causes diseases in soybean seeds. Remarkably it was also found that TRSV-infected seeds have more protein content but lower oil content (Demski et al. 1999; Moyer et al. 1999; Groves et al. 2016).

To control viruses, seed lots can be treated with trisodium phosphate and dry heat (54–58 °C for 1–2 days) to reduce the virus loads of *Pepper mild mottle virus* (PMMoV) and *Maize dwarf mosaic virus*, respectively. Likewise TYLCV infection can be controlled by a number of ways and methods like the using of virus and whitefly free transplants. Insecticides, whitefly repellent, eliminating the infected plants were also successful (Blanc et al. 2011). *Maize dwarf mosaic virus* transmitted by aphids can be managed in corns by employing chemical insecticides or aphicides (Redinbaugh and Zambrano 2014).

To avoid the use of harmful pesticides on the environment and on beneficial insects, biocontrol methods are emerging with time as an alternative to it. Numerous beneficial microorganisms were identified as potent against plant viruses. Supernatant/filtrate extracts of *Aerobacter aerogenes*, *Aspergillus niger*, *Trichothecium roseum*, *Acinetobacter*, *Enterobacter asburiae* and *Neurospora sitophila* when applied significantly reduce the *Tobacco mosaic virus* (TMV) and *Tomato yellow leaf curl virus* (TYLCV) titre in the seeds. Efforts to control viral diseases in crop production include several types of antiviral compounds (Marwal et al. 2017). *Pseudomonas oleovorans* strain KBPF-004 revealed antiviral activity against seedborne tobamoviruses. Similar studies were conducted in seeds of watermelon and pepper against Cucumber green mottle mosaic virus (CGMMV) and Pepper mild mottle virus (PMMoV) (Lee et al. 2009; Li et al. 2016; Kim et al. 2017).

9.5 Concluding Remark

There is a continuous mounting of the global call for food/nourishment, along with the archetypes of food security, altogether seeking sustainable in agriculture and also thinking betterment for the environment. That's the purpose of food industry to challenge with sufficient quantities of food for humans, indeed economically inexpensive and safe (Lakra et al. 2019). Plants as whole are a favourable ground of microorganisms (harmful and beneficial) which intricate in the vertical transmission of microorganisms to next generation and also support a wonderful field of studies related to host-pathogen interactions (Sharma et al. 2019; Marwal et al. 2014d, e). The vertically transmitted seed beneficial microorganisms can endorse germination of seeds via production of growth hormones and also assist in the upsurge of nutrient accessibility in roots and shoots (Björk et al. 2019), whereas the harmful/pathogenic microorganisms (viruses, bacteria and fungi identified in the late 1800s to early 1900s) are responsible for devastating disease emergence and spread (Muthupandi et al. 2019; Sahu et al. 2014b, 2015; Singh et al. 2019). In this review we conclude that the management of seedborne pathogen and arthropods are a challenging job, where microbes are lasting by mining nutrientsfrom seeds, and the seeds itself are continuously battling with the outbreak of pathogen in order to survive. This scuffle for survival is a natural and an incessant process for both disease-causing microbes and seed species. Understanding this plant-microbe affiliation will help the agriculturists, farmer, policy makers, etc. in formulating better management practices for better crop health, better production and constant supply of food and even lessens the use of pesticides that create an environmental risk.

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References

Adam E, Bernhart M, Müller H, Winkler J, Berg G (2018) The Cucurbita pepo seed microbiome: genotype-specific composition and implications for breeding. Plant Soil 422:35. https://doi.org/10.1007/s11104-016-3113-9

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM (2016) Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol 14:e1002352

Al-Askar AA, Ghoneem KM, Rashad YM, Abdulkhair WM, Hafez EE, Shabana YM, Baka ZA (2014) Occurrence and distribution of tomato seed-borne mycoflora in Saudi Arabia and its correlation with the climatic variables. Microb Biotechnol 7(6):556–569

Al-Kassim MY, Monawar MN (2000) Seed-borne fungi of some vegetable seeds in Gazan province and their chemical control. Saudi J Biol Sci. 7:179–184

Anabestani A, Behjatnia S, Izadpanah K, Tabein S, Accotto G (2017) Seed transmission of beet curly top virus and beet curly top iran virus in a local cultivar of petunia in iran. Viruses 9 (10):299

Antignus Y, Lachman O, Pearlsman M (2007) Spread of Tomato apical stunt viroid (TASVd) in greenhouse tomato crops is associated with seed transmission and bumble bee activity. Plant Dis 91:47–50

Aviña-Padilla K, Rivera-Bustamante R, Kovalskaya N, Hammond R (2018) Pospiviroid infection of tomato regulates the expression of genes involved in flower and fruit development. Viruses 10(10):516

Bamisile BS, Dash CK, Akutse KS, Keppanan R, Wang L (2018) Fungal endophytes: beyond herbivore management. Front Microbiol 9:544

Bansil R, Turner BS (2006) Mucin structure, aggregation, physiological functions and biomedical applications. Curr Opin Colloid Interface Sci 11:164–170

Beckstead J, Meyer SE, Reinhart KO, Bergen KM, Dooley SR, Boekweg H (2014) Factors affecting host range in a generalist seed pathogen of semi-arid shrublands. Plant Ecol 215:427–440

Beckstead J, Meyer SE, Ishizuka TS, McEvoy KM, Coleman CE (2016) Lack of host specialization on winter annual grasses in the fungal seed bank pathogen *Pyrenophora semeniperda*. PLoS One 11(3):e0151058

Björk JR, Díez-Vives C, Astudillo-García C, Archie EA, Montoya JM (2019) Vertical transmission of sponge microbiota is inconsistent and unfaithful. Nat Ecol Evol 3(8):1172–1183

Blanc S, Uzest M, Drucker M (2011) New research horizons in vector-transmission of plant viruses. Curr Opin Microbiol 14:483–491

Boddy L, Büntgen U, Egli S, Gange AC, Heegaard E, Kirk PM et al (2014) Climate variation effects on fungal fruiting. Fungal Ecol 10:20–33. https://doi.org/10.1016/j.funeco.2013.10.006

Boudyach EH, Fatmi M, Akhayat O, Benizri E, Aoumar AAB (2010) Selection of antagonistic bacteria of *Clavibacter michiganensis* subsp. Michiganensis and evaluation of their efficiency against bacterial canker of tomato. Biocontrol Sci Tech 11:141–149

Burdman S, Walcott RR (2012) *Acidovorax citrulli*: generating basic and applied knowledge to tackle a global threat to the cucurbit industry. Mol Plant Pathol 13:805–815

Carter LL, Leslie JF, Webster RK (2008) Population structure of *Fusarium fujikuroi* from California rice and water grass. Phytopathology 98:992–998

Chen K, Wang Y, Zhang R, Zhang H, Gao C (2019) CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol 70:667–697

Cody WB, Scholthof HB (2019) Plant virus vectors 3.0: transitioning into synthetic genomics. Annu Rev Phytopathol 57:211–230

Collemare J, O'Connell R, Lebrun MH (2019) Non proteinaceous effectors: the terra incognita of plant–fungal interactions. New Phytol 223(2):590–596

Constable F, Chambers G, Penrose L, Daly A, Mackie J, Davis K, Rodoni B, Gibbs M (2019) Viroid-infected tomato and capsicum seed shipments to Australia. Viruses 11(2):98

Córdoba-Sellés MC, García-Rández A, Alfaro-Fernández A, Jordá-Gutiérrez C (2007) Seed transmission of Pepino mosaic virus and efficacy of tomato seed disinfection treatments. Plant Dis 91:1250–1254

Daferera DJ, Ziogas BN, Polissiou MG (2003) The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, Fusarium sp. and *Clavibacter michiganensis* subsp. michiganensis. Crop Prot 22:39–44

Darrasse A, Bureau C, Samson R, Morris C, Jacques M-A (2007) Contamination of bean seeds by *Xanthomonas axonopodis* pv. phaseoli associated with low bacterial densities in the phyllosphere under field and greenhouse conditions. Eur J Plant Pathol 119:203–215

Darrasse A, Darsonval A, Boureau T, Brisset MN, Durand K, Jacques MA (2010) Transmission of plant-pathogenic bacteria by nonhost seeds without induction of an associated defense reaction at emergence. Appl Environ Microbiol 76:6787–6796

Darsonval A et al (2008) The Type III secretion system of *Xanthomonas fuscans* subsp. fuscans is involved in the phyllosphere colonization process and in transmission to seeds of susceptible beans. Appl Environ Microbiol 74:2669–2678

Das S, Marwal A, Choudhary DK, Gupta VK, Gaur RK (2011) Mechanism of RNA interference (RNAi): current concept. Int Proc Chem Biol Environ Eng 9:240–245

Das AK, Marwal A, Sain D (2014) One-step green synthesis and characterization of flower extract mediated mercuric oxide (HgO) nanoparticles from *Callistemon viminalis*. Research and Reviews. J Pharmaceut Nanotechnol 2:25–28

Das AK, Marwal A, Pareek V, Joshi Y (2016) Surface engineering of magnetite nanoparticles by plant protein: investigation into magnetic properties. Nano Hyb Compos 11:38–44. Trans Tech Publications

Demski JW, Kuhn CW, Hartman GL (1999) Tobacco ringspot. In: Hartman GL, Sinclair JB, Rupe JC (eds) Compendium of soybean diseases, 4th edn. American Phytopathological Society Press, St. Paul, MN

Diniz Cavalcante R, Waléria Guerreiro L, Brainer Martins R, Tovar-Pedraza JM, Saraiva Cãmara MP (2014) Thiophanate-methyl sensitivity and fitness in *Lasiodiplodia theobromae* populations from papaya in Brazil. Eur J Plant Pathol 140:251–259

Divsalar M, Shakeri M, Khandan A (2014) Study on thermotherapy treatment effects on seed germination and vigor of tomato cultivars. Int J Plant Soil Sci 3:799–809

Dutta B, Gitaitis R, Smith S, Langston D Jr (2014) Interactions of seedborne bacterial pathogens with host and non-host plants in relation to seed infestation and seedling transmission. PLoS One 9(6):e99215

Fabre F, Moury B, Johansen EI, Simon V, Jacquemond M, Senoussi R (2014) Narrow bottlenecks affect pea seedborne mosaic virus populations during vertical seed transmission but not during leaf colonization. PLoS Pathog 10(1):e1003833

Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M (2015) Silver nanoparticles as potential antibacterial agents. Molecules 20:8856–8874

Frenkel O, Bornestein M, Shulhani R, Sharabani G, Sofer M, Abo-Moch F et al (2016) Secondary spread of *Clavibacter michiganensis* subsp. michiganensis in nurseries and the conditions leading to infection of tomato seedlings. Eur J Plant Pathol 144:569–579

Gaur RK, Prajapat R, Marwal A, Sahu A, Rathore MS (2011) First report of a begomovirus infecting *Mimosa pudica* in India. J Plant Pathol 93(4, Suppl):80

Gitaitis R, Walcott R (2007) The epidemiology and management of seedborne bacterial diseases. Annu Rev Phytopathol 45:371–397

Goggin DE, Emery RJ, Kurepin LV, Powles SB (2015) A potential role for endogenous microflora in dormancy release, cytokinin metabolism and the response to fluridone in Lolium rigidum seeds. Ann Bot 115:293–301

Groves C, German T, Dasgupta R, Mueller D, Smith DL (2016) Seed transmission of Soybean vein necrosis virus: the first Tospovirus implicated in seed transmission. PLoS One 11(1):e0147342

Hadas R et al (2004) Indexing system for Tomato mosaic virus (ToMV) in commercial tomato seed lots. Phytoparasitica 32:421–424

Han LL, Wang JT, Yang SH, Chen WF, Zhang LM, He JZ (2017) Temporal dynamics of fungal communities in soybean rhizosphere. J Soils Sediments 17:491–498

Hausbeck M, Bell J, Medina-Mora C, Podolsky R, Fulbright D (2000) Effect of bactericides on population sizes and spread of *Clavibacter michiganensis* subsp. michiganensis on tomatoes in the greenhouse and on disease development and crop yield in the field. Phytopathology 90:38–44

Hou YP, Chen YL, Qu XP, Wang JX, Zhou MG (2018) Effects of a novel SDHI fungicide pyraziflumid on the biology of the plant pathogenic fungi Bipolaris maydis. Pestic Biochem Physiol 149:20–25

Jimeânez O, Korpelainen H, Rojas A, Elomaa P, Valkonen JPT (2012) Genetic purity of the common bean seed generations (*Phaseolus vulgaris* L. cv. 'INTA ROJO') as tested with microsatellite markers. Seed Sci Technol 40:73–85

Johnson KL, Minsavage GV, Le T, Jones JB, Walcott RR (2011) Efficacy of a non-pathogenic *Acidovorax citrulli* strain as a biocontrol seed treatment for bacterial fruit blotch of cucurbits. Plant Dis 95:697–704

Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. PLoS One 6(6):e20396

Jones J, Jackson L, Balogh B, Obradovic A, Iriarte F, Momol M (2007) Bacteriophages for plant disease control. Annu Rev Phytopathol 45:245–262

Kang MH, Pengkit A, Choi K, Jeon SS, Choi HW, Shin DB, Choi EH, Uhm HS, Park G (2015) Differential inactivation of fungal spores in water and on seeds by ozone and arc discharge plasma. PLoS One 10(9):e0139263

Kannan M, Ismail I, Bunawan H (2018) Maize Dwarf mosaic virus: from genome to disease management. Viruses 10(9):492

Kasselaki AM, Goumas D, Tamm L, Fuchs J, Cooper J, Leifert C (2011) Effect of alternative strategies for the disinfection of tomato seed infected with bacterial canker (*Clavibacter michiganensis* subsp. michiganensis). NJAS – Wageningen J Life Sci 58:145–147

Kil EJ, Kim S, Lee YJ, Byun HS, Prak J, Seo H, Kim CS, Shim JK, Lee JH, Kim JK et al (2016) Tomato yellow leaf curl virus (TYLCV-IL): a seed-transmissible geminivirus in tomatoes. Sci Rep 6:19013

Kim J, Kil EJ, Kim S, Seo H, Byun HS, Park J, Chung MN, Kwak HR, Kim MK, Kim CS et al (2015) Seed transmission of Sweet potato leaf curl virus in sweet potato (*Ipomoea batatas*). Plant Pathol 64:1284–1291

Kim NG, Seo EY, Han SH, Gong JS, Park CN, Park HS, Domier LL, Hammond J, Lim HS (2017) *Pseudomonas oleovorans* Strain KBPF-004 Culture Supernatants Reduced Seed Transmission of Cucumber green mottle mosaic virus and Pepper mild mottle virus, and Remodeled Aggregation of 126 kDa and Subcellular Localization of Movement Protein of Pepper mild mottle virus. Plant Pathol J 33(4):393

Kitazaki S, Tanaka A, Hayashi N (2014) Sterilization of narrow tube inner surface using discharge plasma, ozone, and UV light irradiation. Vacuum 110:217–220

Kothandaraman SV, Devadason A, Ganesan MV (2016) Seed-borne nature of a begomovirus, Mung bean yellow mosaic virus in black gram. Appl Microbiol Biotechnol 100:1925–1933

Kozieł E, Otulak-Kozieł K, Bujarski J (2018) Ultrastructural analysis of Prune dwarf virus intercellular transport and pathogenesis. Int J Mol Sci 19(9):2570

Kwack Y, Kim KK, Hwang H, Chun C (2014) An ozone micro-bubble technique for seed sterilization in Alfalfa sprouts. Korean J Hortic Sci Technol 32:901–905

Lakra N, Mahmood S, Marwal A, Sudheep NM, Anwar K (2019) Bioengineered plants can be an alternative source of omega-3 fatty acids for human health. In: Plant and human health, vol 2. Springer, Cham, pp 361–382

Lamichhane JR, Dachbrodt-Saaydeh S, Kudsk P, Messean A (2016) Toward a reduced reliance on conventional pesticides in European agriculture. Plant Dis 100:10–24

Lebeis SL, Paredes SH, Lundberg DS, Breakfield N, Gehring J, McDonald M et al (2015) Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. Science 349 (6250):860–864

Lee SW, Han SW, Sririyanum M, Park CJ, Seo YS, Ronald PC (2009) A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity. Science 326:850–853

Li H, Ding X, Wang C, Ke H, Wu Z, Wang Y, Liu H, Guo J (2016) Control of Tomato yellow leaf curl virus disease by *Enterobacter asburiae* BQ9 as a result of priming plant resistance in tomatoes. Turk J Biol 40:150–159

Long RL, Gorecki MJ, Renton M, Scott JK, Colville L, Goggin DE, Commander LE, Westcott DA, Cherry H, Finch-Savage WE (2015) The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. Biol Rev 90(1):31–59

Ludmerszki E, Chounramany S, Oláh C, Kátay G, Rácz I, Almási A, Solti Á, Bélai I, Rudnóy S (2017) Protective role of S-methylmethionine-salicylate in maize plants infected with Maize dwarf mosaic virus. Eur J Plant Pathol 149:145–156

Mahmood S, Lakra N, Marwal A, Sudheep NM, Anwar K (2017) Crop genetic engineering: an approach to improve fungal resistance in plant system. In: Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 581–591

Makarovsky D, Fadeev L, Salam BB, Zelinger E, Matan O, Inbar J, Jurkevitch E, Gozin M, Burdman S (2018) Silver nanoparticles complexed with bovine submaxillary mucin possess strong antibacterial activity and protect against seedling infection. Appl Environ Microbiol 84 (4):e02212–e02217

Mancini V, Murolo S, Romanazzi G (2016) Diagnostic methods for detecting fungal pathogens on vegetable seeds. Plant Pathol 65:691–703

Manhas RK, Kaur T (2016) Biocontrol potential of *Streptomyces hydrogenans* strain DH16 toward *Alternaria brassicicola* to control damping off and black leaf spot of *Raphanus sativus*. Front Plant Sci 7:1869

Marcenaro D, Valkonen JP (2016) Seedborne pathogenic fungi in common bean (*Phaseolus vulgaris* cv. INTA Rojo) in Nicaragua. PLoS One 11(12):e0168662

Marwal A, Gaur RK (2017) Understanding functional genomics of PTGS silencing mechanisms for Tobacco streak virus and other Ilarviruses mediated by RNAi and VIGS. In: Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 489–499

Marwal A, Gaur RK (2019) Nanophytovirology: an emerging field for disease management. In: Plant pathology and management of plant diseases. IntechOpen, Rijeka

Marwal A, Meena S, Chandra S, Sharma A (2012a) In vitro study of antidermatophytic activity of mint. J Med Sci 12(6):182–187

Marwal A, Parjapat R, Sahu AK, Gaur RK (2012b) Current status of Geminivirus in India: RNAi technology, a challenging cure. Asian J Biol Sci 5:273–293

Marwal A, Prajapat R, Gaur RK (2014a) Estimating GC profile and restriction map analysis of three begomovirus components infecting an ornamental plant Marigold (*Tagetes patula*) in India. Int J Adv Res 2(1):773–779

Marwal A, Meena S, Chandra S, Sharma A (2014b) Review on dermatophytes highlighting the status at national and international level: a critical appraisal. J Adv Biotechnol 1(1)

Marwal A, Sahu AK, Gaur RK (2014c) New insights in the functional genomics of plants responding to abiotic stress. In: Molecular approaches in plant abiotic stress. CRC Press, Science Publishers, Taylor & Francis Group, Boca Raton, FL, pp 158–180

Marwal A, Sahu AK, Gaur RK (2014d) Transmission and host interaction of geminivirus in weeds. In: Plant virus–host interaction. Academic Press, New York, NY, pp 143–161

Marwal A, Verma SS, Trivedi S, Prajapat R (2014e) Reactive oxygen species: its effects on various diseases. J Adv Biotechnol 3(1):132–134. https://doi.org/10.24297/jbt.v3i1.1684

Marwal A, Mishra M, Sekhsaria C, Gaur RK (2017) Computational analysis and predicting ligand binding site in the rose leaf curl virus and its betasatellite proteins: a step forward for antiviral agent designing. In: Begomoviruses: occurrence and management in Asia and Africa. Springer, Singapore, pp 157–168

Marwal A, Verma RK, Khurana SMP, Gaur RK (2018) Molecular interactions between plant viruses and their biological vectors. Chapter 12. In: Gaur RK, Khurana SMP, Dorokhov Y (eds) Plant viruses: diversity, interaction and management. CRC Press, Taylor & Francis Group, Boca Raton, FL, pp 205–216. ISBN: 9781138061514

Marwal A, Kumar R, Verma RK, Mishra M, Gaur RK, Khurana SMP (2019a) Genomics and molecular mechanisms of plant's response to abiotic and biotic stresses. Chapter 6. In: Khurana SMP, Gaur RK (eds) Plant biotechnology: progress in genomic era. Springer Nature, Singapore. https://doi.org/10.1007/978-981-13-8499-8_6. ISBN: 9789811384981

Marwal A, Verma RK, Mishra M, Kumar R, Gaur RK (2019b) Mastreviruses in the African World: harbouring both monocot and dicot species. In: Geminiviruses. Springer, Cham, pp 85–102

Mehrotra RS, Agarwal A (2003) Plant pathology, 2nd edn. Teta McGraw-Hill Publishing Company, New Delhi

Meyer SE, Merrill KT, Beckstead J, Allen PS (2014) Indirect effects of a seed bank pathogen on the interactions between *Bromus tectorum* and two native perennial grasses. Oecologia 174:1401–1413

Montesinos L, Bundó M, Badosa E, San Segundo B, Coca M, Montesinos E (2017) Production of BP178, a derivative of the synthetic antibacterial peptide BP100, in the rice seed endosperm. BMC Plant Biol 17(1):63

Moyer JW, German T, Sherwood JL, Ullman D (1999) An update on tomato spotted wilt virus and related tospoviruses. APSnet Feat. https://doi.org/10.1094/APSnetFeatures-1999-0499

Muthupandi K, Marwal A, Tennyson J (2019) Genome of a known but distinct begomovirus associated with a novel satellite molecule infecting a new host bitter gourd (*Momordica charantia*). Biotech 9(6):247

Nandi M, Macdonald J, Liu P, Weselowski B, Yuan ZC (2018) *Clavibacter michiganensis* ssp. michiganensis: bacterial canker of tomato, molecular interactions and disease management. Mol Plant Pathol 19(8):2036–2050

Nehra C, Marwal A, Verma RK, Gaur RK (2019a) Molecular characterization of begomoviruses DNA-A and associated beta satellites with new host *Ocimum sanctum* in India. Proc Natl Acad Sci India Sec B Biol Sci 89(3):903–910

Nehra C, Marwal A, Verma RK, Mishra M, Sharma P, Gaur RK (2019b) Papaya yellow leaf curl virus: a newly identified begomovirus infecting *Carica papaya* L. from the Indian Subcontinent. J Hortic Sci Biotechnol 94(4):475–480

Nishikawa J, Kobayashi T, Shirata K, Chibana T, Natsuaki KT (2006) Seed-borne fungi detected on stored solanaceous berry seeds and their biological activities. J Gen Plant Pathol 72:305–313

Nowicki M, Nowakowska M, Niezgoda A, Kozik EU (2012) Alternaria black spot of crucifers: symptoms, importance of disease, and perspectives of resistance breeding. Veg Crops Res Bull 76:5–19

Ortigosa A, Gimenez-Ibanez S, Leonhardt N, Solano R (2019) Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of Sl JAZ 2. Plant Biotechnol J 17(3):665–673

Orton ES, Deller S, Brown JK (2011) *Mycosphaerella graminicola*: from genomics to disease control. Mol Plant Pathol 12(5):413–424

Paterson RM, Sariah M, Lima N (2013) How will climate change affect oil palm fungal diseases? Crop Prot 46:113–120

Popovski S, Celar FA (2013) The impact of environmental factors on the infection of cereals with Fusarium species and mycotoxin production – a review. Acta Agric Slov 101:105–116

Prajapat R, Marwal A, Gaur RK (2013a) Molecular and in silico characterization of two coat protein of weed associated begomovirus isolates. Sci Int 1(12):408–414

Prajapat R, Marwal A, Jha PN (2013b) *Erwinia carotovora* associated with potato: a critical appraisal with respect to Indian perspective. Int J Curr Microbiol App Sci 2(10):83–89

Prajapat R, Marwal A, Gaur RK (2014) Datura leaf curl betasatellite associated for the first time with *Datura inoxia* leaf curl syndrome in India. J Adv Biotechnol 1(1):1–7

Redinbaugh MG (2003) Transmission of Maize streak virus by vascular puncture inoculation with unit-length genomic DNA. J Virol Methods 109:95–98

Redinbaugh MG, Zambrano JL (2014) Control of virus diseases in maize. Adv Virus Res 90:391–429

Reingold V, Lachman O, Blaosov E, Dombrovsky A (2015) Seed disinfection treatments do not sufficiently eliminate the infectivity of Cucumber green mottle mosaic virus (CGMMV) on cucurbit seeds. Plant Pathol 64:245–255

Reis A, Boiteux LS (2010) Alternaria species infecting brassicaceae in the Brazilian neotropics: geographical distribution, host range and specificity. J Plant Pathol 92:661–668

Rioux RA, Shultz J, Garcia M, Willis DK, Casler M, Bonos S, Smith D, Kerns J (2014) *Sclerotinia homoeocarpa* overwinters in turfgrass and is present in commercial seed. PLoS One 9(10): e110897

Roberts EL, Mormile B, Adamchek C (2019) Fitness attributes of bacterial and fungal seed endophytes of tall fescue. In: Seed endophytes. Springer, Cham, pp 259–271

Rubio M, Martínez-Gómez P, Pallás V, Dicenta F, Sánchez-Navarro JA (2017) Molecular characterization of ilarvirus Prunus necrotic ring spot virus (PNRSV) and Prune dwarf virus (PDV) in

Spanish autochthonous almond cultivars. In: VII International Symposium on Almonds and Pistachios, vol 1219. International Society for Horticultural Science, Leuven, pp 353–356
Rybakova D, Mancinelli R, Wikström M, Birch-Jensen AS, Postma J, Ehlers RU, Goertz S, Berg G (2017) The structure of the *Brassica napus* seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome 5(1):104
Sahu AK, Nehra C, Marwal A, Gaur RK (2013) First report of a betasatellite associated with Radish leaf curl virus infecting new host *Cyamopsis tetragonoloba* (Guar bean) in India. Int J Adv Res 1 (8):740–743
Sahu AK, Marwal A, Nehra C, Choudhary DK, Sharma P, Gaur RK (2014a) RNAi mediated gene silencing against betasatellite associated with Croton yellow vein mosaic begomovirus. Mol Biol Rep 41(11):7631–7638
Sahu AK, Marwal A, Shahid MS, Nehra C, Gaur RK (2014b) First report of a begomovirus and associated betasatellite in *Rosa indica* and in India. Aust Plant Dis Notes 9(1):147
Sahu AK, Nehra C, Marwal A, Gaur RK (2015) First report of a begomovirus associated with betasatellites infecting spinach (*Spinacia oleracea*) in India. J Gen Plant Pathol 81(2):146–150
Schwartz HF, Steadman JR, Hall R, Forster RL (2005) Compendium of bean diseases. The American Phytopathological Society, St Paul, MN
Sen Y, van der Wolf J, Visser RGF, van Heusden S (2015) Bacterial canker of tomato: current knowledge of detection, management, resistance, and interactions. Plant Dis 99:4–13
Shade A, Jacques MA, Barret M (2017) Ecological patterns of seed microbiome diversity, transmission, and assembly. Curr Opin Microbiol 37:15–22
Sharma M, Ghosh R, Pande S (2015) Dry root rot [*Rhizoctonia bataticola* (Taub.) Butler]: an emerging disease of chickpea where do we stand? Arch Phytopathol Plant Protect 48:797–812
Sharma H, Sharma K, Koshal AK (2019) Role of microorganism for eco-friendly agriculture. J Plant Dev Sci 11(8):441–444
Simonsen AK, Dinnage R, Barrett LG, Prober SM, Thrall PH (2017) Symbiosis limits establishment of legumes outside their native range at a global scale. Nat Commun 8:14790
Singh RP, Dilworth AD (2009) Tomato chlorotic dwarf viroid in the ornamental plant *Vinca minor* and its transmission through tomato seed. Eur J Plant Pathol 123:111–116
Singh J, Bhatnagar SK, Tomar A (2019) Study on fungicidal effect of plant extracts on plant pathogenic fungi and the economy of extract preparation and efficacy in comparison to synthetic/chemical fungicides. J Appl Nat Sci 11(2):333–337
Sistani NR, Kaul HP, Desalegn G, Wienkoop S (2017) Rhizobium impacts on seed productivity, quality, and protection of *Pisum sativum* upon disease stress caused by didymella pinodes: phenotypic, proteomic, and metabolomic traits. Front Plant Sci 8:1961
Stergiopoulos I, Burg HA, Okmen B, Beenen HG, van Liere S, Kema GHJ, de Wit P (2010) Tomato Cf resistance proteins mediate recognition of cognate homologous effectors from fungi pathogenic on dicots and monocots. Proc Natl Acad Sci U S A 107:7610–7615
Sudheep NM, Marwal A, Lakra N, Anwar K, Mahmood S (2017) Fascinating fungal endophytes role and possible beneficial applications: an overview. In: Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp 255–273
Syed-Ab-Rahman SF, Xiao Y, Carvalhais LC, Ferguson BJ, Schenk PM (2019) Suppression of *Phytophthora capsici* infection and promotion of tomato growth by soil bacteria. Rhizosphere 9:72–75
Takaiwa F, Wakasa Y, Takagi H, Hiroi T (2015) Rice seed for delivery of vaccines to gut mucosal immune tissues. Plant Biotechnol J 13:1041–1055
Torres-Cortes G, Garcia BJ, Compant S, Rezki S, Jones P, Preveaux A, Briand M, Roulet A, Bouchez O, Jacobson D, Barret M (2019) Differences in resource use lead to coexistence of seed-transmitted microbial populations. Sci Rep 9(1):6648
Umer M, Liu J, You H, Xu C, Dong K, Luo N, Kong L, Li X, Hong N, Wang G, Fan X (2019) Genomic, morphological and biological traits of the viruses infecting major fruit trees. Viruses 11(6):515

Valeria M, Gianfranco R (2013) Seed treatments to control seedborne fungal pathogens of vegetable crops. Pest Manag Sci 70:860–868

Vázquez-de-Aldana BR, García-Ciudad A, García-Criado B, Vicente-Tavera S, Zabalgogeazcoa I (2013) Fungal endophyte (*Epichloë festucae*) alters the nutrient content of Festuca rubra regardless of water availability. PLoS One 8:e84539

Viswanathan A, Ghazoul J, Honwad G, Kumar NA, Bagchi R (2019) The effects of rainforest fragment area on the strength of plant–pathogen interactions. Biol Lett 15(1):20180493

Werner NA, Fulbright DW, Podolsky R, Bell J, Hausbeck MK (2002) Limiting populations and spread of *Clavibacter michiganensis* subsp. michiganensis on seedling tomatoes in the greenhouse. Plant Dis 86:535–542

Wightwick A, Walters R, Allinson G, Reichman S, Menzies N (2010) Environmental risks of fungicides used in horticultural production systems. Fungicides 14:274. https://doi.org/10.5772/13032

Wittmann J, Brancato C, Berendzen KW, Dreiseikelmann B (2016) Development of a tomato plant resistant to *Clavibacter michiganensis* using the endolysin gene of bacteriophage CMP1 as a transgene. Plant Pathol 65:496–450

Yan L, Hu B, Chen G, Zhao M, Walcott RR (2017) Further evidence of cucurbit host specificity among *Acidovorax citrulli* groups based on a detached melon fruit pathogenicity assay. Phytopathology 107(11):1305–1311

Zhang Y, Chen T, Nan Z, Christensen MJ (2019) Cattle grazing alters the interaction of seed-borne fungi and two foliar pathogens of *Leymus chinensis* in a meadow steppe. Eur J Plant Pathol 155:207–218

Weed Management in Sugarcane for Quality Seed Production

10

S. P. Singh, A. K. Tiwari, and A. A. Khan

Abstract

Weeds that compete for light, space and food with the main crop are responsible for crop losses, especially in sugarcane. The most common aspects in sugarcane cultivation are multiple cropping system, timely irrigation, fertilization and weed control. In case of weed control, earlier manual weeding and hoeing are effective but in recent times following the conventional methods has become difficult due to scarcity of labour and higher wages.

Another way to control weeds is by using tractor drawn adjustable cultivator in a wide row spacing planted sugarcane field. As the majority of the farmers have small lands, mechanization is not convenient. The chemical method of weed control is therefore easier, cheaper and quicker than weeding by hired labours.

Keywords

Weed · Sugarcane · Losses · Control

Weeds are undersized plants that compete for light, space, moisture and nutrients for their existence, and this directly influences the main crop. They can survive and reproduce in the face of many obstacles such as drought, frost and low temperature and can grow under varying soil and climatic conditions. They are strong competitors and may develop special adaptation that affect the survival of the plant (Klingman 1963). The weeds grown near plants beats propogate and

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disseminate the seed rhizones, stolons, roots, tubers etc. for example, purpule nutsedge (*Cyperus rotundus* L.) a native to Indian is known to be the worst weed (MeIntyre et al. 1980). Nutsedge is difficult to control because of its ability to proliferate rapidly by its system of tuber chains in which many tubers are dormant due to apical dominance (Richard and Arnold 1979). Sugarcane is an important agro-industrial crop commodity, with sugar being the only component of food consumed without exception in all countries of the world (Shuwell 1999). One of the most important reasons for low productivity of sugarcane is the spread of weeds, especially during the tillering stage, which of course must be free from crop-weed competition. Keeping this phase free of weeds has a positive impact in the early stimulation to form primary tillers and consequently increase in the number of millable canes (Almubark 2011). The opportunity for initial tillers emerging to become effective millable cane latter may be achieved either by reducing weed growth through the chemical herbicide (Richard 1995) or by stimulating and increasing the number of tillers using plant growth regulators (Hayamichi 1999). Losses due to weeds in sugarcane are sometimes exceedingly higher and beyond expectations. Reduction in cane yield due to weeds ranges from 40% to 60% (Chauhan and Srivastava 2002; Tomar et al. 2003; Singh et al. 2019a, b).

In recent past, the problem of weed has aggravated more and more with the introduction of high yielding sugarcane varieties. Multiple cropping increased fertilization, and irrigation control of weeds is one of the most important aspects in sugarcane cultivation. Conventional methods of weed control such as hoeing and manual weeding are the most effective in controlling weeds in sugarcane (Chauhan and Das 1990). However, at present the labourers are becoming fewer and consistently increasing higher wages paid to them further narrow down the profit of farmers. Besides this often in dumpy soils, it is difficult to operate hoeing and weeding manually (Singh et al. 2019c). The chemical method of weed control is therefore easier, cheaper and quicker than weeding by hired labours.

10.1 Weed Identification

Identification of weeds is as essential as the control of weeds itself because it aids in their effective control (Isom 1976). In sugarcane, weeds get optimum conditions for their rapid growth as the crop is grown in well-spaced moist and fertilized soil. Surveys for the identification of sugarcane weeds have been done time to time by many workers. Sadruddin and Srivastava (1983) identified 60 weed species in sugarcane crop in eastern Uttar Pradesh. Rathi and Tiwari (1982), while studying the weed flora in sugarcane intercropped with potato at Kanpur, reported that the winter, summer and rainy season weeds were predominantly: *Chenopodium album* L. (61.3%), *Heliotropium indicum* (46.3%) and *Trianthema monogyna* L. (44.8%), respectively. Johari and Singh (1990) surveyed and classified 45 species of weeds associated with sugarcane in Uttar Pradesh. Dicot weeds dominated in winter and summer while monocots in monsoon season (Singh et al. 2016, 2018).

10.2 Losses Due to Weeds

Like sugarcane, weeds also have the basic requirements for light, moisture, nutrient, space, etc., for their existence. Therefore, the yield is influenced by the density and competitive ability of the two for these inputs. Van Heemst (1958), while studying the competitive ability of 26 crop species with weeds, placed sugarcane at number five after wheat, peas, potato and soybean in descending order.

Singh and Verma (1969) and Agrawal et al. (1977) reported that the summer weeds cause maximum loss to the sugarcane crop. The weed competition during germination did not reduce the yield, but at tillering, it had the most deleterious effects. Singh et al. (1980) observed that sugarcane required weed-free condition up to 120 days. Rolin and Cristoffoleti (1982) reported this period from 30 to 90 days after planting. Blanco et al. (1982) found that controlling the weeds in sugarcane during the first and seconds after sprouting gave as good yield as controlled until second month, the yield was depressed.

Verma and Bhardwaj (1958), Mathur (1962) and Johari and Singh (1989) reported losses in cane yield due to weeds varied from 8% to 63%; Singh and Verma (1969) obtained 48.4% more yield of cane by controlling weeds in sugarcane. Sant and Mane (1970) reported that whereas weeds infestation in sugarcane did not affect germination, it affected tillering which, in turn, reduced the yield of cane by January plantings, respectively. Singh and Singh (1978) found that weeds during monsoon reduced the yield of cane by 35%. Johari et al. (2012) and Mishra et al. (2012) reported that the control of weeds with different combination of (Sempra 75 WG was sprayed with metribuzin 70 WP) herbicides significantly enhanced the yield of sugarcane. Singh et al. (2019a, b, c) also reported that UPH-114B (weedicide) @400 g gave highest yield. Almubarak et al. (2012) reported that the effect of herbicide 2,4-D, however, significantly increased the number of millable canes, which ultimately led to an increase in cane yield.

10.3 Methods of Weed Control

10.3.1 Cultural Methods

Khan and Ran (1956) reported cultural methods to be most effective for controlling weeds in sugarcane, leading to an improvement in yield and quality. Johari and Singh (1990) also obtained better yield of sugarcane by applying four hoeings; however, the quality was not affected. Gill (1963) observed that 17-cm-thick trash applied between the rows of sugarcane suppressed all types of weeds and conserved moisture. Effective weed control by trash mulch has been reported by many workers (Mathur 1960; Kirtikar et al. 1966; Negi and Yadav 1967). Babu et al. (1963) found suppressed weeds by the use of black polythene film.

Parihar and Mukherji (1969) reported that pre- and post-emergence application of 2,4-D was not better than hand weeding in terms of cane growth and yield. Saini et al. (1983) obtained 47.8% increased yield by three hoeing, while one hoeing

between pre- and post-emergence spray sensor produced 84.7% higher cane yield over check (unweeded). Agrawal et al. (1986) reported that preparatory operations are not very essential, if intercultural operations or 10-cm-thick trash mulch or pre- and post-emergence spray of 2.25 kg simonize per hectare is followed.

10.3.2 Chemical Methods

10.3.2.1 Use of Phenoxyacetic

Arkeri (1951, 1953, 1954), while working under Indian conditions, reported that most of the annual weeds in sugarcane could be controlled by spraying 1.68 kg 2,4-D per hectare after 4–5 and 20–28 days of planting. 2,4-D and MCPA were found to have no adverse effect on germination, tillering, growth and final yield of sugarcane (Thakur 1952; Thakur and Singh 1953). However, Narsinga Rao and Dutt (1954) observed that a pre-emergence application of 2,4-D had no injurious effect on cane but post-emergence sprays of 2,4-D affected the cane adversely. Mathur (1964) further observed that three spraying of 2,4-D, each with 3.65 kg/ha, had deleterious effect on cane crop and reduced the yield by about 7.41 MT/ha. Two sprays had no such deleterious effect.

Shrikhande and Mathur (1960) observed that one pre-emergence spray of 2,4-D @ 2.27 kg acid equivalent per hectare after 12–20 days of planting suppressed the growth of weeds for a period of 2 months. Mathur (1954) and Tendon and Mathur (1956) reported that 2,4-D sodium salt @ 1.18 kg/ha readily killed most of the dicot weeds. Daniel and Sugumaran (1976) observed that the pre- and post-emergence spray of 2,4-D sodium salt each @ 3 kg/ha effectively reduced the number of *Cyperus rotundus* L. by percent of nut grass up to 30 days.

Agrawal et al. (1977) reported that the application of 3 kg planotox per hectare as post-emergence spray was effective in controlling *Cyperus rotundus* L. Singh and Gupta (1977) obtained satisfactory weed control by post-emergence application of 3 kg 2,4-D sodium salt and 2.5 kg paraquat per ha after 15–22 days of planting. Dutta et al. (1965) noted that, from yield point of view, 2,4-D was superior to the unweeded control. Maximum improvement in sucrose% in juice was observed within the first week after the crop was sprayed. Thereafter, the difference between sucrose content in juice from the treated cane and untreated control gradually narrowed down (Mathur 1954).

Huinsigi and Dwarkanath (1978) opined that among the herbicide, 2,4-D continued to be the best option of chemical in view of its easy availability and low cost. Johari et al. (2012) reported that the control of weeds by Sampra 75% WG mixed with metribuzin 70 WP @ 7.50 g/ha at 30 DAP expressed higher weed control efficiency. Kadam et al. (2011) also observed that chemical weed control in sugarcane increases the yield of sugarcane.

10.3.2.2 Use of Triazines

Mathur and Singh (1965) found that atrazine applied @ 1.48 kg/ha as pre- and post-emergence was effective against both dicot and monocot weeds, except nutsedge. Mathur (1966) further reported that a pre-emergence application of 4.5 kg simonize per hectare followed by another post-emergence application at 2 months interval resulted in maximum control of nut grass and broad leaved weeds.

Negi and Yadav (1967) compared the effectiveness of a pre- and/or post-emergence atrazine at 1.1 and 2.2 kg with normal cultivation, trash mulching and a control treatment (no hoeing or weeding). The highest cane yield resulted from pre-emergence + post-emergence atrazine at a lower rate, significantly from each other.

Atrazine at 2 kg/ha applied as pre-emergence was as efficient as pre-emergence combined spray of 2,4-D and simonize (2 kg/ha each) in controlling annual grasses and non-grass weeds and the perennial nutsedge (Choudhary and Mani 1970). Mani and Gautam (1970) observed that a pre-emergence atrazine application had long duration effect, and Mani et al. (1973) using atrataf (50 WP) at 1–2 kg/ha, observed that chemical weed control was also advantageous in the uptake of nutrients by sugarcane besides controlling the weeds.

Choudhary and Mani (1972) observed that a pre-emergence application of atrazine of simonize + 2,4-D conserved moisture in the root zone by paralysing weed growth. Gill and Sidhu (1977) found that a mixture of atrazine and diuron in equal ratio applied as pre-emergence of weeds which controled the almost all the weeds and enhanced the yield of cane. Claus (1980) reported that the mixture of ametryne and atrazine successfully controlled the weeds in sugarcane without being phytotoxic to plants. Yeh et al. (1984) found that the pre-emergence application of primextra and atrazine (2:1) gave excellent control of weeds in sugarcane. These herbicides also killed some grasses, such as *Dactyloctenium aegyptium* and Cenchrus Sp., which were not controlled by atrazine alone.

Agrawal et al. (1977) found that the application of simazine + 2,4-D (2.24 kg/ha each) as pre and post emergence spray proved effective against weeds such as *Dactyloctenium aegyptium* L. *Andropogon annulatus* L., *Eragrostis poacoides* L., *Ipomoea pestigrides* L. and *Trianthema portulacastrum* L., while pre- and post-emergence of atrazine @ 2.25 kg/ha found effective against *Corchorus actitangula*, *Andropogon annulatus*, *Eragrostis tenella*, *Ipomoea pestigrides* L., *Trianthema portulacastrum* L., etc. Singh and Singh (1978) noted that simazine @ 2 kg ai/ha applied as pre-emergence proved very effective and gave statistically the same cane yield as recorded under weed-free treatment.

Narwal (1981) found that the application of atrazine produced higher weed killing efficiency (87.6%) which, in turn, increased the number of millable cane and cane yield. Chauhan et al. (1982) obtained higher yield under simazine @ 1.5 kg ai/ha, which recorded 94.6% more yield over unweeded control.

10.3.2.3 Use of Other Chemicals

Sant and Mane (1970), while working at padegoan, observed that 'Lasso' (2-Chloro-2′,6′-diethyl-N-(methoxymethyl)acetanilide) @ 2 kg ai/ha when applied as

pre-emergence in sugarcane controlled most of the grasses. Negi and Agnihotri (1972) also emphasized the usefulnnes of these chemical in controling the weeds. Lasso was further adjudged as one of the most effective herbicide at 14.2 I/ha dose, as it resulted in 62% germination, 76.64 tonnes/ha yield and 16.96% sucrose. Richardson (1977) reported that velpar alone at 0.68 kg ai/ha or in combination with diuron at 2.0 kg ai/ha, when applied as pre- and early post-emergence, had a residual effect on weed control, which lasted for about 4 months.

Ramamoorthi and Bhattacharya (1978) obtained effective control of almost all types of weeds with post-emergence application of Dosanex at 2.3 and 4 kg/ha on the 25th day of planting. Singh and Gupta (1977) screened several herbicides in sugarcane and observed that either atrazine or alachlor controlled weeds, resulting in increased tonnage and juice quality.

At U.P. Council of sugarcane Research, Shahjahanpur, pre- and post-emergence combined application of asulox-40 at 10 kg/ha + actril-D at 5 kg/ha was most effective against weeds resulting in increased tonnage at Golacentre followed by normal cultivation (Anon 1975–1976, 1976–1977). Post-emergence spray of asulox-40 at 10.1/ha gave the maximum sucrose percent followed by pre-emergence spray of actril-D at 61/ha (Anon 1977–1979). Combined spray of atrazine and karmex (2 kg/ha each) as pre- and post-emergence gave the maximum sucrose percent in juice (Anon 1977–1978).

Peng (1980), while screening the pre- and post-emergence herbicides, viz., bladed, isouron and DP x 410 (at 13.2%: 46.8% formulation of velpar and diuron), reported that they gave excellent control of weeds in sugarcane intercropped with peanuts. They have no injurious effect on crop plants. Alves et al. (1977) found that 0.6 kg/ha Tebuthiuron to have control over 85% without causing phytotoxicity to sugarcane plants. The yield was also increased.

Meintyre et al. (1980) reported the effective control of *Digitaria horizontalis*, the most dominant sugarcane weed of Mauritius, by pre-emergence application of Sencor or a mixture of diuron and Sencor. Pre-emergence application of diuron and Sencor @ 1.0 kg ai/ha and 2.0 kg ai/ha, respectively, controlled all types of weeds in sugarcane.

Yeh (1980) could control both dicot and monocot weeds more effectively by pre-emergence applications of 1–1.5 kg oxyfluorfen per hectare than its post-emergence application. Marcondes et al. (1980) recommended glyphosate for post-emergence control of *Cyperus rotundus* and *Cynodon dactylon* (L) pers in sugarcane. Johari and Singh (1989) reported that pre-emergence application of germination followed by 2,4-D alone or mixed with gramoxone as post-emergence controlled most of the weeds and increased the yield of sugarcane.

10.4 Suggestions for Future Studies

1. To control weeds in sugarcane, a comprehensive survey for identification of dominant weed flora has to be made. After that, the effective herbicide should be selected after thorough testing.
2. When sugarcane is intercropped with other economic crops, research experiments should be conducted to understand the herbicidal efficiency, selectivity and residual toxicity of chemical.
3. It is also essential to note the amount of herbicides in the juice of sugarcane and in the commercial product of intercrops grown with sugarcane.
4. The effect of continued use of herbicide in sugarcane on soil microflora should be studied.

References

Agrawal ML, Verma ID, Saxena MMS, Singh ND (1977) Weed control in sugarcane crop. Crop Sug 8:499–501

Agrawal ML, Malik JPS, Ali SA (1986) Effect of tillage on productivity in sugarcane. Ind Sug Crop J 12:5–8

Almubark NF (2011) Effect of methods of weed control and varieties in quality characters, sugar yield, and companion weeds of sugarcane. In: Twelth scientific congress. Technical Education Commission, Bagdad, pp 309–320

Almubarak NF, Calabi FT-AL, Al-Janaby A (2012) Effect of growth regulators and herbicide on growth and yield of sugarcane in central region of Iraq. Ind J Sugarcane Technol 27(02):79–82

Alves A, Buss A, Honda T, Pompeu RM, Silva SA, Vender C (1977) Effect of "Tibuthiuron" applied at full and half rates and reapplied at various rates for weed control in sugarcane. In: Proc Int Soc Sugarcane Technol, vol 16, pp 1123–1140

Anon (1975–1976) To study the effect of different herbicidal combinations on weed flora in sugarcane. Ann Rep U.P. Council of Sugarcane Research. U.P. Council of Sugarcane Research, Shahjahnapur, pp 77–78

Anon (1976–1977) To assess the utility of asulox-40 and actril-D weedicides. Ann Rep U.P. Council of Sugarcane Research. U.P. Council of Sugarcane Research, Shahjahnapur, p 82

Anon (1977–1978) To assess the utility of asulox-40 and actril-D weedicides. Ann Rep U.P. Council of Sugarcane Research. U.P. Council of Sugarcane Research, Shahjahnapur, pp 91–92

Anon (1977–1979) Control of winter and summer seasons, weed through cultural and chemical through autumn planted cane. UPCSR Ann Rep 98

Arkeri HR (1951) Preliminary studies on weed control with chemicals in sugarcane crops. In: Proc Deccan Sugar Technol Assoc India, vol 8, pp 55–61

Arkeri HR (1953) Effect of pre emergence treatment with chemicals used for control of weeds on growth and yield of sugarcane. Proc Deccan Sugar Technol 10:121–127

Arkeri HR (1954) Further studies on pre emergence treatment with 2, 4-D in sugarcane. Proc Deccan Sugar Technol Assoc India 11:192–195

Babu CN, Srinivasan K, Narayanan V (1963) Use of black polythene film as mulch for sugarcane crop. Ind J Sugarcane Res Dev 8:76–77

Blanco HG, Barbosa JC, Oliveira DA (1982) Competition of natural weed community with sugarcane (Saccharum spp.) with an eighteen months old cycle. In: Abstract of the 14th Brazilian Cong. Latin American Weed Association (ALAM), Campinas, pp 30–31

Chauhan DR, Behl KN, Bhatia NL (1982) Coop Sug 13:225–228

Chauhan RS, Das FC (1990) Weed management in sugarcane. Indian Sugar:231–233
Chauhan RS, Srivastava TK (2002) Influence of weed management practices on weed growth and yield of sugarcane. Indian J Weed Sci 34:318–319
Choudhary PN, Mani VS (1970) A field study on the efficiency of herbicide as affected by pre monsoon irrigation in sugarcane cultivation. Ind J Weed Sci 2(1):15–25
Choudhary PN, Mani VS (1972) Water use efficiency as influenced by chemical weed control in sugarcane cultivation. Proc Deccan Sugar Technol Assoc India 34:24–42
Claus R (1980) Contribution of chemical weed control in sugarcane in Ivory Coast. Anon Trop (French) 35:86–91. (Cpr. Int. Sugar Jour, 88: 304)
Daniel KV, Sugumaran T (1976) A study of the control of weeds in sugarcane fields. Coop Sug 7:482–486
Dutta DK, Sriram K, Medley GJ (1965) Ind Sugarcane J 9(4):226–228
Gill HS (1963) Influence of hot weather and transmission on the incidence of certain sugarcane borers and weeds. Ind J Sugarcane Res Dev 63:163–168
Gill HS, Sidhu MS (1977) Studies on chemical weed control on spring planted sugarcane. In: Proc Weed Sci Conf Hyderabad, D.P., 1971, p 20
Hayamichi Y (1999) Effect of plant growth retardant on growth and sugar accumulation of sugarcane. J Agric Sci. Tokyo-Nogoyo-Daigaku 43(4):197–201
Huinsigi GPN, Dwarkanath N (1978) Abs Weed Sci Conf Coimbatore, p 25
Isom WH (1976) Identification as related to effective weed control. In: Proc Ann California Weed Conf, vol 28, pp 9–15. (cf. weed abstracts, 1977, 26:84)
Johari D, Singh RG (1989) Relative efficacy of herbicides on weed control in sugarcane, Bharatiya Sugar, pp 65–68
Johari D, Singh RG (1990) Relative efficacy of chemical and cultural method of weed control in sugarcane. In: Paper presented in the National Seminar of Indian Society for plant Physiology, Bombay, 1989–1990, p 10
Johari D, Sharma ML, Singh SP, Priyanka S (2012) Relative efficacy of herbicides on weed control in sugarcane. In: 71th Annual Convention of STAI, pp 168–172
Kadam BS, Suryavanshi MM, Veer DM, Patil KB, More SM, Khot RB (2011) Influence of weed management practices in cane yield and weed incant city of ratoon crop of sugarcane (Co 86032). Coop Sug 42(7):41–46
Khan AR, Ran SK (1956) Studies in the relative efficacy of different forms and doses of nitrogen inn conjunction with and without herbicides on growth, yield and quality of sugarcane. In: Proc Int Soc Sugarcane Technol India, vol 9, pp 339–345
Kirtikar, Negi VS, Singh RP (1966) The use of simazine with 2, 4-D as weedicide in sugarcane field. In: Proc Sug Technol Assoc India, p A-43
Klingman GC (1963) Weed control as a science. John Wiley & Sons, New York, NY; London
Mani VS, Gautam KC (1970) Paper read at the Summer Institute in Weed Science held at I.A.R.I., New Delhi
Mani VS, Pandita ML, Gautam KC, Wandas B (1973) Weed hilling chemicals in potato cultivation. PANS 23:17–18
Marcondes DA, Brinholi O, Thacekfilho S (1980) Effect of glyphosate on sugarcane. In: Resumos 13th Congresso Brasieiro de Herbicides e Ervas Daninhas Bahia, pp 36–37. (cf. weed abstracts, 1981, 30:356)
Mathur PS (1954) Proc 2nd Bien Conf Sug Res Dev Workers, vol 8, Part III, pp 637–638
Mathur PS (1960) On the utility of track mulching in sugarcane field. Ind J Sugarcane Res Dev 4:4
Mathur PS (1962) Use of chemicals in sugarcane culture in India, chemicals as weedicides. Ind Sug 12:475–477
Mathur PS (1964) On the time of application of 2, 4-D for weed control in sugarcane field. Ind J Sugarcane Res Dev 2:21
Mathur PS (1966) Recent trends in weed control in sugarcane crop. In: Proc 2nd Weed Control Seminar, Hissar, pp 101–104

Mathur Ps, Singh RP (1965) Further observation on the use of simazine as weedicide in sugarcane field. Ind Sug 9:143–146
MeIntyre G, Pitcher J, Barbe C, Yerriah M (1980) The effect of various herbicidal treatments on Digitaria horizonntalis growing in cane plants. In: Proc Int Soc Sugarcane Technol, vol 17, pp 21–24
Mishra MM, Mishra SS, Mishra KN, Nayab PK (2012) Effect of different weed management practices on yield of sugarcane ratoon. Ind J Sugarcane Technol 27(02):76–78
Narsinga Rao G, Dutt NL (1954) Preliminary weed control studies at Coimbatore. In: Proc 8th Cong Ind Soc Sugarcane Technol, p 270
Narwal SS (1981) Coop Sug 13(4):181–184
Negi NS, Agnihotri BS (1972) Control of weeds in sugarcane by pre emergence herbicide Lasso. In: Proc Deccan Sugar Technol Assoc India, vol 24, pp 66–70
Negi NS, Yadav RK (1967) Atrazine as a herbicide in sugarcane fields. Ind Sug 4:31–34
Parihar NS, Mukherji N (1969) Control of weeds in sugarcane fields in Chambal Commanded Area of Rajasthan. Ind Sug 19:525–528
Peng SY (1980) Isouron and Velpar in a new criterion for field evaluation of herbicide. Taiwan Sugar 27:94–99
Ramamoorthi K, Bhattacharya K (1978) Use of dosanex-80 WP for weed control in sugarcane. In: Abs Weed Sci Conf Coimbatore, p 26
Rathi KS, Tiwari AN (1982) Studies on weed control in the companion cropping of potato with autumn planted sugarcane. Ind J Weed Sci 14:141–145
Richard LC, Arnold P (1979) Effects of humidity and moisture stress on glyphosate control of Cyperus rotundus L. Weed Res 19:241–246
Richard EP (1995) Sugarcane response to simulated fluazifop-butyl drift. Weed Sci 43(4):660–665
Richardson FE (1977) Velpar-A new herbicide for use in sugarcane. Proc Int Soc Sugarcane Technol 16:1341–1352
Rolin JC, Cristoffoleti JC (1982) Critical period for weed competition in sugarcane (Saccharum sp.). In: 14th Brazilian Cong on Herbicides and Herbaceous Weeds (SBHED) and 6th Cong of the Latin American Weed Assoc. (ALAM) Campinas, p 30
Sadruddin, Srivastava SN (1983) Survey of weed flora in sugarcane fields in eastern U.P. In: Proc Sug Technol Assoc India, vol 47, p 186
Saini SK, Singh PP, Negi PS, Kumar K (1983) Studies on weed control in autumn planted sugarcane. In: Proc Sug Technol Assoc India, vol 4, pp 122–124
Sant MV, Mane VS (1970) Weed control in sugarcane. In: Proc 4th Jt Conv S. T. A., D. S.T. A. and S. I. S. T. A
Shrikhande JG, Mathur PS (1960) Weed control in sugarcane fields. In: Proc 4th All India Conf Sugarcane Res Dev Workers, Indian Union, pp 209–220
Shuwell S (1999) Sugar industry in clean and unpolluted environment. Executives of Egyptian Sugar. 30th Annual Conference
Singh RP, Verma ID (1969) Studies on weed flora of sugarcane fields and their influence on growth and yield of the crop. Indian Sugar 19:268
Singh C, Gupta M (1977) Present position of chemical weed control in sugarcane. Proc Weed Sci Conf Hyderabad 209
Singh G, Pant PC, Bhan VM (1980) Studies of critical period of weed control in spring planted sugarcane. Indian J Weed Sci 12(2):35–41
Singh SP, Lal M, Shahi VK, Sharma BL (2016) Efficacy of dormex (*hydrogen cyanamide*) on germination, growth, yield and quality of sugarcane. Agrica 5:66–68
Singh G, Singh PP (1978) Chemical weed control in spring planted sugarcane. Indian J Sugar Technol 1:23–28
Singh SP, Singh RP, Tiwari AK (2018) Effect of plant growth regulators on yield contributing traits and quality of sugarcane. Agrica 7(1):60–65
Singh SP, Singh RP, Tiwari AK (2019a) Efficacy of weedicides on growth, yield and quality of sugarcane. Agrica 8(2):88–91

Singh SP, Singh P, Tiwari AK (2019b) Factors associated with sugarcane germination. Agrica 8 (2):57–60
Singh SP, Singh RP, Yadav SP, Singh SC (2019c) Effect of plant growth regulators on morphological characteristics, yield, and quality of sugarcane. Agrica 8(1):41–44
Tendon RK, Mathur PS (1956) On the use of 2, 4-D as weedicide in sugarcane fields. In: Proc Sug Technol Assoc India, vol 24: Part I, pp 139–144
Thakur C (1952) Effect of MCPA on nut grass. Agron J 44:589
Thakur C, Singh HN (1953) Chemical control of nut grass (Cyperus rotundus L.). Curr Sci 22:287
Tomar RK, Singh JP, Garg RN, Gupta VK, Sahoo RN, Arora RP (2003) Effect of weed management practices on weed growth and yield of wheat in rice based cropping system under varying levels of tillage. Ann Pl Prot Sci 11:123–128
Van Heemst HD (1958) The influence of weed competition on crop yield. Agric Syst 18:81–93
Verma RD, Bhardwaj RBL (1958) A better way of tracking weeds in sugarcane. Ind Firm 8:81–93
Yeh HJ (1980) Weed control by new weedicide Oxyfluorfen in sugarcane field. Taiwan Sugar 27:125–129
Yeh HJ, Jaung CB, Wang T (1984) Effect of Primextra on weed control and sugarcane growth. Rep Taiwan Sug Res Inst 106:1–6

Insect Pest Management for Healthy Seed Production

11

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Abstract

Seeds are affected by majority of insects throughout the crop age. The majority of insect pests causing losses to seeds belong to order Coleoptera along with some species of Lepidoptera, Psocoptera, Hymenoptera, and Diptera. Apart from this, some mites species (class Acari) are also associated with seed. However few mites are beneficial insects and called as biocontrol agents. Hence, in order to develop and implement seed inset pest (SIP) management programs, knowing pests and beneficial insects is important. Generally, the integrated pest management (IPM) is a systemic approach in which different control measures act together to suppress economic control of pests. Consequently, the present chapter provides an overview of the important SIP, and then, the different SIP control methods are mentioned to choose them for an integrated SIP management program. Accordingly, different control strategies are used in the management of the SIP including resistant plant varieties, healthy seeds, sanitation and exclusion, seed treatment (using chemical and botanical pesticides), physical treatments (aeration, extreme temperatures and relative humidity, impact and removal, ionizing radiation, modified atmospheres), biological control, mating disruption, trapping, and packaging, and sealing will be discussed. Based on the conditions and available facilities, an appropriate control method or integration of several control measures could be selected in the SIP control programs.

Keywords

Seed treatment · Biological control · Pesticide · Physical control · IPM

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11.1 Introduction

Insects belong to a group of commonly small animals named Arthropods. Of over a million various insect species that are present throughout the world, only a fairly small number have adapted themselves to feeding in the rather dry environment which we call seeds (Pedersen 1984; Pal et al. 2016). Seeds represented 22% of the 1105 commodities among the different forms of stored commodities (i.e., wheat, wheat flour, wheat bran, wheat germ, etc.). Seeds must be protected by various insect infestations from the harvesting to planting. Maintaining the seed quality is dependent on abiotic and biotic factors. Some of biotic factors are moisture, temperature, humidity, and storage conditions. Seeds may be stored in the different conditions for varying periods after the production process (Hagstrum and Subramanyam 2006). However, the seed quality may also be reduced by biotic factors (TeKrony 1976). The main pests that commonly affect the seed quality are disease-causing pathogens and insects. These pests may attack both seed and seedlings (Hefner et al. 2018). The mobility and the ability of many insect species to find and reproduce on many commodities, in particular seeds, throughout the marketing system make them especially difficult to manage (TeKrony 1976; Hagstrum and Subramanyam 2006). Up to now, a number of literature has been published on the various aspects of stored seed pests (e.g., Lieberman et al. 1961; Howe 1973; TeKrony 1976; Pedersen 1984; Rees 2004; Hagstrum and Subramanyam 2006, 2009; Mew and Hossain 2008; Hubert 2012; Hagstrum et al. 2013; Stejskal et al. 2014; Pal et al. 2016; FAO 2017). However, it is stated that 218 insect species have been found in stored wheat, 256 species have been found in stored maize, 167 species have been found in stored rice, and 505 species have been found in at least one stored cereal grain (barley, grain, oat, maize, rye, rice, sorghum, and wheat) (Hagstrum and Subramanyam 2009). Also, the seed samples (mostly vegetable and grass seeds) analyzed in Czech Republic laboratory showed 60% arthropod infestation (Kučerová and Horak 2004). Therefore, it is important to consider the seed insect pests (SIP) due to their high economic importance in the seed production and management.

In order to develop and implement SIP management programs, knowing which species of pests and beneficial insects are present is important. Also, information regarding their life histories, biology, ecology, and behavior are needed. A key gap in our knowledge is the availability of detailed management programs that consolidate all the existing methods in a unified program to manage SIP populations. Generally, the integrated pest management (IPM) is a systemic approach in which interacting components (mostly control measures) act together to maximize the advantages (mostly enhancing the yield) and minimize the disadvantages (mostly causing risk to human and environment) of pest management programs (Fathipour and Maleknia 2016). Hence, the present chapter provides an overview of the important SIP, and then the different SIP control methods are mentioned to choose them for integrated SIP management (ISM) programs.

11.2 Seed Insect Pests (SIP)

Many insect species have adapted to survive, feed, and reproduce on seeds. The majority of SIP belonging to order Coleoptera, but some species of Lepidoptera, Psocoptera, Hymenoptera, and Diptera cause economic losses when they reach high population (Rees 2004; Hagstrum and Subramanyam 2006). In addition, some mite species (class Acari) have been determined to be associated with seeds. The proportion of the important SIP species in different orders is shown in Fig. 11.1. In general, mite infestations are less likely to result in economic losses than insect infestations. However, mites can damage seed and reduce germination (Hubert 2012). The numbers of the important families of SIP in different groups are shown in Fig. 11.2. In congruence, the three most important families of stored-product pests ranking by number of species are reported the Tenebrionidae, Dermestidae, and Bruchidae by Hagstrum and Subramanyam (2009). Also, the numbers of the important SIP species in different families are shown in Fig. 11.3. In this chapter, we considered the Acari (mites) pest species in addition to insect species. The important SIP species with their host range and some details are shown in Table 11.1. With regard to an investigation performed in the Czech Republic, *Acarus siro* L. (Sarcoptiformes: Acaridae) has been a dominant mite pest from all aspects (frequency, abundance, and diversity infestation), followed by *Tyrophagus putrescentiae* (Schrank) (Sarcoptiformes: Acaridae), *Tarsonemus granarius* Lindquist (Acarina: Tarsonemidae), and *Lepidoglyphus destructor* (Acari: Astigmata). Also, *Lepinotus patruelis* Pearman (Psocoptera: Trogiidae) has been the most frequent psocid pest on seeds (Kučerová and Horak 2004). In addition, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae), *Stegobium paniceum* (L.) (Coleoptera: Anobiidae) and *Oryzaephilus*

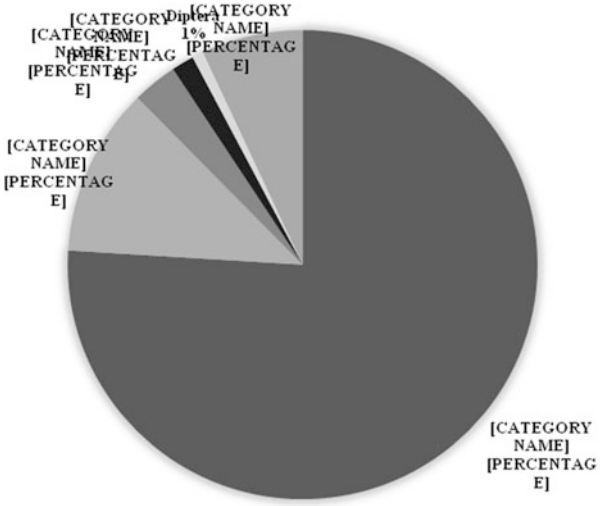

Fig. 11.1 The proportion of the important seed insect pest (SIP) species in different groups

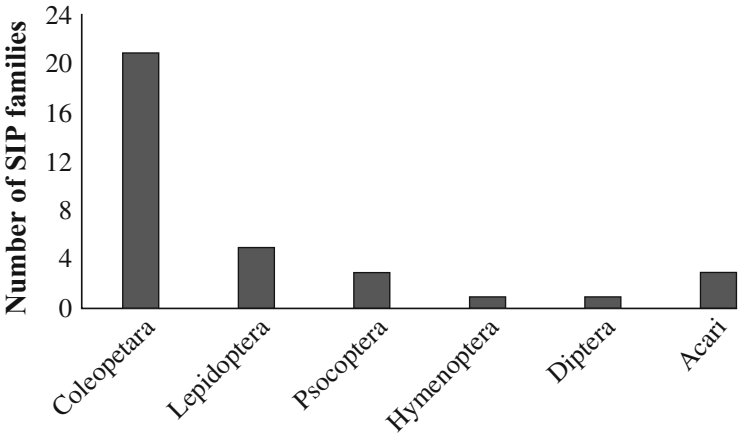

Fig. 11.2 The numbers of the important families of seed insect pests (SIP) in different groups

surinamensis (L.) (Coleoptera: Silvanidae) are reported as the five important pest of stored-product which attacked 246, 207, 177, 143, and 140 commodities, respectively (Hagstrum and Subramanyam 2009). On the other hand, many species of Hymenoptera and Hemiptera and mites are beneficial and called as biocontrol agents (Hubert 2012). *Cheyletus eruditus* (Schrank) (Acarina: Cheyletidae) has been a dominant predatory mite in 21 types of seed samples in the Czech Republic (Kučerová and Horak 2004). In the biological control section of this chapter, different biocontrol agents of SIP will be explained.

Generally, SIP same as the stored-product pests can be classified into different groups based on the feeding and injury patterns. Insect species that feed directly on a seed are mostly classified into primary pests, those that attack intact seeds, and secondary pests, which require the seed to be damaged before they can attack it. In reality, seeds accumulate damage among different processes including harvesting, handling, transporting, cleaning, and drying. Such a damage can notably increase the attack rate by secondary pests to exemplify *Tribolium* spp., *Oryzaephilus* spp., *Cryptolestes* spp., and psocids (*Liposcelis* spp.). The damage formerly caused by pre-harvest pests and by primary storage pests will also encourage secondary pests. Commonly, primary pests tend to have a more restricted host range than secondary pests. Many secondary pests to exemplify *Trogoderma* spp. and *Tribolium* spp. attack a very wide range of seeds (Rees 2004, 2007). However, in a classification based on the seed injury pattern, secondary pests are externally feeding species which cause damage largely to the seed germ. Conversely, primary pests are internally feeding species that causing hidden infestation inside the seed kernels. In some instances, they remove the endosperm while leaving the germ uninjured (Stejskal et al. 2014). Actually, each pest species requires a various level of damage before it can successfully breed on a seed. It depends on the species; for some pests the level is minimal, although for others it may be substantial (Rees 2007). Ignoring the pest group, every type of pests can be equally economically important for the

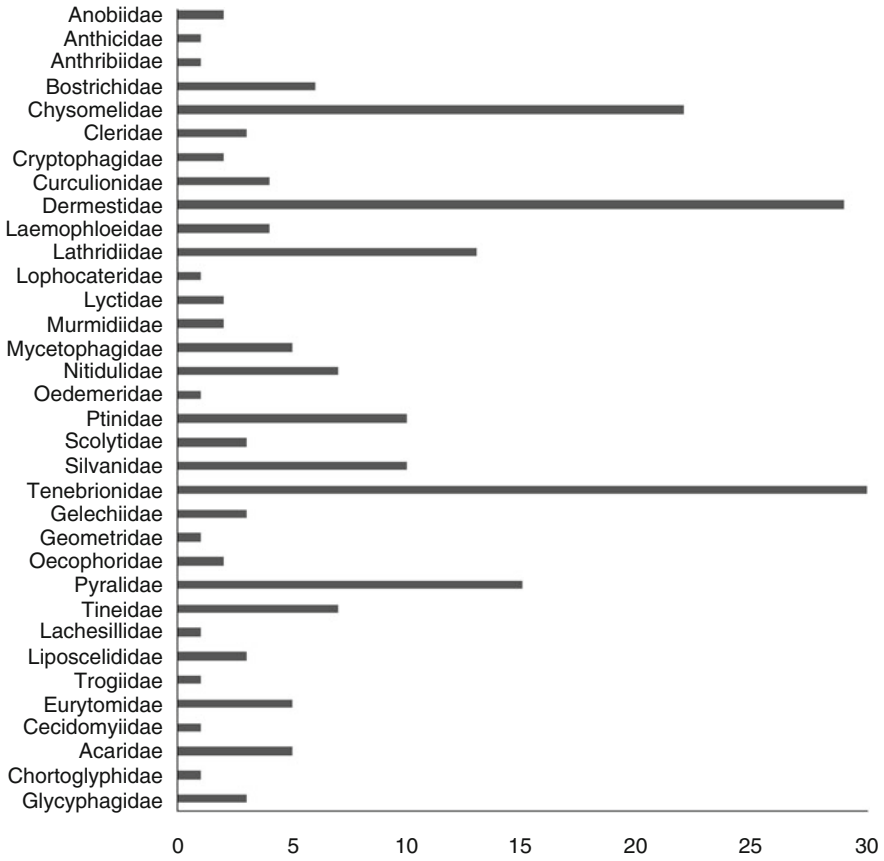

Fig. 11.3 The numbers of the important seed insect pest (SIP) species in different families

seed production depending on the situation (Stejskal et al. 2014). Temperature, relative humidity, and seed moisture content profoundly affect the rate at which insects can reproduce and hence become pests. The ability of an insect to multiply or survive is notably dependent upon the environmental temperature. Generally, SIP survive within a temperature range of 15–42 °C. No species completely covers this range, and each species varies in its tolerance to either the high or low extremes of this range (Rees 2004). In addition, seeds vary in their susceptibility to attack by the pests. Seeds contain toxins, and antifeedant chemicals tend to have fewer and more specific pests, such as the bruchid beetles that have evolved to attack pulses. In comparison, cereal seeds are attacked by a wide range of pest species.

Table 11.1 The important seed insect pests (SIP) classified by different orders

Family	Scientific name	Host seed	Distribution	Remark	Biology
Order Coleoptera					
Anobiidae	*Lasioderma serricorne*	Alfalfa, bagaruwa, beans, caraway, carrot, cauliflower, cereals, cotton, dill, eggplant, fennel, fenugreek, grass, juniper, lettuce, mango, mustard, oilseed, pulse, pumpkin, sesame, sunflower, tobacco	Cosmopolitan	Larvae internal feeders, adults do not feed, the beetle is well adopted to developing on tobacco	Ashworth (1993b)
	Stegobium paniceum	Polyphagous such as, bagaruwa, beet, caraway, carrot, cucumber, eggplant, fennel, fenugreek, grass, guava, lettuce, mustard, nasturtium	Cosmopolitan	Larvae internal feeders, adults do not feed, rapid development is at 30 °C and 80–95% r.h.	Lefkovitch (1967)
Anthicidae	*Omonadus floralis*	Grain, oilseed, pulse, other seeds	Cosmopolitan	Larvae soon appear	Hemp and Dettner (2003)
Anthribiidae	*Araecerus fasciculatus*	Polyphagous such as cotton, sesame, sunflower	Tropical and subtropical	Larvae feeding inside seed	Childers and Woodruff (1980)
Bostrichidae	*Dinoderus* spp.	Grain, oilseed, pulse, other seed	Cosmopolitan	Egg production increase with increasing relative humidity	Norhisham et al. (2013)
	Prostephanus truncatus	Maize, sorghum, wheat	South USA to northern South America, Africa	Larvae internal feeders producing lots of flour	Nansen and Meikle (2002)
	Rhyzopertha dominica	Annatto, barley, cantaloupe, caraway, cowpea, grass, maize, oat, pumpkin, rice, rye, sorghum, wheat, other seeds	Cosmopolitan	Larvae and adults internal feeders producing lots of flours, primary pest	Edde (2012)

Chysomelidae	*Acanthoscelides obtectus*	Bean, chickpea, grain, lentil, lupine, pulse, soybean	Cosmopolitan except east and south-east Asia	Larvae born directly into seed and develop concealed within seed	Pfaffenberger (1985)
	Bruchidius spp.	Bagaruwa, barseem, bean, chickpea, clover, cowpea, cowpea, fenugreek, grain, lentil, mint, pulse, soybean, other seeds	Cosmopolitan	Larvae on hatching bore directly into seed and develop only in seed	Southgate (1979)
	Bruchus spp.	Cotton, grain, lentil, pea, pulse, other seeds	Cosmopolitan	Larvae on hatching bore directly to seed	Southgate (1979)
	Callosobruchus spp.	Chickpea, cotton, cowpea, fenugreek, green gram, lentil, pulse	Cosmopolitan	Larvae on hatching bore directly into seed, adults do not feed	Adedire and Akinneye (2004)
	Caryedon serratus	Bagaruwa, cassia, cotton, grain, oilseed, pulse, sesame, other seeds	Cosmopolitan	Larvae on hatching bore directly into seed, develop completely within seed	Gagnepain and Rasplus (1989)
	Sennius morosus	Pulse	Central and North America Mexico	A larva consumes several seeds and pupate inside the seed	Center and Johnson (1973)
	Zabrotes subfasciatus	Grain, bean, cowpea, mung bean	Tropics, absent in Australasia	Larvae on hatching bore directly into seed, develop concealed within seed	Golob and Kilminster (1982)
Cleridae	*Necrobia rufipes*	Cotton, sesame, maize	Asia, Africa, North America, Europe	Larvae mobile, active external feeders	Simmons and Ellington (1925)
Cryptophagidae	*Cryptophagus* spp.	Grain, oilseed, pulse	Cosmopolitan	Larvae mobile, external feeders	Hinton and Stephens (1941)
Curculionidae	*Sitophilus granarius*	Grain, peanut, pulse, safflower, spinach, sunflower, other seeds	Cosmopolitan	Larvae immobile, develop concealed within single grain	Longstaff (1981)
	Sitophilus oryzae	Alfalfa, broad bean, caraway, cereal, cotton, safflower, sesame, watermelon, other seeds	Cosmopolitan	Same as *Sitophilus granarius*	Longstaff (1981)
	Sitophilus zeamais	Cereal, pulse, sesame, other seeds	Subtropical and tropics	Same as *Sitophilus granarius*	Longstaff (1981)

(continued)

Table 11.1 (continued)

Family	Scientific name	Host seed	Distribution	Remark	Biology
Dermestidae	*Anthrenus* spp.	Clover, cotton, grain, melon, oilseed, pulse, other seeds	Cosmopolitan	Adults live ca. 2–8 weeks	Beal (1998)
	Attagenus spp.	Alfalfa, beet, cotton, fennel, grain, grass, nut, oilseed, pulse, safflower, spinach, sunflower, tobacco, other seeds	Cosmopolitan	Larvae feeding, adults feed only on pollen and nectar	Veer et al. (1991)
	Dermestes spp.	Cotton, grain, oilseed, pulse, sesame, other seeds	Cosmopolitan	Both larva and adult feed on a seed	Zanetti et al. (2016)
	Orphinus fulvipes	Grain, oilseed, pulse, tobacco, other seed	Cosmopolitan		Háva (2015)
	Phradonoma nobile	Cotton, grain, oilseed	Africa, Asia and Middle east, Europe		Háva et al. (2011)
	Reesa vespulae	Broccoli, grain, lettuce, pepper, pulse, tomato, other seeds	Cosmopolitan	Larvae cause serious damage	Kadej et al. (2017)
	Thorictodes heydeni	Grain, oilseed, sesame, pulse, other seeds	Cosmopolitan		Chatterjl and Sarup (1959)
	Thylodrias contractus	Cantaloupe, grain, pulse, other seeds	Africa, Asia, Europe, North America, Oceania	Adults have great sexual dimorphism	Mertins (1981)
	Trogoderma granarium	Alfalfa, beet, cauliflower, coriander, cotton, cucumber, cumin, eggplant, fennel, flax, grain, grass, oilseed, pulse, pumpkin, sesame, squash, tomato, watermelon	Cosmopolitan	Larvae mobile, cast skins left in infested material	Hadaway (1956)
	Trogoderma inclusum	Alfalfa, burr clover, clover, cotton, cumin, fenugreek, grain, melon, oilseed, pulse, safflower, watermelon, other seeds	North America, Europa and Northern and central Asia	Same as *Trogoderma granarium*	Beal (1961)
	Trogoderma variabile	Alfalfa, beet, burnet, burr clover, cantaloupe, clover, cotton, cucumber, cumin, eggplant, flax, grain, lettuce, muskmelon, oilseed, onion, pepper, pumpkin, safflower, squash, sunflower, tomato, turnip, watermelon	Cosmopolitan	Same as *Trogoderma granarium*	Beal (1961)

Laemophloeidae	*Cryptolestes* spp.	Cereal, cotton, fennel, grass, maize, melon, oilseed, pulse, rice, sesame, sorghum, sunflower, watermelon, wheat,	Cosmopolitan	Larvae mobile, external feeders	Howe and Lefkovitch (1957)
Lathridiidae	*Cartodere* ssp.	Grain, oilseed, sunflower	Cosmopolitan	Temperature range 17–37 °C and r.h. above 40%	Hagstrum et al. (2013)
Lophocateridae	*Lophocateres pusillus*	Annatto, bean, cantaloupe, cotton, grain, oilseed, pulse, rice, sesame, watermelon, other seeds	Cosmopolitan	Larvae mobile, external feeders	Halstead (1968b)
Lyctidae	*Lyctus africanus*	Cotton, oilseed	Africa, Asia and Middle east, Europe, Oceania, North America	Cellulose-based diet give positive response to approve *L. africanus* growth	Rosel (1969)
Murmidiidae	*Murmidius ovalis*	Grain	Cosmopolitan	Eggs fail to hatch at 15 °C	Halstead (1968a)
Mycetophagidae	*Typhaea stercorea*	Barley, cotton, flax, grain, grass, maize, oilseed, pulse, rice, sesame, sorghum, sunflower, wheat, other seeds	Cosmopolitan	Larvae mobile, external feeders, eggs hatch at 17.5–30 °C	Jacob (1988)
Nitidulidae	*Carpophilus* spp.	Cereal, cotton, maize, oilseed, pulse, sesame, sorghum, sunflower, sunflower, other seeds	Cosmopolitan	Larvae mobile, external feeders	Williams et al. (1983)
	Stelidota geminate	Grain, oilseed	Africa, Asia, Europe, North America	Larvae 5 days feeding at 23 °C	Weber and Connell (1975)
Oedemeridae	*Nacerdes melanura*	Grain, pulse	Cosmopolitan	20 °C and 100% r.h. is optimize for rearing	Pitman et al. (2003)

(continued)

Table 11.1 (continued)

Family	Scientific name	Host seed	Distribution	Remark	Biology
Ptinidae	*Gibbium aequinoctiale*	Grain	Cosmopolitan	Larvae internal feeders, immobile when mature	Belles and Halstead (1985)
	Gibbium psylloides	Cotton, fennel, grain, oilseed, pulse, other seeds	Cosmopolitan	Same as *Gibbium aequinoctiale*	Belles and Halstead (1985)
	Mezium americanum	Grain, melon, oilseed, tobacco, other seeds	Cosmopolitan	Same as *Gibbium aequinoctiale*	Borowski and Sławski (2017)
	Ptinus spp.	Alfalfa, chamomile, cotton, grain, oilseed, pulse, spinach, sunflower, tobacco, wheat, other seeds	Cosmopolitan	Larvae internal feeders, immobile when mature, adults feed on seeds	Bentley (1944) Howe and Burges (1951)
Scolytidae	*Hypothenemus hampei*	Grain, coffee, pulse	Cosmopolitan	Larvae and adults feeders	Damon (2000)
	Pagiocerus frontalis	Avocado, grain, other seeds	Central, North, and South America	Higher number of emerged adults observe at 20 °C and 80% r.h.	Eidt-Wendt and Schulz (1990)
Silvanidae	*Ahasverus advena*	Cereal, cotton, fennel, grass, groundnut, oilseed, peanut, pulse, safflower, sesame, sunflower, other seeds	Cosmopolitan	Larvae mobile, external feeders, optimum conditions is 90% r.h. and 25 °C	Jacob (1996)
	Cathartus quadricollis	Annatto, cereal, especially maize, cowpea, grain, melon, oilseed, pulse, sunflower, other seeds	Tropics, absent from Australia	Larvae mobile, external feeders	Allotey and Morris (1993)
	Nausibius clavicornis	Grain, oilseed	Cosmopolitan		Breese and Wise (1959)
	Oryzaephilus mercator	Cereal, coriander, cotton, fennel, mango, melon, oilseed, pulse, sesame, sunflower, watermelon, other seeds	Cosmopolitan	Larvae mobile, external feeders, 74% r.h. is optimum to growth	Howe (1956) Arbogast (1976)
	Oryzaephilus surinamensis	Alfalfa, annatto, caraway, cereal, cotton, oilseed, pulse, safflower, sunflower, other seeds	Cosmopolitan, cold tolerant	Larvae mobile, external feeders, 74% r.h. is optimum to growth	Howe (1956) Arbogast (1976)

Tenebrionidae	*Alphitobius* spp.	Cereal, cotton, flax, grass, groundnut, oilseed, other seeds	Cosmopolitan	Larvae mobile, external feeders	
	Cynaeus angustus	Cereal, maize	Mexico, USA, Canada, Europe	Larvae mobile, external feeders	Dunkel et al. (1982)
	Gnatocerus cornutus	Beet, cereal, oilseed, pulse, other seeds	Cosmopolitan	Larvae mobile, external feeders	Okada et al. (2015)
	Gnatocerus maxillosis	Grain, oilseed, pulse, pumpkin, other seeds	Tropical	Larvae mobile, external feeders	
	Latheticus oryzae	Bagaruwa, cereal, cotton, oilseed, pulse, other seeds	Cosmopolitan	Larvae mobile, external feeders	Hafeez and Chapman (1966)
	Palorus subdepressus	Black sesame, caraway, cereal, cotton, flax, oilseed, pulse	Cosmopolitan	Larvae mobile, external feeders	Halstead (1967)
	Somaticus spp.	Maize	South Africa	Larvae feed on maize	Drinkwater et al. (1990)
	Tenebrio molitor	Barley, cotton, pulse, wheat, other seeds	Cosmopolitan	Adults relatively short live	Cotton (1927)
	Tenebrio obscurus	Cotton, grain, oilseed, pulse, other seeds	Cosmopolitan	Same as *T. molitor*	Cotton (1927)
	Tenebroides mauritanicus	Annatto, cereal, cotton, oilseed, pulse, sesame, sunflower, other seeds	Cosmopolitan	Larvae mobile, external feeders, adults feeding	Coskuncu and Kovanci (2005)
	Tribolium spp.	Polyphagous such as cotton, dill, eggplant, fennel, flax, grass, mango, mustard, okra, radish, safflower, sesame, sunflower, tomato, tobacco	Cosmopolitan	Larvae mobile, external feeders, adults feeding	Sokoloff (1977)
Order Lepidoptera					
Gelechiidae	*Sitotroga cerealella*	Cereal, cotton, lettuce, oilseed, pulse, radish, other seeds	Cosmopolitan	Larvae—excavate cavity in grain, remain concealed there	Consoli and Amaral Filho (1995)

(continued)

Table 11.1 (continued)

Family	Scientific name	Host seed	Distribution	Remark	Biology
Geometridae	*Idaea inquinata*	Grain, pulse	West-Palearctic species, Africa, Europe, Asia	Highest number of hatched eggs observe at 29 °C and 70% r.h.	Limonta and Locatelli (2015)
Oecophoridae	*Endrosis sarcitrella*	Grain, oilseed, pulse, other seed	Cosmopolitan	Female-biased sex ratio	Woodroffe (1951a)
Oecophoridae	*Hofmannophila pseudospretella*	Cantaloupe, cauliflower, grain, oilseed, pulse, other seeds	Asia and Middle east, Europe, Oceania, North America, and Mexico	The duration of the feeding larval stage is 71 days at 25 °C and 90 r.h.	Woodroffe (1951b)
Pyralidae	*Achroia grisella*	Grain, tobacco	Cosmopolitan	Larvae also pest in beehives, feed on pollen	Uçkan et al. (2007)
	Cadra cautella	Cereal, cotton, oilseed, pulse, sesame, sunflower, mango, other seeds	Cosmopolitan	Larvae—external feeders, produce silk webbing, adults do not feed	Allotey and Goswami (1990)
	Corcyra cephalonica	Cabbage, cauliflower, cotton, eggplant, flax, grain, oilseed, radish, tomato, turnip	Cosmopolitan	Larvae—external feeders, produce lots of silk webbing, adults do not feed	Allotey and Azalekor (2000)
	Ephestia elutella	Cereal, cotton, oilseed, sugar beet, sunflower	Cosmopolitan	Same as *Cadra cautella*	Ashworth (1993a)
	Ephestia kuehniella	Cereal, cotton, hemp, oilseed, pulse, safflower, sesame, other seeds	Cosmopolitan	Same as *Cadra cautella*	Jacob and Cox (1977)
	Etiella zinckenella	Chickpea, cowpea, grain, lentil, pulse	Cosmopolitan	Seeds partially or entirely eaten by larvae	Edmonds et al. (2000)
	Euzophera sagax	Tea	Africa		Benjamin (1968)
	Mussidia nigrivenella	Polyphagous such as cotton, maize,	Tropical regions, Africa, Europe		Sétamou et al. (1999)

	Plodia interpunctella	Cantaloupe, caraway, cereal, clover, cotton, cucumber, hemp, juniper, lettuce, lupine, mustard, oilseed, pepper, pulse, pumpkin, safflower, sesame, squash, sunflower, turnip, watermelon, other seeds	Cosmopolitan	Larvae—external feeders, produce silk webbing, adults do not feed	Mohandass et al. (2007)
	Pyralis farinalis	Cotton, grain, oilseed, pulse, sunflower, other seeds	Africa, Asia, Europe, Oceania, North and South America	Optimum conditions for larvae are 25 °C and 88% r.h.	Shang et al. (2013)
Tineidae	*Tineola bisselliella*	Grain, lettuce, other seeds	Cosmopolitan	At 80% r.h. and 30 °C, about 100 eggs are laid per mated female	Cox and Pinniger (2007)
Order Psocoptera					
Lachesillidae	*Lachesilla pedicularia*	Beet, caraway, grain	Cosmopolitan		Sommerman (1946)
Liposcelididae	*Liposcelis* spp.	Cereal, coriander, cotton, oilseed	Cosmopolitan	Adult and nymphs feeders, long-lived	Broadhead and Hobby (1944)
Trogiidae	*Lepinotus reticulatus*	Beet, coriander, grass, maize, rice, squash	North America, China	Adult and nymphs feeders, short-lived	Opit and Throne (2014)
Order Hymenoptera					
Eurytomidae	*Bruchophagus roddi*	Alfalfa, carrot, clover	Cosmopolitan	Larvae feed within the seed for 8–12 days	Strong (1962)
Eurytomidae	*Systole albipennis*	Cumin, coriander, dill, fennel, lovage, parsley	Asia, Europe, South America	The larvae infest the seeds at both field and storage stages	Meena et al. (2015)
Order Diptera					
Cecidomyiidae	*Contarinia wattsi*	Big bluestem	Widespread	The total loss of larvae was close to 40%	Carter et al. (1988)

(continued)

Table 11.1 (continued)

Family	Scientific name	Host seed	Distribution	Remark	Biology
Class Acari					
Acaridae	*Acarus farris*	Barley, cereal, grain, grass, oat, radish, soybean, sugar beet, wheat	Widespread	Immature stages are susceptible to low and high temperatures (7–29.7 °C)	Sánchez-Ramos et al. (2007)
	Acarus siro	Polyphagous such as bean, beet, clover, cotton, grain, grass, hemp, kale, lentil, lettuce, lupine, maize, oilseed, onion, pea, poppy, radish, soybean, spinach, sugar beet, sunflower	Cosmopolitan	Adults and nymphs are external feeding	CAB International (2005)
	Aleuroglyphus ovatus	Barley, bean, grain, lentil, maize, rice, sunflower, wheat	Widespread	Optimum temperature to breed is 28 °C	Xia et al. (2009)
	Tyrophagus longior	Barley, flax, grain, grass, rape, sugar beet, wheat	Cosmopolitan	Adults and nymphs are external feeding	CAB International (2005)
	Tyrophagus putrescentiae	Barley, beet, carrot, cotton, flax, grain, grass, lettuce, onion, parsnip, poppy, radish, rape, rice, soybean, sugar beet, sunflower, tobacco	Cosmopolitan	The most rapid development take place at 32.2 °C	Barker (1967)
Chortoglyphidae	*Chortoglyphus arcuatus*	Barley, cereal, clover, grass, lentil, maize, poppy, rice, sugar beet, wheat	Widespread	The mite is also frequently in mattress dust	CAB International (2005)
Glycyphagidae	*Glycyphagus domesticus*	Barley, bean, cereal, flax, grain, grass, hemp, oat, poppy, sugar beet, sunflower, wheat	Cosmopolitan	Humidity has a large controlling influence in mite life (above 70%)	Hora (1934)
	Gohieria fusca	Barley, flax, grain, grass, hemp, poppy, rice, sugar beet, wheat	Widespread	Female deposits 111.5 eggs on crushed wheat	Taha et al. (2010)
	Lepidoglyphus destructor	Barley, beet, cereal, cotton, cotton, flax, grass, hemp, kale, lettuce, maize, onion, poppy, radish, rice, sorghum, sugar beet, sunflower	Cosmopolitan	High r.h. in combination with temperatures at about 20 °C will lead to the highest breeding	Danielsen et al. (2004)

11.3 Seed Insect Pest (SIP) Control Strategies

Seed are commonly stored in silos, flat stores, as well as in small bulk containers or different packages (boxes, large, and small bags) which such a storage inherently includes a high risk of pest infestation. Also, it is documented a notably higher infestation of stored wheat and barley by arthropod pests in flat stores than silos in the Czech Republic (Stejskal et al. 2014). However, in order to reduce the damage of pests, selecting an appropriate control method or integration of several control methods is a vital step in the SIP control programs.

Currently, use of chemical pesticides is the main component employed for insect pest management. However, the intensive and indiscriminate use of chemical insecticides has resulted in insect resistance to many common pesticides. Additionally, the overuse of chemical pesticides has destroyed the non-target organism in particular natural enemy's population (Fathipour and Maleknia 2016; Tazerouni et al. 2019a). Hence, it is important to consider the biocompatible control methods for the SIP management programs (Pal et al. 2016). Different control measures are used in the management of the SIP. So, integration of various control methods can improve the management of the SIP. The Food and Agriculture Organization of the United Nations (FAO) has divided the options that may be considered when determining SIP risk management into three sections including pre-planting, pre-harvesting, and harvesting and post-harvest handling (FAO 2017). The processes and their subdivisions are presented in Fig. 11.4. Then, the important control methods of the SIP are discussed as follows.

11.3.1 Resistant Plant Varieties

Host plant resistance (HPR) is an economical and environmental method of pest control. This is one of the most successful techniques for suppressing pest populations or damage (Tazerouni et al. 2019a). Modern breeding programs may produce plant varieties that have a level of resistance to pests, which may include resistance to regulated pests (FAO 2017). The method is innate quality of the plant that renders it unsuitable as food or shelter for insect pests either through antibiosis or non-preference (antixenosis) and confers on the plant the ability to withstand without loss in yield (tolerance). Resistance genes may be effective against all or some races, strains, biotypes, or pathotypes of the targeted pest, but the emergence of new races, strains, biotypes, or pathotypes may affect the level of resistance (Jackai and Singh 1983; FAO 2017; Tazerouni et al. 2019a). Host plant resistance to insect pests can be inherited either vertically (controlled by a single gene) or horizontally (controlled by many genes). Since the large number of genes involved, it is much more difficult to breed cultivars with horizontal resistance to insect pests (Fathipour and Mirhosseini 2017).

To characterize which mechanism is active in particular cases, experiments can be planned to determine the life history parameters of the pest among different varieties as well as their nutritional indices and susceptibility index (Dobie's formula) (Dobie

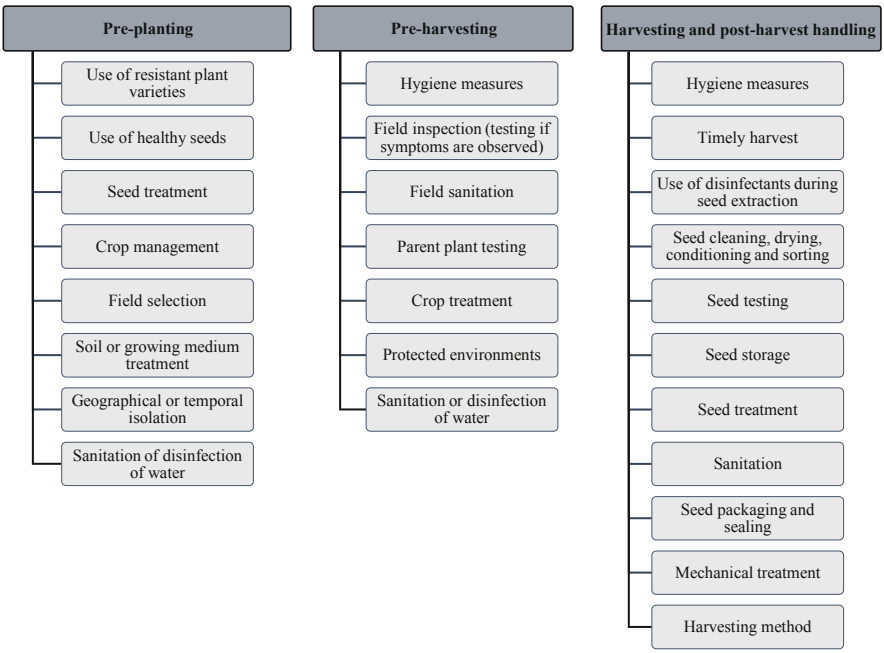

Fig. 11.4 The various processes and their subdivisions that may be considered when determining seed insect pest (SIP) control management (FAO 2017)

1974). A considerable amount of investigations has been published on the resistance plant varieties to various SIP, as reviewed in Table 11.2. In these experiments, different biological parameters including developmental period and mortality, adult mortality, sex ratio, and number of F1 progeny were measured for the pests grown on each seed variety. Also, a number of researchers have determined the susceptibility of different seeds to the pest damage based on the demographic parameters (e.g., Golizadeh and Abedi 2016; Karimi-Pormehr et al. 2018; Namin et al. 2018). All the equations of demographic parameters and life table construction are described by Birch (1948) and Carey (1993, 2001).

The literature review demonstrated that various methods are utilized by researchers to evaluate the levels of seed resistance to the SIP. For instance, Ahmed et al. (1989) stated that the number of emergence holes is a better indicator of seed resistance than the number of eggs present on the chickpea seed (*Cicer arietinum* L.) for *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). Also, the procedure to determine the Dobie index of susceptibility could be shortened by replacing progeny yield with the number of eggs laid per sample and days to mid-emergence with days to first emergence (Urrelo et al. 1990). In addition, the rapid analysis of phenolic content by quantitative imaging could provide a reasonable prediction of susceptibility of maize genotypes that may assist breeders in the selection of less susceptible cultivars (Arnason et al. 1992; Ramputh et al. 1999).

Table 11.2 The resistant and susceptible varieties of host plants to various seed insect pests (SIP)

Host seed	Pest scientific name	Resistant variety	Susceptible variety	Reference
Maize	*Sitophilus zeamais*	Aseda, TZE-Y POP STR	Aburohemaa, Obaatanpa, Omankwa	Acheampong et al. (2019)
		EV8430DMRSR, Kandjerendjere		Nhamucho et al. (2017)
		2000SYNEE-WSTR, TZBRELD3C5		Nwosu (2016)
		ACR.97 TZL COMP.1-W, TZL COMP.4C2, ADV. NCRESTR, BG 97 TZE COMP.3XL	Akparike, Bende, Ogbia muno	Zakka et al. (2015)
		Poza-Rica 8761, S 8662	Tlalti 8767, Ejura 7843	Meikle et al. (1998)
		Sinaloa 35	Cacahuacintle	Arnason et al. (1992)
	Prostephanus truncatus	P8c3	CML244 × CML349	Bergvinson and García-Lara (2011)
		Poza-Rica 8761, S 8662	Tlalti 8767, Ejura 7843	Meikle et al. (1998)
		Ilogna 8032	Mexico 212	Arnason et al. (1992)
	Plodia interpunctella	A509 × ND203, A508 × W33		Abdel-Rahman et al. (1968)
Cowpea	*Callosobruchus maculatus*	VBRL 02, VSNA 02	VRDR 02, VRBC 02	Lopes et al. (2018)
		TVu-11953		Amusa et al. (2018)
		24-125B-3, IT89KD-288	CB-3, CB-5, CB-46, UC-27	Mbata et al. (2009)
		IT84S-2246-4		Appleby and Credland (2004)
		Maiduguri-A, Maiduguri-B, TVu 2027	Ife Brown	Jackai and Asante (2003)
		IT84S-275-9, IT85F-2205		Ofuya and Credland (1995)
		TVu 2027, IT81D-1064, IT81D-1157, IT86-498	TVu 310, TVu 2896, TVu 9944, TVu 9836	Mbata (1993)

(continued)

Table 11.2 (continued)

Host seed	Pest scientific name	Resistant variety	Susceptible variety	Reference
		TVu 2027	Californian	Dick and Credland (1986)
	Callosobruchus subinnotatus	TVu 2027, IT81D-1064, IT81D-1157, IT86-498	TVu 18, TVu 36, TVu 2896, IT81D-1020	Mbata (1993)
Sorghum	*Sitophilus oryzae*	LTR108	654	Zhai et al. (2016)
		Tuba, Abula Gorad	Wogere, Merabete	Ramputh et al. (1999)
		Nigerian cultivar (CSH5)	Nigerian cultivar (FD1)	Adetunji (1988)
		Tanzanian cultivar (336)	Tanzanian cultivar (303)	Adetunji (1988)
Chickpea	*Callosobruchus maculatus*	CM-68	ILC-varieties	Ahmed et al. (1989)
		G109-1		Raina (1971)
	Callosobruchus analis	G109-1		Raina (1971)
	Callosobruchus chinensis	G109-1		Raina (1971)
Common bean	*Zabrotes subfasciatus*	RAZ 94, RAZ 104		Schmale et al. (2003)
		Bayocel-1, Flor de Mayo Bajio, Bayo Victoria	FM M-38, Mayocoba, G-4523	Maldonado et al. (1996)
Barley	*Tribolium castaneum*	Makuyi, Fajr30		Namin et al. (2018)
	Sitotroga cerealella	Bahman, Fajr30	$19A_1$	Karimi-Pormehr et al. (2018)
Rice	*Rhyzopertha dominica*	Wells, Jupiter, Pirogue	Rico, Francis	Chanbang et al. (2008)
Wheat	*Trogoderma granarium*	Kouhdasht	Gaskojen	Golizadeh and Abedi (2016)
Mung bean	*Callosobruchus maculatus*	Mutant 71-27, Pak-22	Mutants 121-25, 19-19, M-28	Khattak et al. (1987)

However, Jackai and Asante (2003) proposed a method by using 40 seeds and two pairs of adults of *C. maculatus* in screening cowpea for resistance. They concluded that percent adult emergence, growth index, and percent weight loss are the most reliable indicators for resistance of cowpea to damage by *C. maculatus*. Besides, Amusa et al. (2018) mentioned that mean development period and percentage adult emergence are two of the more important traits measuring bruchid (e.g.,

C. maculatus) resistance in cowpea. Accordingly, the appropriate method to evaluate the seed resistance is completely dependent on the pest and host seed species.

As mentioned before, various factors participate in seeds to withstand the pest pressure. For example, Abdel-Rahman et al. (1968) stated that the least favorable varieties for *P. interpunctella* possess the smallest kernels and the high mortalities on them are caused by the inability of newly hatched larvae to enter undamaged grains. In addition, Chanbang et al. (2008) showed that the characteristics of the rough rice hull (solid, split, and cracked hulls) are important for conferring susceptibility of different varieties to *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and the tolerant varieties appear to have thicker hulls than the susceptible varieties. However, it is suggested that nutritional quality, especially seed hardness, may be the major factor responsible for the susceptibility of barley cultivars to *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) (Karimi-Pormehr et al. 2018). Hence, the identification of these factors is pivotal to develop new resistant seed varieties.

11.3.2 Healthy Seeds

The presence of insects, weed seeds, and seeds of other varieties or other crops is not hygienic for the seed production. The existence of storage insects and fungi may result in considerable losses through direct damage to the seeds, reduction in germination, and less market value because of discoloration and mustiness of seeds. The appearance of abnormal seeds looks like discolored seeds, spotted seeds, deformed seeds, insect damaged seeds, germinated seeds, and seeds with smut. The SIP may not only damage the seeds physically but also encourage the growth of storage molds, thus reducing seed germination. For instance, the high prevalence of four species of storage insects, including *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *T. castaneum* (Coleoptera: Tenebrionidae), and *R. dominica* (Coleoptera: Bostrychidae) in stored rice seeds, implies poor seed quality in terms of insect infestation. However, there is the Seed Health Improvement Subproject (SHIP). In the SHIP, seed health refers to the total health status of a seed lot for planting, which involves both seed health conditions and seed contaminants. It is stated that seed health has two components: (1) the health conditions, is well developed, filled, or unfilled and (2) the seed contaminants (do seeds for planting contain weed seeds; seeds of other varieties; other inert materials such as plant debris, soil particles, scletoria of a pathogen, etc.). An appropriate and sustainable technology is needed to improve seed health and quality of stored seeds. Such a technology can be developed by reducing or eliminating the initial inocula of seedborne pathogens, seed contaminants (destructive insects and weed seeds), and abnormal seeds (spotted, discolored, and deformed) present in storedseeds; minimizing pest and disease pressure on the crop; and maintaining the quality of storedseeds in storage process. Conversely, very few technologies have been developed through research or are available in the country to solve seed health-related

problems. At the moment, the technologies such as treatment of seed with seed dressing chemicals and spraying growing crops with pesticides cannot be advocated because of the high cost and the attendant health and environmental hazards (Fakir and Mia 2004). Additionally, hidden infestations are often hard to detect. So, radio photography using X-rays is effective. Other approaches including the development of PCR and ELISA tests and use of near-infrared spectroscopy and electronic nose detectors are being researched (Rees 2004).

11.3.3 Sanitation and Exclusion

Sanitation prevents insect problems by reducing, through routine cleaning, the seed that is available to SIP. Sanitation, the cleaning, and removal of seeds that harbors an insect pest is the first line of defense in SIP control program (Phillips and Throne 2010). The design and maintenance of the building, grounds, and equipment can make an IPM program more effective by preventing insects from entering a facility. Other important exclusion methods include insect-resistant packaging and the inspection of incoming ingredients for insects (Hagstrum and Subramanyam 2006). Since SIP can develop, survive, and reproduce on very small amounts of available resources, it is imperative that newly harvested seeds be stored in clean bins and not be loaded into bins that contain older seeds that may harbor SIP. Harvesting equipment, transportation containers, loading areas, and storage bins need to be as clean as possible before harvest and storage of the new seeds, and sometimes it is prudent to treat the surfaces of inside walls, floors, and ceilings of such structures and machinery with a residual insecticide to kill any insects that may remain following the previous storage season (Phillips and Throne 2010).

Exclusion through design and maintenance of building, grounds, and equipment includes (1) the placement of outside lights and ornamental plants so as not to attract insects; (2) the screening and sealing of windows, vents, utility lines, and doors so that insects cannot enter a building; (3) sealing cracks and crevices around the bases of equipment and floor-wall junctions; (4) maintaining a vegetation-free barrier zone around the perimeter of a facility; (5) adequate drainage to prevent the accumulation of water on the grounds; (6) proper maintenance of the garbage-disposal area; and (7) maintaining a clutter-free roof. Hence, sanitation and exclusion can reduce the numbers of many SIP, processing and marketing facilities, and transportation vehicles and make other insect pest management methods more effective. Also, the sanitation and exclusion methods are low risk, inexpensive and without creating resistance. On the other hand, sanitation and exclusion require continuous effort, high labor, and organized effort (Hagstrum and Subramanyam 2006). So, seed facilities or packages need to be sealed very well to prevent SIP and to be constructed of durable materials to resist penetrators.

11.3.4 Seed Treatment

Seeds may be treated to eliminate an infestation by a pest; however, they may be treated even if not infested, either as a precaution by a general disinfection or to protect the seedlings growing from the seeds when exposed to pests in the environment. Seed treatments may also be unrelated to pests; for example, seeds may be treated with seedling growth enhancer (Pal et al. 2016; FAO 2017). Seed treatment for pest control includes the application of a pesticide to the seed surface to reduce, control, or repel a pest. Pest management in seed treatment relies on two steps of management principles including protection and eradication. Protection consists of applying a chemical barrier to the seed that protects the seed of young seedling from SIP. Eradication includes methods to eliminate, inactivate, repel, or destroy insects and pathogens from the seed or seedling. Seed treatment generally controls insect pests and fungal diseases (TeKrony 1976; Hefner et al. 2018). However, seed treatment with broad-spectrum fungicide such as thiram and carboxin hold promise. They are not only effective against a wide range of pathogens but also are thought to delay seed deterioration during storage (Adebisi et al. 2004). Treated seed must have a statement on the seed container that indicates important information including (1) the seed has been treated; (2) the name of the pesticide used for the seed treatment; (3) a precautionary statement indicating that the treated seed cannot be used for food, feed, or oil under any circumstances; and (4) seed treated with highly toxic substances requires a skull and crossbones label and the word *poison* within the precautionary statement (Hefner et al. 2018).

In the seed treatment, good coverage is essential for appropriate results. There are a number of manners for applying pesticides to seed including dust, dip slurry, mist, and pelleted. Dust is a dry powder formulation of pesticide that is applied to the seed in the planter box. Dip slurry is a suspension of pesticide in water that the seed is dipped into or mixed with. Mist is a pesticide that is sprayed or misted onto the seed, usually resulting in good coverage. Pelleted pesticide is a two-step process. The seed is misted with a pesticide, and then the treated seed is coated with a fine layer of clay or calcium material. This is the most effective method of pesticide seed treatment because the pesticide is contained and protected by the pellet coating (TeKrony 1976; Hefner et al. 2018).

Seed should only be treated with a pesticide once, either commercially or by the grower. If the application rate is too high or if the pesticide is applied more than once, it may result in reduced or complete lack of germination due to chemical toxicity (Hefner et al. 2018). Also, widespread experience has proven that repeated use of the single chemical pesticides in poorly sealed warehouses leads to develop strong resistance by the insects. For example, continuous and discriminate use of phosphine has resulted in the evolution of chemical resistance in SIP (Kumar et al. 2017). It is important to use high-quality certified seeds for the seed treatment. Hence, damaged seed or seed of poor quality would be a poor investment (TeKrony 1976; Hefner et al. 2018).

11.3.5 Pesticides

Pesticides play a major role in SIP control, but it is important to consider the side effects of chemical pesticides to SIP natural enemies and other non-target organisms. Also, indiscriminate use of chemical pesticides against SIP in high-value seed production has repeatedly led to pest resistance and environmental pollution. Hence, it is important to consider that when the use of chemical pesticides is needed. Calendar-based applications performed without any assessment of whether or not they will produce an economic gain for the seed productions are mostly an ineffective use of chemical pesticides. On the contrary, responsive (need-based) applications are a more appropriate tool. Practically, the responsive use of chemical pesticides is often dependent on the availability of an appropriate pest monitoring system. Pesticides applications should be based on efficient decision-making tools, one of which is economic injury level (EIL). EIL is the smallest number of insects (amount of injury) that will cause yield losses equal to the pest management costs and thus the pest population density or extent of crop damage at which the value of the crop destroyed exceeds the cost of controlling the pest. The pest density at which management action should be taken to prevent an increasing pest population from reaching the economic injury level is called the economic threshold (ET) or action threshold (AT) (Fathipour and Mirhosseini 2017). Further, choosing selective pesticides is important to minimize the side effects on non-target organisms. Additionally, a finer point of effective chemical control is applying at the most susceptible stage of pest in order to achieve the highest possible pest mortality rate. It is depend on the species, but commonly, the susceptible stage of SIP is first-instar larvae or nymphs. However, a great number of pesticides are used against SIP around the world. Various pesticides are discussed as follow.

Synthetic chemical pesticides, particularly the fumigant gas hydrogen phosphide, commonly referred to as phosphine, are mostly used in SIP control and will continue to be important tools (Phillips and Throne 2010). In addition, organophosphorus compounds are widely used; increasing concerns over the use of organophosphorus linked to health and environmental fears (Hagstrum and Subramanyam 2006). In the Czech Republic, several formulations of pyrethrins (pyrethrum), pyrethroids (deltamethrin, cypermethrin), and organophosphates (chlorpyrifos, pirimiphos-methyl) are available to SIP control (Stejskal et al. 2014). However, organophosphate insecticides, spinosad, juvenile hormone, and diatomaceous earth have been recommended for direct application on seed against SIP. Moreover, residual insecticides are applied either as a liquid or a dust to specific areas of a processing facility, storage structure, transportation vehicle, or stored commodity to suppress insect pest populations for a period of time ranging from months to a year or more. Residual insecticides also have been applied as a fog, mist, aerosol, or smoke to kill flying insects or exposed crawling insects. Also, organophosphates, pyrethroids, and chlorinated hydrocarbons have been recommended as empty-bin sprays. Besides, Dichlorvos (DDVP) commonly has been used as a space treatment (Hagstrum and Subramanyam 2006). However, biological control agents often are more susceptible to residual pesticides than pest insects are, and these pesticides also kill their host or

prey, depleting their food supply. Therefore, it is important to consider the side effects of pesticides on the life history traits of the natural enemies.

11.3.5.1 Insect Growth Regulators (IGRs)

IGRs are synthetic insecticides that mimic insect hormones and act by disrupting the normal development of immature stages of SIP such as fenoxycarb, methoprene, pyriproxyfen, diflubenzuron, and flufenoxuron (Phillips and Throne 2010). These compounds can disrupt the normal development of insects by mimicking the action of insect hormones and/or by interfering with hormone-regulated processes. They have been used in a variety of practical applications and are effective against a range of SIP (Collins 2006). The IGRs are not directly toxic to adult stage, although their potential effects on reproductive sterility have not been fully investigated. Another key attribute to these IGRs is their low levels of toxicity to mammals and inherent high level of food safety (Phillips and Throne 2010). The different IGRs compounds are explained as below:

Juvenile hormone analouge (JHA) substances have been identified in the insects. They appear to affect pest development by disrupting normal morphogenesis to the adult, resulting in abnormal insects with combined juvenile and adult characteristics. These abnormal insects cannot feed or reproduce and eventually die. The JHAs also appear to affect embryonic development resulting in failure of egg hatch (Collins 2006).

Chitin synthesis inhibitors affect immature stages of insects and prevent hatching of eggs. They interfere with the moulting process by inhibiting chitin incorporation into the insect cuticle, but their precise mode of action is unknown. They do not appear to inhibit chitin synthetase (Collins 2006).

Moulting hormone (ecdysone) agonists promote a premature and ultimately lethal moult. Ecdysone is a steroidal hormone secreted by the prothoracic gland in insects, which after release is converted to 20-hydroxyecdysone, inducing moulting and metamorphosis (Collins 2006).

11.3.5.2 Inert Dusts

Inert dusts have been used traditionally as SIP protectants. The products are based on inert materials such as silica gel or diatomaceous earth. They have proved effective as grain protectants, as structural treatments in empty stores, and as surface treatments in conjunction with aeration. Inert dusts have a physical mode of action and are picked up as pests walk over a treated surface. The most widely accepted view of their mode of action is that damage to the cuticle is caused by removal or sorption of cuticular waxes resulting in loss of water from the body, depending on the relative humidity of the air, leading to death through desiccation. The main advantages of inert dusts are that they have low mammalian toxicity, do not contain chemical insecticide or knockdown agents, do not leave harmful residues, are effective against chemical resistant species, are persistent, and are stable at high and low temperatures. It is postulated that because inert dusts do not affect metabolic pathways by chemical action, physiological resistance is unlikely to occur (Collins 2006). In this case, diatomaceous earth are very promising alternatives to the

currently used neurotoxic insecticides. Diatomaceous earth are particularly effective against a wide range of SIP. Athanassiou et al. (2003) found that the diatomaceous earth SilicoSec® is effective for the control *S. oryzae* of paddy rice and barley at 1000 ppm. The diatomaceous earth efficacy varies remarkably according to dose, exposure, and temperature against *S. oryzae* and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (Athanassiou et al. 2006). It is stated that the diatomaceous earth effectiveness was generally less at lower temperatures against larvae of the *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Also, the increase of relative humidity decreases diatomaceous earth efficacy. In addition to this, diatomaceous earth efficiency varies according to the type of grain from which insects have emerged, the strain of insects (adults or larvae), the assessed variety of grain, and the geographical origin of diatomaceous earth. Diatomaceous earth can also provide long-term efficacy against SIP. Also, the commercially available DE Keepdry® is effective against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), *O. surinamensis* and *S. oryzae*, *Sitophilus zeamais* Motschulsky (Coleoptera: Cuculionidae), and *T. castaneum*. Recently, Athanassiou et al. (2014) showed that Keepdry® is effective at doses that are lower than other commercially available diatomaceous earth, for the control of *R. dominica* and *S. oryzae*, again, taking into consideration that its efficacy is influenced by the abiotic factors temperature and relative humidity (Athanassiou et al. 2014). As with insects, different mite species show various tolerances to inert dusts, with *L. destructor* more tolerant than *A. siro* and *T. putrescentiae*. *Lepidoglyphus destructor* has long stiff hairs over its body which may prevent some of the dust adhering to the mite cuticle. Of those inert dusts assessed against storage mites, "Protect-it" appears the most effective with 0.5–1 g/kg reducing population development of *A. siro* and *T. putrescentiae* by 495%, and 1–3 g/kg required to have the same effect on *L. destructor* (Collins 2006). Additionally, it is suggested that alumina powder can be considered for seed protection against *A. obtectus*, particularly during long-term storage, as it is economically cost-effective, exerts limited toxicity to humans, and demands no repetitive use like conventional pesticides (Lazarević et al. 2018). Hence, two review papers are presented on diatomaceous earths and inert dusts by Kuronic (1998) and Golob (1997), respectively. They could be useful for obtaining more information regarding this matter.

11.3.5.3 Fumigants

Fumigants are toxic gases that penetrate into commodities. Maintaining an adequate concentration of a fumigant for sufficient time kills most SIP. Commonly, some of the pest management methods that are used for SIP are preventive. Because fumigants work quickly and are effective against most insect pests, they can be used in response to discovering an insect problem. Fumigations are sometimes done on a calendar schedule but can be used only when a sampling program indicates that the insect pest population is likely to reach an unacceptable level. Popular liquid fumigants such as carbon disulfide and ethylene dibromide are registered for general use in the United States, and methyl bromide is scheduled to be phased out by the year 2005 because of its ozone-depleting properties (Hagstrum and Subramanyam

2006). Under Montreal Protocol, 2002, the use and production of methyl bromide was discontinued in developed countries by the year 2005 and worldwide by 2015. Also, the DDVP vaporization strips (dichlorvos, Vapona) banned in 2013 due to EU legislative restrictions (Stejskal et al. 2014).

Phosphine is currently the most widely used fumigant for SIP control. In the Czech Republic, phosphine (PH_3)-based fumigants are the most commonly used procedure to control seed infestation by internally feeding pests (Stejskal et al. 2014). Phostoxin (aluminium phosphide) should be applied at the rate of 1 tablet/100 kg cowpea in an air-tight container. Place the tablet in a paper envelop or wrap securely in a tissue paper. After 4 days, remove and bury the paper and its contents. Phostoxin tablets may be used at the rate of 1–3 tablets/ton. The treatment should be repeated after 4–6 months (Tazerouni et al. 2019a). The effectiveness of fumigants generally increases as insect metabolic rates increase with temperature. Phosphine is not released from pellets below 4.5 °C (Stejskal et al. 2014). There are different chemical compounds that can be used as fumigation measure such as sulfuryl fluoride (sold commercially as ProFume), tetrachloride, chloropicrin, ethylene dichloride, and hydrogen cyanide. Different SIP shows various degree of resistant to the compounds. For example, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) is more resistant to carbon tetrachloride, chloropicrin, ethylene dichloride, and hydrogen cyanide, while *T. confusum* is more resistant to carbon disulfide, ethylene oxide, methyl bromide, and sulfuryl fluoride (Hagstrum and Subramanyam 2006). However, the fumigant activity of botanical compounds will be discussed in the botanical section.

It is important to consider fumigant atmospheres need to be used with care because it can be lethal to humans. Also, phosphine is corrosive to copper, silver, and gold (Hagstrum and Subramanyam 2006). Further, ozone depletion, insect resistance, and residues on grain surface are the more problems with the use of chemical fumigants (Kumar et al. 2017). Additionally, it is reported that phosphine (PH_3) has no adverse effect on most tested seeds; hydrogen cyanide (HCN) did not decrease or increase germination of the treated seeds, while dichloroethane decreased germination dramatically (Stejskal et al. 2014).

11.3.5.4 Botanicals

Many plants and minerals have insecticidal properties; that is, they are toxic to SIP. Botanical insecticides are extracted or derived from plants or minerals. They are also called natural insecticides. Plant parts and extracts have been, and still are, used in many parts of the world to kill or repel insects. Plants are known to produce a range of secondary metabolites which can possess multiple modes of action, including acute toxicity, repellency, antifeedant or antioviposition effects, and inhibition of growth, development, or reproduction. Different aspects of botanical pesticides against SIP have been reviewed by Jacobson (1989). Essential oils, obtained by steam distillation of plant foliage and even the foliage itself of certain aromatic plants (notably in the families Myrtaceae and Lamiaceae but in other plant families as well), have traditionally been used to protect stored grain and legumes and to repel flying insects in the home. The insecticidal constituents of many plant extracts and

essential oils are mainly monoterpenoids. The contact and fumigant insecticidal actions of plant essential oils have been well demonstrated against SIP (Isman 2000; Pal et al. 2016). There are various review articles about the plant essential oils and botanical pesticides (e.g., Isman 2000; Bakkali et al. 2008; Moharramipour and Negahban 2014). A vast array of plant essential oils, extracts, and their components has been evaluated against SIP as reviewed in Table 11.3. Some commercial botanical pesticides are explained as follow.

Azadirachta indica A. Juss (neem) is one of the most extensively studied botanical sources of pesticidal compounds which is native to semiarid areas of Asia and Africa. Extracts of various parts of the tree, but especially of the seeds, have been shown to possess insecticidal properties. Azadirachtin is effective as a feeding deterrent, repellent, toxicant, sterilant, and growth disruptant in insects, acting primarily as an oral poison. Several tetranortriterpenoids have been isolated and identified, but the major entomologically active component is azadirachtin (Jacobson 1989). There have been few studies on the acaricidal activity of azadirachtin, but its efficacy against insects indicates its potential (Collins 2006).

Pyrethrum, a commercial mixture of compounds derived from *Chrysanthemum* sp., is perhaps the most successful botanical insecticide throughout all modern pest control, and this is certainly the case for SIP (Phillips and Throne 2010). Although natural pyrethrum is still used commercially, it has been largely superseded by the synthetic pyrethroids, which have greatly improved insecticidal properties and photostability. Pyrethroids are effective against SIP and degrade slowly on wheat under normal conditions of storage with increased rate of loss at higher temperatures and moisture levels. Pyrethroids act on the ion channels in insect nerve synapses by keeping sodium channels open, resulting in a continuous slow depolarization which eventually blocks nerve conduction and causes paralysis (Collins 2006).

Spinosad is registered in different countries as a grain protectant at a maximum labeled use rate of 1 ppm (1 mg a.i./kg of grain) and with the Maximum Residue Level or tolerance on grains set at 1 or 1.5 ppm. Spinosad effectively controls economically important beetle and moth pests associated with SIP and is also effective against certain psocid species. Spinosad provides grain protection through control of adult and/or immature life stages of SIP. Different aspects of spinosad were reviewed by Hertlein et al. (2011). Also, novel compounds have also been evaluated as insecticides and acaricides against SIP such as chemosterilants, fatty acids, and inorganic salts (Collins 2006).

11.3.6 Physical Treatments

Physical control of SIP involves the manipulation of physical factors to eliminate or reduce their populations to a tolerable level. Physical measures are safer alternatives as it can be applied directly to the seeds (Banks and Field 1995). These methods include aeration, extreme temperatures and relative humidity, impact and removal, ionizing radiation, and modified atmospheres. Also, combination of these technologies has been practiced. For example, major Czech Republic seed-

Table 11.3 The important plant compounds that are developed to control seed insect pests (SIP)

Plant compounds	Target pest	Stage[a]	Concentration	References
1,8-cineole	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 9.5 μL/L	Lee et al. (2004)
	Sitophilus oryzae	A	Fumigant LD_{50}: 22.8 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 15.3 μL/L	Lee et al. (2004)
Achillea fragrantissima	*Callosobruchus maculatus*	E	Fumigant LC_{50}: 96.3 μL/L	Nenaah et al. (2015)
	Callosobruchus maculatus	A	Fumigant LC_{50}: 33.6 μL/L	Nenaah et al. (2015)
Ageratum conyzoides	*Callosobruchus maculatus*	E	Fumigant LC_{50}: 71.6 μL/L	Nenaah et al. (2015)
	Callosobruchus maculatus	A	Fumigant LC_{50}: 19.2 μL/L	Nenaah et al. (2015)
Alpinia conchigera	*Sitophilus zeamais*	A	Fumigant: LC_{50}: 85 μL/L	Suthisut et al. (2011)
	Tribolium castaneum	A	Fumigant: LC_{50}: 73 μL/L	Suthisut et al. (2011)
Anethum graveolens	*Ephestia kuehniella*	L	Contact LC_{50}: 18.35%	Mikhaiel (2011)
	Tribolium castaneum	L	Contact LC_{50}: 15.38%	Mikhaiel (2011)
Aphanamixis polystachya (Ethanol)	*Tribolium castaneum*	A	Contact LD_{50}: 32 μg/insect	Talukder and Howse (1995)
Artemisia herba-alba	*Bruchus rufimanus*	A	Fumigant: LC_{50}: 8.3 μL/L	Titouhi et al. (2017)
	Callosobruchus maculatus	A	Fumigant: LC_{50}: 7.7 μL/L	Titouhi et al. (2017)
Artemisia scoparia	*Callosobruchus maculatus*	A	Fumigant: LC_{50}: 1.46 μL/L	Negahban et al. (2006)
	Sitophilus oryzae	A	Fumigant: LC_{50}: 1.87 μL/L	Negahban et al. (2006)
	Tribolium castaneum	A	Fumigant: LC_{50}: 2.05 μL/L	Negahban et al. (2006)
Artemisia sieberi	*Callosobruchus maculatus*	A	Fumigant LC_{50}: 1.45 μL/L	Negahban et al. (2007)
	Sitophilus oryzae	A	Fumigant LC_{50}: 3.86 μL/L	Negahban et al. (2007)
	Tribolium castaneum	A	Fumigant LC_{50}: 16.76 μL/L	Negahban et al. (2007)
Astrocarym aculeatum	*Sitophilus zeamais*	A	Fumigant LC_{50}: 53.4%	Santos et al. (2015)
Callistemon sieberi	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 7.8 μL/L	Lee et al. (2004)

(continued)

Table 11.3 (continued)

Plant compounds	Target pest	Stage[a]	Concentration	References
	Sitophilus oryzae	A	Fumigant LD_{50}: 27.3 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 17.4 μL/L	Lee et al. (2004)
Carum copitum (loaded nanogel)	*Sitophilus granarius*	A	Fumigant: LC_{50}: 4.65 μL/L	Ziaee et al. (2014)
	Tribolium confusum	A	Fumigant: LC_{50}: 14.85 μL/L	Ziaee et al. (2014)
Carum copticum	*Callosobruchus maculatus*	E	Fumigant: LC_{50}: 1.01 μL/L	Sahaf and Moharramipour (2008)
	Callosobruchus maculatus	L	Fumigant: LC_{50}: 2.50 μL/L	Sahaf and Moharramipour (2008)
	Callosobruchus maculatus	A	Fumigant: LC_{50}: 0.90 μL/L	Sahaf and Moharramipour (2008)
	Plodia interpunctella	E	Fumigant: LC_{50}: 184.61 μL/L	Shojaaddini et al. (2008)
	Plodia interpunctella	L	Fumigant: LC_{50}: 91.36 μL/L	Shojaaddini et al. (2008)
	Plodia interpunctella	P	Fumigant: LC_{50}: 105.69 μL/L	Shojaaddini et al. (2008)
	Plodia interpunctella	A	Fumigant: LC_{50}: 257.83 μL/L	Shojaaddini et al. (2008)
	Sitophilus oryzae	A	Fumigant: LC_{50}: 0.91 μL/L	Sahaf et al. (2007)
	Tribolium castaneum	A	Fumigant: LC_{50}: 33.14 μL/L	Sahaf et al. (2007)
Cinnamomum aromaticum	*Situphilus zeamais*	A	Contact LC_{50}: 0.66 mg/cm^2 Fumigant LC_{50}: 0.54 mg/cm^2	Huang and Ho (1998)
	Tribolium castaneum	A	Contact LC_{50}: 0.70 mg/cm^2 Fumigant LC_{50}: 0.28 mg/cm^2	Huang and Ho (1998)
Citrus latifolia	*Callosobruchus maculatus*	A	Contact LC_{50}: 943.9 ppm Fumigant LC_{50}: 10.2 μL/L	de Andrade et al. (2016)
Citrus sinensis	*Corcyra cephalonica*	L	2.5 g/40 g legume seed	Allotey and Azalekor (2000)
Coffea arabica (dichloromethane)	*Tribolium castaneum*	A	Fumigant LC_{50}: 5555.4 ppm	Phankaen et al. (2017)
Eucalyptus blakelyi	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 9.7 μL/L	Lee et al. (2004)

(continued)

Table 11.3 (continued)

Plant compounds	Target pest	Stage[a]	Concentration	References
	Sitophilus oryzae	A	Fumigant LD_{50}: 31.2 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 15.5 μL/L	Lee et al. (2004)
Eucalyptus codonocarpa	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 10.4 μL/L	Lee et al. (2004)
	Sitophilus oryzae	A	Fumigant LD_{50}: 19 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 18.2 μL/L	Lee et al. (2004)
Eucalyptus intertexta	*Callosobruchus maculatus*	A	Fumigant: LC_{50}: 2.55 μL/L	Negahban and Moharramipour (2007)
	Sitophilus oryzae	A	Fumigant: LC_{50}: 6.93 μL/L	Negahban and Moharramipour (2007)
	Tribolium castaneum	A	Fumigant: LC_{50}: 11.59 μL/L	Negahban and Moharramipour (2007)
Eucalyptus leucoxylon	*Callosobruchus maculatus*	A	Fumigant: LC_{50}: 2.76 μL/L	Kambouzia et al. (2009)
	Sitophilus oryzae	A	Fumigant: LC_{50}: 8.48 μL/L	Kambouzia et al. (2009)
	Tribolium castaneum	A	Fumigant: LC_{50}: 13.15 μL/L	Kambouzia et al. (2009)
Eucalyptus nicholii	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 9.5 μL/L	Lee et al. (2004)
	Sitophilus oryzae	A	Fumigant LD_{50}: 29 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 13.7 μL/L	Lee et al. (2004)
Foeniculum vulgare	*Callosobruchus maculatus*	A	Contact LC_{50}: 178.13 ppm Fumigant LC_{50}: 2.58 μL/L	Gusmão et al. (2013)
Jatropha curcas (Goncalo genotype)	*Sitophilus zeamais*	A	Contact LT_{50}: 8 (day)	Silva et al. (2012)
	Rhyzopertha dominica	A	Contact LT_{50}: 8 (day)	Silva et al. (2012)
Laurus nobilis (from Morocco)	*Rhyzopertha dominica*	A	Fumigant LC_{50}: 68 μL/L	Jemâa et al. (2012)
	Tribolium castaneum	A	Fumigant LC_{50}: 172 μL/L	Jemâa et al. (2012)

(continued)

Table 11.3 (continued)

Plant compounds	Target pest	Stage[a]	Concentration	References
Melaleuca armillaris	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 8.2 μL/L	Lee et al. (2004)
	Sitophilus oryzae	A	Fumigant LD_{50}: 30.6 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 13.7 μL/L	Lee et al. (2004)
Melaleuca fulgens	*Rhyzopertha dominica*	A	Fumigant LD_{50}: 7.8 μL/L	Lee et al. (2004)
	Sitophilus oryzae	A	Fumigant LD_{50}: 28.6 μL/L	Lee et al. (2004)
	Tribolium castaneum	A	Fumigant LD_{50}: 14.1 μL/L	Lee et al. (2004)
Melissa officinalis	*Plodia interpunctella*	L	Fumigant: LC_{50}: 5.57 μL/L	Rafiei-Karahroodi et al. (2011)
Mentha microphylla	*Acanthoscelides obtectus*	A	Fumigant LC_{50}: 1.1 μL/L	Papachristos and Stamopoulos (2002)
Mentha viridis	*Acanthoscelides obtectus*	A	Fumigant LC_{50}: 1.2 μL/L	Papachristos and Stamopoulos (2002)
Perovskia abrotanoides	*Sitophilus oryzae*	A	Fumigant: LC_{50}: 18.75 μL/L	Arabi et al. (2008)
	Tribolium castaneum	A	Fumigant: LC_{50}: 11.39 μL/L	Arabi et al. (2008)
Potato glycoalkaloids	*Trogoderma granarium*	A	Contact LD_{50}: 16.7 μg/mg insect	Nenaah (2011)
Tagetes minuta	*Callosobruchus maculatus*	E	Fumigant LC_{50}: 161.9 μL/L	Nenaah et al. (2015)
	Callosobruchus maculatus	A	Fumigant LC_{50}: 77.8 μL/L	Nenaah et al. (2015)
Terpinen-4-ol	*Tribolium confusum*	E	Fumigant: LC_{50}: 109.4 μL/L	Stamopoulos et al. (2007)
	Tribolium confusum	L	Fumigant: LC_{50}: 2.3 μL/L	Stamopoulos et al. (2007)
	Tribolium confusum	P	Fumigant: LC_{50}: 2.3 μL/L	Stamopoulos et al. (2007)
	Tribolium confusum	A	Fumigant: LC_{50}: 5.5 μL/L	Stamopoulos et al. (2007)
Thymus persicus	*Callosobruchus maculatus*	A	Fumigant: LC_{50}: 2.39 μL/L	Moharramipour et al. (2008)
	Sitophilus oryzae	A	Fumigant: LC_{50}: 3.34 μL/L	Saroukolai et al. (2010)
	Tribolium castaneum	A	Fumigant: LC_{50}: 234.42 μL/L	Moharramipour et al. (2008)
	Tribolium castaneum	A	Fumigant: LC_{50}: 236.9 μL/L	Saroukolai et al. (2010)

[a]E = egg, L = larva, P = pupal stage, and A = adult

producing companies are well equipped with sieving and aspirating machines that are used to remove dust, impurities, and pests before accepting new seed batches into the facility for further processing (dry or moist sowing seed pickling) and storage (Stejskal et al. 2014). However, the merits and demerits of most physical methods for the insect control of stored grain are listed by Kumar et al. (2017). For instance, sun drying has merits in terms of ecological, widespread, no need of skilled labor, use of renewable energy source, and environmentally friendly, but the method has some disadvantages such as weather dependent and unhygienic conditions and requires prolonged exposure time. The different physical methods are explained as below.

11.3.6.1 Aeration

Aeration involves forced movement of outside air through a seed store to create a uniform temperature. Aeration is an effective and safe method which it can slow the growth populations of many species of SIP. Low-temperature aeration results in acceptable mortality of SIP when the cold temperatures maintain for several months. It is reported that aeration is shown to be a feasible and effective tool for stored grain management. With utilization of aeration, losses from *R. dominica*, *P. interpunctella*, *Cryptolestes* spp., and *Tribolium* spp. considerably reduced in Oklahoma (Cuperus et al. 1986). The aeration performed at 10 m^3/h/ton with an 0.02 kW fan resulted in decreasing the populations of some SIP to exemplify, *O. surinamensis* and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Armitage et al. 1994). However, the cost of aeration method is low which the electricity to run aeration fans commonly costs no more that fumigation. Simple and inexpensive equipments are needed to monitor the temperature of the exhaust air or the number of hours that an aeration fan has run. Additionally, the seed quality is maintained by equilibrating seed temperature throughout the seed mass and thus preventing moisture migration. In addition, cooling also slows the breakdown of residual insecticides so that they are effective in suppressing insect populations for a long time. On the contrary, some disadvantages are mentioned for the aeration including (1) the method provides only short-term protection against SIP; (2) poor aeration can reduce quality; (3) capital investment is high; (4) the method may seem to be more complex than other insect pest management; and (5) aeration can reduce seed moisture (Hagstrum and Subramanyam 2006).

11.3.6.2 Extreme Temperatures and Relative Humidity

Insects generally do not regulate their body temperature and so extreme temperatures reduce their survival rate. Commonly, for most SIP, 25–35 °C is optimal for growth and reproduction (Fields 1992). Commonly, mortality causes from extreme temperatures depends upon the temperature and the exposure time. Temperatures over 60 °C can cause death in seconds, temperatures between 50 and 60 °C in minutes, and temperatures between 43 and 46 °C in hours (Phillips and Throne 2010). Also, high temperatures can cause insects to move around more. Temperatures between 5 and 15 °C delay insect development and are eventually lethal after very long exposure time. Temperatures between −1 and 3 °C can cause

death in hours or days, and temperatures below −1 °C can cause death more quickly (Rees 2004; Hagstrum and Subramanyam 2006). A number of studies have reported the efficiency of extreme temperatures on the SIP. For instance, exposure of 24 h at −18 °C is sufficient to kill all life stages of the psocid, *Liposcelis* spp. (Arthur et al. 2017). Besides, it is reported that *Acarus farris* (Oudemans) (Acari: Acaridae) density was drastically reduced from 174 mites/cm^2 at the control temperature (15 °C) to 14 mites/cm^2, 11 mites/cm^2, and 1 mites/cm^2 at 6 °C, 4 °C, and 2 °C, respectively, as temperature decreased (Sánchez-Ramos and Castañera 2009). It is stated that, for most of the year, pest mites can be controlled in the Czech Republic stores via low temperatures that are maintained by active ventilation in flat stores and silos (Stejskal et al. 2014). Moreover, high temperatures have been investigated for SIP suppression as reviewed by Fields (1992). Also, in tropical countries, solar heating has been recommended for killing insects infesting cowpeas and grain. Many but not all insects are killed during forced hot-air drying. Further, infrared radiation and dielectric heating by microwave or radio frequencies have been shown to be effective for cereal grain. The susceptibility of different SIP and developmental stages may depend upon the heating rate (Hagstrum and Subramanyam 2006).

The lethal temperatures vary notably and depend on insect species, life stage, acclimation, and relative humidity (Fields 1992). For *T. confusum* at 44 °C, the order of heat tolerance among different life stages is pupae > eggs > larvae > adults. However, at 50 °C, these differences in heat tolerance among various stages are not significant (Hagstrum and Subramanyam 2006). In addition, the eggs of *Liposcelis* spp. are the most tolerant life stage, and the nymphs and adults are far more susceptible than eggs (Arthur et al. 2017). So, in order to design a SIP control program based on the extreme temperatures, it is important to consider the mentioned factors.

Generally, cold temperature kills insects more slowly than heat. Low temperatures are often maintained for the duration of storage. Heat treatments do not provide any long-term protection against SIP and must be repeated periodically, or other insect pest management methods must be used (Hagstrum and Subramanyam 2006). To arrest seed deterioration in the storage, dry-cold conditioned storage structures are recommended (Adebisi et al. 2004). A combination of heat followed by cold treatment (8 days at 10.5 °C) also may reduce the overall high-temperature exposure times required for suppression of *P. interpunctella* eggs. For the combination of heat and cold, only 6.5 h at 42 °C or 7.7 min at 46 °C were required for 99.9% mortality instead of 10.3 h at 42 °C and 34 min at 46 °C for high temperature alone or 19.8 days at 0.5 °C for low temperature alone (Hagstrum and Subramanyam 2006). Moreover, the cost of dry-cold conditioned storage structures is expensive (Adebisi et al. 2004).

Relative to most insects, SIP are highly resistant to moisture loss. However, most species breed fastest under humid conditions, typically 60–80%, which in terms of grain moisture content is roughly equivalent to 13–15%. The impact on population development of humidities below 60% varies considerably among different species (Hagstrum and Subramanyam 2006). For example, high moisture content levels result in the deterioration of cowpea and make them more susceptible to infestation

by SIP and infection by fungi. At harvest, cowpea should be left to dry for some time in order to reduce the moisture content to safe levels. The safe moisture levels of cowpea are reported as 13% or lower (Tazerouni et al. 2019a). Generally, relative humidity below 55% reduces population growth of many SIP, but some species can survive and reproduce even below 20% relative humidity (Banks and Field 1995). Further, at higher humidities, mould growth can become a problem, and this may hinder the development of some species.

11.3.6.3 Impact and Removal

Internal and external SIP infesting whole grain can be killed by impact when a seed is moved or by using impact machines. Cleaning seed by hand or by sieving and aspiration can remove SIP. Routinely, subsistence farmers in tropical countries separate insect-infested kernels from non-infested kernels by hand and feed them to their animals. However, some methods of killing the insects in the sievings are necessary to prevent these insects from reinfesting seed facilities. For example, major Czech Republic seed-producing companies are well equipped with sieving and aspirating machines that are used to remove dust, impurities, and pests before accepting new seed batches into the facility for further processing and storage (Stejskal et al. 2014). Also, disturbance of insects by tumbling can reduce the infestation levels of at least one insect species. Mostly, multiple impacts are more effective than a single impact. Also, the efficacy of the method may vary according to different life stages of the pest. For example, the fourth instars, prepupae, and pupae of both *S. granarius* and *S. zeamais* are the stages that are most susceptible to impact. Impact does not delay the development of the survivors of either species. A twice-daily 3.3-m (10.8-ft) drop (kernels reaching a velocity of 6.4 m/s) throughout their developmental period caused 100% mortality and a 0.11-m (0.36-ft) drop (kernels reaching a velocity of 1.4 m/s) caused 34% mortality. Death seems to be caused not because damage to the cuticle increases loss of water but by physical damage to the insects, such as that visible in the pupal stage of *S. granarius* as physical distortion of legs (Hagstrum and Subramanyam 2006; Phillips and Throne 2010). There are some advantages to use of impact and removal method including (1) many insects can be killed by impact or removed by sieving or aspiration; (2) impact is safe; (3) operating costs are low; and (4) resistance is not a problem yet. Contrary, some disadvantages were known as (1) impact does not provide long-term protection; (2) impact or removal alters the seed; (3) cost may be high; and (4) market for cleanings is variable (Hagstrum and Subramanyam 2006).

11.3.6.4 Ionizing Radiation

Ionizing radiation sterilizes or kills insects by damaging cells and producing free radicals that break chemical bonds. Radiation sensitivity is directly related to cell reproductive activity and inversely related to the degree of cell differentiation. Within a developmental stage, the susceptibility of an insect to ionizing radiation varies greatly with age. For instance, Aye et al. (2008) reported that the rates of both hatching and pupation of *P. interpunctella* exposed to gamma irradiation were lower when young individuals are irradiated. They suggested that irradiation at 0.5 kGy is

appropriate for the inhibition of development and reproduction of *P. interpunctella*. Also, Matin and Hooper (1974) investigated the effects of ionizing radiation on all stages of *R. dominica*, and the egg and pupal stages were the most susceptible and tolerant stages, respectively. However, ionizing radiation that can be used for insect pest management includes gamma rays from cobalt-60 or cesium-137 isotopes, acceleration of electrons from a tungsten filament, and X-rays derived from accelerated electrons. The doses required to kill adult moths tended to be higher than those required to kill adult beetles (1.0 kilogray vs. 0.5 kilogray [kGy]). The least-susceptible species of beetles tolerate a six to seven times higher dose than the most susceptible species. Bostrichids, tenebrionids, dermestids, and anobiids are the least susceptible, and bruchids, curculionids, and laemophloeids are the most susceptible. Sterilizing doses vary from 50 Gy for bruchids to 1 kGy for moths. Females are generally sterilized at a lower dose than males. Although irradiated insects can live for days or weeks, they tend to feed less and do not reproduce. Additionally, the ionizing radiation method has low operating cost and benefits and in particular without creating resistance. On the contrary, some disadvantages were reported such as (1) the method does not provide long-term protection; (2) mortality is delayed; (3) precautions are needed for worker safety; (4) ionizing radiation kills seeds; and (5) the method requires a large investment (Hagstrum and Subramanyam 2006). It is important to consider irradiation may not be appropriate for seed disinfestations that are to be used for planting or as malting barley. Seed are killed at the same doses that kill insects; although, it is reported that the germination of wheat grain is lowered after treatment with microwave radiation but is not affected by a dose of 1 kGy gamma radiation. Generally, both the number of germinated seeds and the growth rate for the crops decrease with an increase in the radiation dose the seeds were exposed to (El-Naggar and Mikhaiel 2011). Hence, the appropriate combination needs to be established for the safe disinfecting of each seed.

11.3.6.5 Modified Atmospheres (MAs)

Exposure of SIP to toxic concentrations of atmospheric gases, such as oxygen or carbon dioxide, has been practiced for centuries and has been promoted in recent years as an appropriate substitute for chemical fumigation. Target gas concentrations for insect toxicity are 3% or less of oxygen and/or 60% or more of carbon dioxide. Hence, one type of controlled atmosphere would be addition of CO_2 to levels above 60% for 24 h more or flushing an exposed space with an inert gas such as nitrogen to displace O_2 below 3% (Phillips and Throne 2010). A low-oxygen atmosphere can also be achieved and maintained by applying vacuum or low pressure, to the infested seeds. Vacuum hermetic fumigation (VH-F) is a new term for the sealed vacuum flexible container to control SIP. The vacuum pump helps to remove the air from the system to make the negative pressure below 100 mm Hg. Vacuum reduces the partial pressure O_2 as well as the water vapor from the interstitial atmosphere. It causes the insect mortality because of hypoxia and dehydration due to O_2 and water vapor deficit ecosystem, respectively (Kumar et al. 2017). Wong-Corral et al. (2013) reported that the pupae and eggs of three bruchid beetles, including *C. maculatus*, *A. obtectus*, and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae), are

the most tolerant stages to hypercarbia, while adults are less tolerant. Also, the susceptibility of the eggs varies greatly according to their phase of development. Depending on CO_2 concentrations (50%, 70%, and 90%), to achieve total mortality of *Z. subfasciatus* eggs, a maximum of 9–11 days are needed. Besides, MAs can be prevented aflatoxin production by *Aspergillus flavus* on maize since the mycotoxin production is only recorded when maize is infested with *S. zeamais* (Riudavets et al. 2018). Moreover, it is stated that MAs (<1% oxygen or >35% carbon dioxide) act more slowly than methyl bromide or sulfuryl fluoride but as fast as phosphine (Hagstrum and Subramanyam 2006). Also, MAs are more expensive to use than other fumigants. In addition, MAs need to be used with care because both can be lethal to humans (Hagstrum and Subramanyam 2006). So, the various aspects of the method should be considered in SIP management program.

11.3.7 Biological Control

A pest is able to increase to high densities due to the absence of its natural enemies that regulate populations of that pest in its distribution area. Biological control has been defined many times, but a commonly accepted definition is the use of living organisms to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Hajek 2004). It is widely accepted that there are three general approaches to biological control including importation (classical biological control), augmentation, and conservation of natural enemies. However, in the eyes of the general public, augmentation is more visible and recognized as a result of the wide availability of natural enemies (Hajek 2004).

Biological control is an often-underutilized component of integrated pest management of SIP control. Seed managers tend to use chemical pesticides to control insects. The use of natural enemies to control stored-grain insect pests may seem relatively new, but biological control is used as far back as 1911 to control Mediterranean flour moth (Flinn and Hagstrum 2001). There is a wide range of natural enemies, including parasitoids, predators, and pathogens that attack various developmental stages of SIP as shown in Table 11.4. By definition, parasitoids are natural enemies that lay their eggs inside or on the outside of any life stage of its host. Some of the natural enemies are commercially available for augmentative biological control. For example, the *Cheyletus* predators have been mass reared and sold in paper bags, each containing 2000–3000 live specimens of *C. eruditus*, under the commercial name CHEYLETIN® (Stejskal et al. 2014). As shown in Table 11.4, the most abundant natural enemies of SIP are often in the order Hymenoptera. The important families of hymenopteran parasitoids are Ichneumonidae, Bethylidae, Braconidae, Pteromalidae, and Trichogrammatidae.

Life history strategies are extremely diverse among various parasitoid species. For instance, the parasitoid species that parasitizes the host egg and larval stages called egg parasitoid and larval parasitoid, respectively. One of the well-known examples of egg-parasitoid group is Trichogrammatid wasps which are belonged to polyphagous genus *Trichogramma* (Hymenoptera: Trichogrammatidae). Also,

Table 11.4 The important natural enemies of seed insect pests (SIP)

Order	Family	Scientific name	Host pest	Host stage[a]	Main region reported	Reference
Parasitoid						
Hymenoptera	Bethylidae	*Cephalonomia gallicola*	*Lasioderma serricorne*, *Oryzaephilus surinamensis*	L	Cosmopolitan	Lim et al. (2007)
		Cephalonomia stephanoderis	*Hypothenemus hampei*	L-P	Widespread	Abraham et al. (1990)
		Cephalonomia tarsalis	*Oryzaephilus surinamensis*	L-P	Cosmopolitan	Powell (1938)
		Cephalonomia waterstoni	*Cryptolestes ferrugineus*, *C. pusillus*, *C. turcicus*	L	Cosmopolitan	Amante et al. (2017a)
		Holepyris hawaiiensis	Lepidoptera, *Plodia*, *Ephestia*	L	Hawaii, West Africa, Ceylon, Venezuela	Cotton and Good (1937)
		Holepyris sylvanidis	Cucujidae, Tenebrionidae, Curculionidae	L	Cosmopolitan	Amante et al. (2017b)
		Laelius spp.	Coleoptera, Dermestidae	L	Cosmopolitan	Mertins (1980)
		Plastanoxus westwoodi	*Cryptolestes pusillus*	L-P	Columbia, New York	Rahman et al. (2009)
		Prorops nasuta	*Hypothenemus hampei*	L-P	Widespread	Abraham et al. (1990)
		Pteromalus cerealellae	*Acanthoscelides obtectus*, *Callosobruchus maculatus*, *Lasioderma serricorne*, *Sitophilus oryzae*, *Sitotroga cerealella*, etc.	L	Widespread	Onagbola and Fadamiro (2008)
	Braconidae	*Apanteles carpatus*	*Tineola bisselliella*	L	Cosmopolitan	Plarre et al. (1999)
		Blacus humilis	*Blastophagus piniperda*, *Cryptophagus lycoperdi*, *Stegobium paniceum*	L	Widespread	Farahani et al. (2013)
		Habrobracon hebetor	Lepidotera	L	Cosmopolitan	Adarkwah and Scholler (2012)

	Chalcididae	*Antrocephalus mitys*	*Corcyra cephalonica*, *Opisina arenosella*, *Galleria mellonella*	L-P	Cosmopolitan	Pereira et al. (2013)
	Eulophidae	*Baryscapus bruchophagi*	*Bruchophagus roddi*, *B. bruchophagi*, *B. funebris*	L	Widespread	Marouf and Ebrahimi (2018)
		Phymastichus coffea	Hypothenemus hampei	A	Widespread	Espinoza et al. (2009)
	Eupelmidae	*Eupelmus vuilleti*	Bruchidae	L-P	West Africa	Darrouzet et al. (2002)
	Ichneumonidae	*Diadegma chrysostictos*	*Achroia grisella*, *Ephestia kuehniella*, *E. elutella*	L	Cosmopolitan	Horstmann and Shaw (1984)
		Venturia canescens	Pyralid	L	Cosmopolitan	Adarkwah and Scholler (2012)
	Pteromalidae	*Anisopteromalus calandrae*	Stored-product beetles	L	Cosmopolitan	Bodlah et al. (2016)
		Choetospila elegans	Coleoptera	L	Cosmopolitan	Flinn (1998)
		Dinarmus basalis	Bruchid	L-P	Widespread	Amevoin et al. (2007)
		Dinarmus vagabundus	*Callosobruchus* spp.	L	Widespread	Lotfalizadeh and Gharali (2008)
		Lariophagus distinguendus	Stored-product beetles	L-P	Cosmopolitan	Ryoo et al. (1991)
		Theocolax elegans	*Rhyzopertha dominica*, *Sitophilus* spp.	L-P	Cosmopolitan	Flinn and Hagstrum (2002)
	Trichogrammatidae	*Trichogramma* spp.	Lepidoptera	E	Cosmopolitan	Hassan (1993)
		Uscana mukerjii	Bruchid	E	India	Sood and Pajni (2006)

(continued)

Table 11.4 (continued)

Order	Family	Scientific name	Host pest	Host stage[a]	Main region reported	Reference
Diptera	Tachinidae	*Exorista larvarum*	Lepidoptera	L	Widespread	Dindo et al. (2019)
Predator						
Coleoptera	Histeridae	*Teretriosoma nigrescens*	Prostephanus truncatus	L	America, Africa	Oussou et al. (1998)
Hemiptera	Anthocoridae	*Lyctocoris campestris*	Polyphagous	E-L-A	Cosmopolitan	Parajulee et al. (1994)
		Xylocoris flavipes	Coleoptera, Lepidoptera	E-L-P	Widespread, temperate regions	Russo et al. (2004)
		Xylocoris sordidus	Polyphagous	L	Western hemisphere	Arbogast et al. (1983)
	Reduviidae	*Amphibolus venator*	Polyphagous	L-A	Cosmopolitan	Murata et al. (2007)
		Peregrinator biannulipes	Polyphagous	T	Cosmopolitan	Imamura et al. (2008)
Mesostigmata	Ascidae	*Blattisocius tarsalis*	Lepidoptera, Coleoptera	E	Cosmopolitan	Nielsen (2003)
Prostigmata	Acarophenacidae	*Acarophenax lacunatus*	*R. dominica*, *T. castaneum*, *C. ferrugineus*	E		Oliveira et al. (2003)
	Cheyletidae	*Cheyletus* spp.	Grain mites, stored-product insect	E-L-A	Cosmopolitan	Žďárková (1998)
Pathogen						
Hypocreales	Clavicipitaceae	*Metarhizium anisopliae*	Polyphagous	T	Cosmopolitan	Cherry et al. (2005)
	Cordycipitaceae	*Beauveria bassiana*	Polyphagous	T	Cosmopolitan	Cherry et al. (2005)

Nematoda	Steinernematidae	*Steinernema feltiae*	Polyphagous	L-P-A	Cosmopolitan	Athanassiou et al. (2008)
		Steinernema carpocapsae	Polyphagous	L-P-A	Cosmopolitan	Rumbos and Athanassiou (2012)
Nematoda	Heterorhabditidae	*Heterorhabditis bacteriophora*	Polyphagous	L-P-A	Cosmopolitan	Rumbos and Athanassiou (2012)
		Heterorhabditis megidis	Polyphagous	L-P-A	Cosmopolitan	Rumbos and Athanassiou (2012)
Sphingobacteria	Flexibacteraceae	*Bacillus thuringiensis*	Polyphagous	T	Cosmopolitan	CAB International (2005)

[a]E = egg, L = larva, P = pupal stage, A = adult and T = total stage

parasitoids can be categorized as either endo- or ectoparasitoids. Endoparasitoid lives within the host body such as *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae). On the contrary, ectoparasitoid feeds on the host from outside, for example, *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae). However, predators include some species of Hemiptera and Coleoptera and in particular Acarina (mites) as shown in Table 11.4. Some biocontrol agents attack a single insect pest species (monophagous), while other attack several species (oligophagous and polyphagous). Generally, predators tend to attack more species than parasitoids do. Pathogens that cause diseases in SIP consisted of bacteria, viruses, fungi, and nematodes (Flinn and Hagstrum 2001). Also, some pathogens such as *Bacillus thuringiensis* (Berliner) (Eubacteriales: Bacillaceae) and granulosis viruses that commonly infect moth larvae are commercially available for some SIP.

The use of high-quality biological control agents for release is a pivotal step in the successful implementation of biological control programs. Before using a biocontrol agent in an integrated pest management program, it is necessary to know about the efficacy of the biocontrol agents (Fathipour and Maleknia 2016). The efficiency of a biocontrol agent depends on many factors such as temperature, pest species, pest stage, pest density, etc. (Pekár and Hubert 2008). The main measures that are used in the evaluation of biocontrol agents are life table parameters, foraging behavior, and multiple interactions. Studying the characteristics of biological control agents helps to understand their influence on the population dynamics of pest and their influence on the structure of SIP communities in which they exist (Fathipour and Maleknia 2016). Among the life table parameters, the intrinsic rate of increase (r) is a key parameter in the prediction of population growth potential and has been widely used to evaluate efficiency of biocontrol agents (Fathipour and Mirhosseini 2017). For instance, life history parameters of *Xylocoris flavipes* (Reuter) (Heteroptera: Anthocoridae) are investigated on *T. castaneum* at four constant temperatures by Russo et al. (2004). In addition, Bastami et al. (2011) evaluated the life table parameters of three population of *H. hebetor* on *E. kuehniella* in order to find the highest performance population of the parasitoid. In addition to the life table parameters, foraging behaviors including functional response, numerical response, mutual interference, preference, and switching are useful tools for evaluation of SIP natural enemies (for more information, see Rezaei et al. 2019; Tazerouni et al. 2019b). For example, a number of studies determined the host preference and functional response of SIP natural enemies in different conditions (e.g., Parajulee et al. 1994; Plarre et al. 1999; Flinn and Hagstrum 2002; Jarrahi and Safavi 2016). Moreover, studies on multiple interactions like intraguild predation, olfactory response, tritrophic interaction, cannibalism, and competition could be helpful in evaluation of SIP natural enemies (Fathipour and Maleknia 2016). For example, Berger et al. (2017) reported that a combination of the parasitoid *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) and the predator *X. flavipes* is promising component for integrated control of *A. obtectus*. Adarkwah and Scholler (2012) suggested that two-parasitoid combination, *H. hebetor* and *V. canescens*, could be as effective as *H. hebetor* alone to control *P. interpunctella*. The highest

mortality (93%) is achieved with a single release of 20 *H. hebetor* plus 20 *V. canescens* (host-parasitoid ratio was 1:1 for each parasitoid species).

After the selection of the efficient biocontrol agents, it is necessary the pest density and release ratio of biocontrol agents to be evaluated. Monitoring process involves taking multiple samples over time and evaluating biocontrol agent and pest densities, which should be compared with those in the no-release plots over time. Estimating the seed damage level treated and non-treated (control) samples at the end of the biocontrol program would have a key role in selection of efficient natural enemies for SIP management programs (Fathipour and Maleknia 2016). In this case, recommended predator-prey ratios of *Cheyletus* species are 1:10 or 1:100, depending on the moisture content and temperature. Moreover, when the infestation of Acaroid mites is higher than 1000 specimens per kilogram of material or when other insect pests are also present, it is necessary to suppress the population of mites (or insects) chemically (Stejskal et al. 2014).

One of the fundamental processes in biological control programs is the mass rearing and mass release of the biocontrol agents. Generally, a thorough understanding of the biological characteristics of a biocontrol agent is required to achieve successful mass rearing program. Mass production methods usually produce large numbers of the natural enemies, free from contaminants, which can be released immediately in the target site. The goal of mass rearing program is to propagate the maximum number of high-quality insects with minimum labor, space, and cost. However, there are virtually no general rules that can be laid down as to optimum conditions of cage size, environment, feeding, and illumination for breeding these. Each problem demands different treatments, depending on the requirements of individual species and number to be produced, and each scheme is usually based on technique developed in the laboratory and then modified for cheap and large-scale production (Rezaei et al. 2014, 2018). Therefore, quality control parameters are mandatory in the production of biocontrol agents; hence, only the acceptable natural enemies are sold, and the healthy species of natural enemies are released. However, the International Organization for Biological Control Global Working Group on Mass Rearing and Quality Assurance (MRQA) is established in 1980 as the Working Group on Quality Control (WGQC) to assure success of insect mass rearing for pest management programs. Mass rearing of biological control agents consisted of the synchronization of entities including the beneficial species, its host or prey species, and the host plant or food (Singh 1982). For SIP biocontrol agents, some studies have been done to optimize the host specie (e.g., Nayak and Collins 2001; Hasan and Phillips 2010; Jagadeesan et al. 2013), and some research have been investigated the optimization of the beneficial species (e.g., Ayvaz et al. 2008; Ghimire and Phillips 2010). Furthermore, the new and optimized method to mass propagate of SIP natural enemies could be useful in order to perform an acceptable and cost benefit biocontrol programs.

There are various advantages for the natural enemies to exemplify: (1) most pests have an appropriate range of biocontrol agents; (2) they have a suitable ability to find the pests; (3) natural enemies can be self-perpetuating; (4) they commonly are widely distributed geographically; (5) biological control method is an effective in

comparison with other manners; (6) biological control is a low-risk method; (7) biological control is registered for use; and (8) resistance is not a problem for the natural enemies. On the contrary, some disadvantages are reported for the using natural enemies including (1) the shelf life of a biocontrol agents is limited and (2) correct species and timing are essential for biocontrol programs (Hagstrum and Subramanyam 2006). Also, three situations, where the biological control would be a valuable component of integrated pest management, are mentioned by European working group (funded by the COST system) including (1) empty room treatment against stored-product mites, beetles, and moths; (2) preventative treatment of bulk commodities against weevils (*Sitophilus* spp.) and storage mites; and (3) preventative application of egg parasitoids against moths in packaged products. Further, it is stated that the development of a biological control method for bruchids on a large scale must take into account parameters such as (1) the variability of the climatic conditions that determines the level of initial infestation of seeds by the bruchids and their parasitoids and (2) the mass rearing strategy for the parasitoid, its transport, and release at farmer level (Amevoin et al. 2007). It is generally accepted that insecticides are harmful to natural enemies of insect pests. Considerable circumstantial evidence exists regarding the negative effects of pesticides on natural enemies; this subject needs further investigation and validation.

11.3.8 Mating Disruption (MD)

Knowing of the chemical communication of the SIP is valuable for the development of alternative control measures. Mating disruption (MD) is a new biorational method for SIP control. It is based on using high doses of sexual pheromone to confuse males as they try to find and mate with females. According to the literature, the efficiency of sex pheromone is evaluated for different SIP to exemplify, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) (Coffelt and Vick 1987), *T. confusum* (Olsson et al. 2006), *L. serricorne* (Fardisi and Mason 2013), and *C. maculatus* (Shu et al. 1996). However, it is reported that the number of eggs laid and egg viability is considerably reduced when adult male, female, or both male and female *P. interpunctella* are delayed from mating. Delaying females as opposed to males from mating have a greater impact on egg laying and egg viability. It is suggested that techniques such as the use of high-pheromone concentrations or ultrasound, which can delay or disrupt mating, would be an effective behavioral management strategy to suppress populations of *P. interpunctella* (Huang and Subramanyam 2003). Also, MD was concurrently evaluated in the Czech Republic and two other countries. Although it is found to be a promising method, the MD efficacy showed significant variability among the tested buildings, most likely caused by the specific local conditions (Stejskal et al. 2014). However, mass-trapping males with a sex pheromone can theoretically control a population if a large number of males are removed from the population. Moreover, male moths such as *P. interpunctella* can inseminate an average of six females in their lifetimes; thus, a few surviving males in a population under mass-trapping treatment could maintain the reproductive rate of

the population at a level similar to that without mass-trapping (Phillips and Throne 2010). So, MD, in which a treatment area is saturated with an unnaturally high concentration of synthetic sex pheromone and males are unable to locate and successfully mate with females, need to be investigated further for various SIP.

11.3.9 Trapping

The use of traps may assist inspectors in detecting and monitoring insect populations. Being in position for several days or weeks, traps often provide evidence of an infestation that could have occurred between inspection times. Many are highly attractive and can detect insects at level well below what is possible by simple inspection. Traps fall into two types, those that rely on a physical feature to attract and detain insects and those that use a bait to attract insects. Insect species vary considerably in their propensity to be trapped and the type of trap that is most effective. The inspector needs to know which species they are targeting to select the right trap and bait. Species descriptions detail which traps are most useful for the particular group of insects. Before embarking on a trapping program, it is important be clear as to how the results obtained are to be used. For example, there is no point in using traps if the data is not going to be used to direct pest control operations. Equally, trapping may be pointless if insects are so abundant that they can easily be seen (Rees 2004). While traps can often detect SIP at lower densities than other insect-sampling methods, they may not provide reliable information about insect densities. Trap catch is influenced by environmental factors such as temperature, residual food and air movement, and by the physiological states of the insect pests that influence their mobility. Because the environment and the physiological states of the insect pests generally change over time, trap efficiency can change over time. Converting trap catch to absolute insect density can adjust for this variation in trap efficiency and allow managers to better classify insect pest populations as being above or below an insect density threshold at which insect pest suppression is required (Hagstrum and Subramanyam 2006).

There are different types of traps including crevice, pitfall, bait, light, and pheromone-baited traps. A simple crevice trap can be made from a piece of corrugated cardboard. Also, simple pitfall traps can be made from disposable plastic drinking cups or used drink cans. The crevice and pitfall traps are highly effective tools in the detection of free-ranging beetles such as *Tribolium*, *Oryzaephilus*, *Cryptolestes* and the psocid *Liposcelis* in or on grain bulks. Further, a simple bait trap can be made from a mesh bag containing a mix of foodstuffs such as dried carob, dried fruit, groundnuts, and brown rice (Rees 2004).

11.3.10 Packaging and Sealing

Packaging is a system with the objective of protecting the products against different hostile environments in particular SIP. The package is designed to protect the seeds

from the point of manufacture to the point at which it is finally planted. This process often means that the package will have to provide this protection for up to several years (Subramanyam and Hagstrum 2000). The level of SIP resistance depends upon the materials used for packaging, the type of seal, and the package design. *Lasioderma serricorne*, *R. dominica*, and *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) adults and *P. interpunctella* larvae often enter packages by chewing a hole in the packaging material, while *Cryptolestes pusillus* (Schonherr) (Coleoptera: Laemophloeidae), *Oryzaephilus mercator* L. (Coleoptera: Silvanidae), *O. surinamensis*, and *T. castaneum* adults generally enter through existing holes or loose seams. Adults of *C. pusillus*, *L. serricorne*, *O. mercator*, and *T. castaneum* are better penetrators than larvae. Odors emanating from small holes in packaging may cause females to lay eggs near or in these holes. Holes too small for adults may allow newly hatched larvae to enter (Hagstrum and Subramanyam 2006). The retail marketplace is the final points of contact between seed processors and consumers, and studies have been shown SIP are commonly found in retail stores and are capable of infesting packaged seeds (Scheff et al. 2018).

A number of studies have reported on packaging and sealing. For instance, it is reported that the use of methoprene-treated packaging could be a valuable technology that seed manufacturers could utilize to prevent infestations of their products and protect the safety and integrity of their packaged seed. Methoprene-treated packaging prevented penetration of *P. interpunctella* and *T. variabile* into sealed packages, but when presented with a pinhole defect, *P. interpunctella* easily invaded and infested the packaged seed (Scheff et al. 2018). Also, the application of 5% allyl disulfide to multilayered rice films as novel packaging materials with effective anti-insect activity (Lee et al. 2017). Further, seals and closures can often be improved by changing glue patterns or the type of glue used. Generally, a glue pattern that forms a complete seal with no channels for the insect to crawl through may prevent insect entry. Insect resistance can also be improved by overwrapping the packages with a material such as oriented polypropylene films. In order to maximize the effectiveness of overwraps, they should fit tightly around the package (Subramanyam and Hagstrum 2000).

Application of packaging, sanitation, and exclusion methods together is more effective than using any one of these methods alone. The pests will eventually find defects or damage in a package that allows them to enter. Using sanitation to reduce insect numbers will allow packaging to protect a seed longer. Exclusion limits the number of insects entering a facility, and sanitation reduces the reproduction of those that have already entered (Hagstrum and Subramanyam 2006). Also, bin sealing is critical for effective use of chemical fumigants when needed. Hence, packaging seeds for finished products at both wholesale and retail levels of marketing must be resistant to penetration by SIP. So, it is necessary that seeds be sealed very well to prevent SIP and be constructed of durable materials to resist penetration. Insect-resistant packaging has improved over the years, but with the development of new packaging materials, improvements can still be expected (Phillips and Throne 2010).

11.4 Integrated SIP Management (ISM)

Integrated SIP Management (ISM) implies the effective use of as many control measures as are compatible, in order to suppress pest populations below damaging levels and optimize yields with minimum disruption of or damage to the environment. There is the need to search for a combination of control options that blend to produce satisfactory results. Some control strategies are more suitable for use than others under certain conditions. The decision as to what should be the central theme in any IPM program will depend on what is likely to have the greatest pay off in the widest possible sense. No single approach is the best for all situations. Moreover, it is accepted that a single control strategy is unlikely to produce satisfactory results (Jackai 1995; Tazerouni et al. 2019a). It is important to mention that ecology, sampling, cost-benefit analysis, and pest management programs for SIP are likely to be unique for each location in the marketing system and for each time that SIP are managed. Hence, correct identification of SIP is very important because the effectiveness of insect pest management methods differs among species. Also, knowing which insect species are present allows the published information about those species to be used to design and implement a study, a sampling program, or an insect pest management program (Hagstrum and Subramanyam 2006). So, there is a need for monitoring of the SIP incidence and identification of the pest and in particular knowledge of the environment and pest biology in order to make timely and appropriate decisions on the use of control measures. In this case, attractant traps could be useful tools, and they can be a key component of ISM (Phillips and Throne 2010).

Although the use of non-chemical methods such as physical and biological controls has been employed for SIP management, many studies indicate that the seed producers continue to use broad-spectrum insecticides for control of SIP as first, easily accessible and reliable option in most area of the world (Stejskal et al. 2014). The overuse of insecticides not only increases SIP resistance to this chemical compounds but also causes high mortality of natural enemies and non-target organism. Hence, to reduce the risk of SIP resistance to chemical pesticides, the threat to food safety, environmental pollution, human health problems, and dangerous side effects to non-target beneficial organisms such as natural enemies and pollinators, the below strategies are suggested by Fathipour and Mirhosseini (2017) considering the use of insecticides: (1) the use of bio-insecticides instead of synthetic ones; (2) the use of selective insecticides instead of broad-spectrum ones; (3) minimizing insecticide use; (4) mixing insecticides with different modes of action; and (5) rotation of insecticides.

Since ISM is a comprehensive approach that integrates many components to maximize the advantages and minimize the disadvantages of the management plan, all possible interactions among components of the ISM system should be taken into consideration (Fathipour and Sedaratian 2013). Thus, it is important to consider the different trophic interactions in any combination methods, in particular HPR and biological control agents. For success in the integration, it is recommended that the effect of HPR on the population growth and performance of the third trophic level

(biocontrol agents) be tested (Fathipour and Mirhosseini 2017). For instance, Schmale et al. (2003) showed that the combination of resistant bean varieties with the parasitoid *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae) is favorable for suppressing damage by *A. obtectus*. In addition, the P84c3, a resistant variety of maize against *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), in combination with predator *Teretrius nigrescens* Lewis (Coleoptera: Histeridae), causes to reduction of 80% in progeny number, 81% grain weight loss, and 75% frass production arise from *P. truncatus* (Bergvinson and García-Lara 2011). However, HPR can be a valuable component of an ISM system, compatible with other control methods such as physical, sanitation, seed treatment, and biological control. For example, it is suggested that the storage of resistant varieties of cowpea is compatible with application of low pressure application in integrated seed management of *C. maculatus*. Integration of low pressure with resistant plant cultivars ensures that all treated insects die and none survives to propagate tolerance to resistant plant materials (Mbata et al. 2009). Further, combining early planting and early harvest with resistant varieties could be an appropriate tactic for resource-poor farmers to effectively manage *S. zeamais* in the Niger Delta agro-ecological zone (Zakka et al. 2015). Moreover, when a seed is attacked by multiple pests, the resistance status of the variety chosen for the management program should be considered for all SIP. A seed variety that is highly resistant to one pest might be susceptible to another pest in the same conditions. Such conflicts should be taken into account before designing an appropriate program to SIP control.

Some general characteristics of pest management methods for SIP may be useful in selecting a method or methods. Biological control, fumigation, and extreme temperatures of a facility penetrate into places where insects hide. A good sanitation program can discover and eliminate many insect infestations and make other insect pest management methods more effective. Residual insecticides and insect-resistant packaging are the primary methods that provide long-term protection against SIP. However, residual insecticides lose their effectiveness over time, and SIP may eventually penetrate some of the insect-resistant packages. Refrigerated storage, cooling seeds by aeration, and use of natural enemies can provide long-term protection in some situations. Natural enemies can be self-perpetuating, but their effectiveness is often reduced by standard business practices and other insect pest management methods. However, knowledge of SIP mobility, the sources of an insect infestation, and environmental factors can be used to improve the ISM program (Hagstrum and Subramanyam 2006).

Commonly, complexity of control methods can be a disadvantage because complex methods are more difficult to use. On the contrary, complexity can be an advantage because it increases the number of ways in which an insect pest management method can be used and may allow insect pest management to be done more cost-effectively. Careful integration of more than one insect pest management method can make pest management for SIP more effective without increasing the cost (Hagstrum and Subramanyam 2006). For instance, it is reported that at half the label rates or lower, diatomaceous earths-spinosad and diatomaceous earths-deltamethrin combinations are effective alternative grain protectants that are safer

and possibly cheaper against *P. truncatus*, *S. zeamais*, and *T. castaneum* (Machekano et al. 2017). Additionally, the combination of essential oils from *Rosmarinus officinalis* (L.) and *Perovskia atriplicifolia* (Benth) with gamma radiation increases the mortality of *T. castaneum* adults. The mortality is 3–6 times higher than could be expected from the sum of the effects of each of the treatments (Ahmadi et al. 2013).

Integrated pest management is a decision support system for the selection and use of pest management tactics, either alone or harmoniously coordinated into a management strategy, based on cost-benefit analysis that take into account the interests of and impacts on producers, society, and the environment (Fathipour and Mirhosseini 2017). In this case, computer simulation models can be used to compare the effectiveness of different pest management methods for SIP or combinations of insect pest management methods. These models also can be used to evaluate the effectiveness of different implementation options and to optimize the timing of pest management programs for SIP (Hagstrum and Subramanyam 2006).

11.5 Conclusion

Many insects and mites species are well known as SIP. Since the seeds are stored for prolonged period, they are vulnerable to pest attack. Also, seeds are marketed and transported over long geographical distances which elevate their global spread. Currently, protecting seeds against SIP have become more difficult due to the worldwide distribution of the pests, increasing pesticides resistance and decreasing pesticides active ingredients (Stejskal et al. 2014). Although IPM programs for SIP population control can help to reduce the use of harmful pesticides and promote food safety, they have received less attention than they deserve because of a key gap in our knowledge, which is the availability of comprehensive regional management programs that consolidate all the available techniques in an appropriate program to manage SIP populations in such a manner that economic damage is avoided and adverse side effects are minimized (Fathipour and Mirhosseini 2017). Accordingly, this chapter has tried to introduce all the available and potential control measures of SIP that might from different components of an ISM program and to discuss the factors determining their interactions. Also, further research should be done theoretically or experimentally to investigate the integration of various control measures that are cost-effective for ISM programs. However, full-scale testing under a different of conditions is needed to evaluate the efficacy of integrated methods for commercial use. Hence, economic analyses are needed to stablish which integration of SIP management methods is cost-effective.

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References

Abdel-Rahman HA, Hodson AC, Christensen CM (1968) Development of *Plodia interpunctella* (Hb.) (Lepidoptera, Phycitidae) on different varieties of corn at two levels of moisture. J Stored Prod Res 4:127–133

Abraham YJ, Moore D, Godwin G (1990) Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). Bull Entomol Res 80:121–128

Acheampong A, Ayertey JN, Eziah VY, Ifie BE (2019) Susceptibility of selected maize seed genotypes to *Sitophilus zeamais* (Coleoptera: Curculionidae). J Stored Prod Res 81:62–68

Adarkwah C, Scholler M (2012) Biological control of *Plodia interpunctella* (Lepidoptera: Pyralidae) by single and double releases of two larval parasitoids in bulk stored wheat. J Stored Prod Res 51:1–5

Adebisi MA, Daniel IO, Ajala MO (2004) Storage life of soybean (Glycine max L. Merril) seeds after seed dressing. J Trop Agric 42:3–7

Adedire CO, Akinneye JO (2004) Biological activity of tree marigold, *Tithonia diversifolia*, on cowpea seed bruchid, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Ann Appl Biol 144:185–189

Adetunji JF (1988) A study of the resistance of some sorghum seed cultivars to *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J Stored Prod Res 24:67–71

Ahmadi M, Abd-alla AMM, Moharramipour S (2013) Combination of gamma radiation and essential oils from medicinal plants in managing *Tribolium castaneum* contamination of stored products. Appl Radiat Isot 78:16–20

Ahmed K, Khalique F, Afzal M, Tahir ME, Malik BA (1989) Variability in chickpea (*Cicer arietinum* L.) genotypes for resistance to *Callosobruchus maculatus* F.(Bruchidae). J Stored Prod Res 25:97–99

Allotey J, Azalekor W (2000) Some aspects of the biology and control using botanicals of the rice moth, *Corcyra cephalonica* (Stainton), on some pulses. J Stored Prod Res 36:235–243

Allotey J, Goswami L (1990) Comparative biology of two phycitid moths, *Plodia interpunctella* (Hubn.) and *Ephestia cautella* (Wlk.) on some selected food media. Int J Trop Insect Sci 11:209–215

Allotey J, Morris JG (1993) Biology of *Cathartus quadricollis* Guerin-Meneville (Coleoptera: Silvanidae) on some selected food media. Int J Trop Insect Sci 14:61–68

Amante M, Russo A, Schöller M, Steidle JL (2017a) Olfactory host location in the rusty grain beetle parasitoid *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae). J Stored Prod Res 71:1–4

Amante M, Schöller M, Hardy IC, Russo A (2017b) Reproductive biology of *Holepyris sylvanidis* (hymenoptera: Bethylidae). Biol Control 106:1–8

Amevoin K, Sanon A, Apossaba M, Glitho IA (2007) Biological control of bruchids infesting cowpea by the introduction of *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae) adults into farmers' stores in West Africa. J Stored Prod Res 43:240–247

Amusa OD, Ogunkanmi LA, Adetumbi JA, Akinyosoye ST, Ogundipe OT (2018) Genetics of bruchid (*Callosobruchus maculatus* Fab.) resistance in cowpea (*Vigna unguiculata* (L.) Walp.). J Stored Prod Res 75:18–20

de Andrade DK, de Oliveira JV, Navarro DMDAF, Santos JPO (2016) Control of *Callosobruchus maculatus* (FABR.) (Coleoptera: Chrysomelidae: Bruchinae) in *Vigna unguiculata* (L.) WALP. with essential oils from four *Citrus* spp. plants. J Stored Prod Res 68:25–32

Appleby JH, Credland PF (2004) Environmental conditions affect the response of West African *Callosobruchus maculatus* (Coleoptera: Bruchidae) populations to susceptible and resistant cowpeas. J Stored Prod Res 40:269–287

Arabi F, Moharramipour S, Sefidkon F (2008) Chemical composition and insecticidal activity of essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera:

Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Int J Trop Insect Sci 28:144–150
Arbogast RT (1976) Population parameters for *Oryzaephilus surinamensis* and *O. mercator*: effect of relative humidity. Environ Entomol 5:738–742
Arbogast RT, Flaherty BR, Press JW (1983) Demography of the predaceous bug *Xylocoris sordidus* (Reuter). Am Midl Nat 109:398–405
Armitage DM, Cogan PM, Wilkin DR (1994) Integrated pest management in stored grain: combining surface insecticide treatments with aeration. J Stored Prod Res 30:303–319
Arnason JT, Gale J, De Beyssac BC, Sen A, Miller SS, Philogene BJR, Lambert JDH, Fulcher RG, Serratos A, Mihm J (1992) Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). J Stored Prod Res 28:119–126
Arthur FH, Hartzer KL, Throne JE, Flinn PW (2017) Freezing for control of stored-product psocids. J Stored Prod Res 72:166–172
Ashworth JR (1993a) The biology of *Ephestia elutella*. J Stored Prod Res 29:199–205
Ashworth JR (1993b) The biology of *Lasioderma serricorne*. J Stored Prod Res 29:291–303
Athanassiou CG, Kavallieratos NG, Tsaganou FC, Vayias BJ, Dimizas CB, Buchelos CT (2003) Effect of grain type on the insecticidal efficacy of SilicoSec® against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Prot 22:1141–1147
Athanassiou CG, Kavallieratos NG, Tsakiri JB, Xyrafidis SN, Vayias BJ (2006) Effect of temperature and humidity on the insecticidal effect of SilicoSec® against *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae. J Econ Entomol 99:1520–1524
Athanassiou CG, Palyvos NE, Kakouli-Duarte T (2008) Insecticidal effect of *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) against *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) in stored wheat. J Stored Prod Res 44:52–57
Athanassiou CG, Kavallieratos NG, Lazzari FA (2014) Insecticidal effect of Keepdry® for the control of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on wheat under laboratory conditions. J Stored Prod Res 59:133–139
Aye TT, Shim JK, Ha DM, Kwon YJ, Kwon JH, Lee KY (2008) Effects of gamma irradiation on the development and reproduction of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). J Stored Prod Res 44:77–81
Ayvaz A, Karasu E, Karabörklü S, Tunçbilek AŞ (2008) Effects of cold storage, rearing temperature, parasitoid age and irradiation on the performance of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). J Stored Prod Res 44:232–240
Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils–a review. Food Chem Toxicol 46:446–475
Banks HJ, Field JB (1995) Physical methods for insect control in stored-grain ecosystem. In: Jayas DS, White NDG Subramanyam B (eds) Stored grain ecosystem, Marcel Dekker New York, NY, pp 353-409
Barker PS (1967) The effects of high humidity and different temperatures on the biology of *Tyrophagus putrescentiae* (Schrank) (Acarina: Tyrogliphidae). Can J Zool 45:91–96
Bastami FA, Fathipour Y, Talebi AA (2011) Comparison of life table parameters of three populations of braconid wasp, *Habrobracon hebetor* (Hym.: Braconidae) on *Ephestia kuehniella* (Lep.: Pyralidae) in laboratory conditions. Appl Entomol Phytopathol 78:153–176
Beal RS (1961) Descriptions, biology, and notes on the identification of some *Trogoderma* larvae. Technical Bulletin No. 1228. Agricultural Research Service United States Department of Agriculture, Washington, DC
Beal RS Jr (1998) Taxonomy and biology of Nearctic species of *Anthrenus* (Coleoptera: Dermestidae). Trans Am Entomol Soc 124:271–332

Belles X, Halstead DGH (1985) Identification and geographical distribution of *Gibbium aequinoctiale* Boieldieu and *Gibbium psylloides* (Czenpinski) (Coleoptera: Ptinidae). J Stored Prod Res 21:151–155

Benjamin DM (1968) Insects and mites on tea in Africa and adjacent islands. East Afr Agric For J 33:345–357

Bentley EW (1944) The biology and behaviour of *Ptinus Tectus* Boie (Coleoptera, Ptinidae), A pest of stored products: V. Humidity reactions. J Exp Biol 20:152–158

Berger A, Degenkolb T, Vilcinskas A, Schöller M (2017) Evaluating the combination of a parasitoid and a predator for biological control of seed beetles (Chrysomelidae: Bruchinae) in stored beans. J Stored Prod Res 74:22–26

Bergvinson DJ, García-Lara S (2011) Synergistic effects of insect-resistant maize and *Teretrius nigrescens* on the reduction of grain losses caused by *Prostephanus truncatus* (Horn.). J Stored Prod Res 47:95–100

Birch LC (1948) The intrinsic rate of natural increase of an insect population. J Anim Ecol 17:15–26

Bodlah I, Bodlah MA, Naeem M, Khaliq S, Aihetasham A (2016) First record of *Anisopteromalus calandrae* (howard, 1881) (hymenoptera: chalcidoidea: pteromalidae) as a potential bio-control agent of stored grain beans from rawalpindi and Islamabad. Asian J Agric Biol 4:73–76

Borowski J, Sławski M (2017) *Mezium americanum* (Laporte de Castelnau, 1840), a new synonym of *Mezium sulcatum* (Fabricius, 1781) (Coleoptera: Ptinidae, Gibbiinae, Meziini). World Sci News 65:171–175

Breese MH, Wise TE (1959) The biology of *Nausibius clavicornis* (Kug.) (Col., Cucujidae). Bull Entomol Res 50:237–258

Broadhead E, Hobby BM (1944) Studies on a species of *Liposcelis* (Corrodentia, Liposcelidae) occurring in stored products in Britain. Part I. Entomol Mon Mag 80:957–958

CAB International (2005) Crop protection compendium. CAB International, Wallingford

Carey JR (1993) Applied demography for biologists. Oxford University Press, New York, NY

Carey JR (2001) Insect biodemography. Annu Rev Entomol 46:79–110

Carter MR, Manglitz GR, Rethwisch MD, Vogel KP (1988) A seed midge pest of big bluestem. J Range Manag Arch 41:253–254

Center TD, Johnson CD (1973) Comparative life histories of *Sennius* (Coleoptera: Bruchidae). Environ Entomol 2:669–672

Chanbang Y, Arthur FH, Wilde GE, Throne JE (2008) Hull characteristics as related to susceptibility of different varieties of rough rice to *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). J Stored Prod Res 44:205–212

Chatterjl S, Sarup P (1959) Biology of *Thorictodes heydeni* Reitt.(Thorictidae: Coleoptera) a pest of stored cereals and their products in India. Indian J Entomol 21:282–283

Cherry AJ, Abalo P, Hell K (2005) A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. J Stored Prod Res 41:295–309

Childers CC, Woodruff RE (1980) A bibliography of the coffee bean Weevil *Araecerus fasciculatus* (Coleoptera: Anthribidae). Bull Ent Soc Am 26:384–394

Coffelt JA, Vick KW (1987) Sex pheromones of *Ephestia cautella* (Walker) (Lepidoptera Pyralidae): influence of mating on pheromone titer and release rate. J Stored Prod Res 23:119–123

Collins DA (2006) A review of alternatives to organophosphorus compounds for the control of storage mites. J Stored Prod Res 42:395–426

Consoli FL, Amaral Filho BF (1995) Biology of *Sitotroga cerealella* (Oliv.) (Lepidoptera: Gelechiidae) reared on five corn (maize) genotypes. J Stored Prod Res 31:139–143

Coskuncu KS, Kovanci B (2005) Studies on the biology and distribution of cadelle, *Tenebroides mauritanicus* (L.) (Coleoptera: Trogossitidae) in Bursa, Turkey. J Entomol 2:17–20

Cotton RT (1927) Notes on the biology of the meal worms, *Tenebrio Molitor* Linne and *T. Obscurus* Fab. Ann Entomol Soc Am 20:81–86

Cotton RT, Good NE (1937) Annotated list of the insects and mites associated with stored grain and cereal products, and of their arthropod parasites and predators (No. 258). US Dept. of Agriculture, Washington, DC

Cox PD, Pinniger DB (2007) Biology, behaviour and environmentally sustainable control of *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae). J Stored Prod Res 43:2–32

Cuperus GW, Prickett CK, Bloome PD, Pitts JT (1986) Insect populations in aerated and unaerated stored wheat in Oklahoma. J Kansas Entomol Soc 59:620–627

Damon A (2000) A review of the biology and control of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). Bull Entomol Res 90:453–465

Danielsen C, Hansen LS, Nachman G, Herling C (2004) The influence of temperature and relative humidity on the development of *Lepidoglyphus destructor* (Acari: Glycyphagidae) and its production of allergens: a laboratory experiment. Exp Appl Acarol 32:151–170

Darrouzet E, Huignard J, Chevrier C (2002) Effect of differential host exposure on reproduction in the solitary ectoparasitoid *Eupelmus vuilleti*. Entomol Exp Appl 103:73–81

Dick KE, Credland PF (1986) Variation in the response of *Callosobruchus maculatus* (F.) to a resistant variety of cowpea. J Stored Prod Res 22:43–48

Dindo ML, Rezaei M, De Clercq P (2019) Improvements in the rearing of the tachinid parasitoid *Exorista larvarum* (Diptera: Tachinidae): influence of adult food on female longevity and reproduction capacity. J Insect Sci 19:1–6

Dobie P (1974) The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by *Sitophilus zeamais* Motsch.(Coleoptera, Curculionidae). J Stored Prod Res 10:183–197

Drinkwater TW, Giliomee JH, Pringle KL (1990) The geographical distribution of false wireworms, *Somaticus* species (Coleoptera: Tenebrionidae) associated with maize in South Africa. J Entomol Sci South Afr 53:127–136

Dunkel FV, Barak AV, Harein PK (1982) Geographical distribution of *Cynaeus angustus* (LeConte) (Coleoptera: Tenebrionidae) and its association with stored products. J Biogeogr 9:345–352

Edde PA (2012) A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. J Stored Prod Res 48:1–18

Edmonds RP, Borden JH, Angerilli NPD, Rauf A (2000) A comparison of the developmental and reproductive biology of two soybean pod borers, *Etiella* spp. in Indonesia. Entomol Exp Appl 97:137–147

Eidt-Wendt J, Schulz FA (1990) Studies on the biology and ecology of *Pagiocerus frontalis* (Fabricius) (Coleoptera: Scolytidae) infesting stored maize in Ecuador. In: Fifth International Working Conference on Stored-Product Protection, Bordeaux, France, pp 9–14

El-Naggar SM, Mikhaiel AA (2011) Disinfestation of stored wheat grain and flour using gamma rays and microwave heating. J Stored Prod Res 47:191–196

Espinoza JC, Infante F, Castillo A, Pérez J, Nieto G, Pinson EP, Vega FE (2009) The biology of *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) under field conditions. Biol Control 49:227–233

Fakir GA, Mia MAT (2004) The quality of farmer-saved rice seeds in Bangladesh. In: National Workshop on Seed Health Improvement for Pest Management and Crop Protection, Dhaka, Bangladesh, pp 12–36

FAO (2017) International movement of seeds. International standard for phytosanitary measures. Produced by the Secretariat of the International Plant Protection Convention. IPPC, Rome

Farahani S, Talebi AA, Rakhshani E, van Achterberg C (2013) A study of the genus *Blacus nees* (Hymenoptera: Braconidae: Blacinae) in Iran, with three new records. Ukrainska Entomofaunistyka 4:3–11

Fardisi M, Mason LJ (2013) Influence of lure (food/sex pheromone) on young mated cigarette beetle (*Lasioderma serricorne* (F.)) (Coleoptera: Anobiidae) flight initiation. J Stored Prod Res 53:15–18

Fathipour Y, Maleknia B (2016) Mite predators. In: Omkar (ed) Ecofriendly pest management for food security. Academic Press, New York, NY, pp 329–366

Fathipour Y, Mirhosseini MA (2017) Diamondback moth (*Plutella xylostella*) management. In: Reddy GVP (ed) Integrated management of insect pests on canola and other *Brassica* oilseed crops. CABI, Croydon, pp 13–43

Fathipour Y, Sedaratian A (2013) Integrated management of *Helicoverpa armigera* in soybean cropping systems. In: El-Shemy H (ed) Soybean-pest resistance. INTECH Open Access Publisher, Rijeka, pp 231–280

Fields PG (1992) The control of stored-product insects and mites with extreme temperatures. J Stored Prod Res 28:89–118

Flinn PW (1998) Temperature effects on efficacy of *Choetospila elegans* (Hymenoptera: Pteromalidae) to suppress *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat. J Econ Entomol 91:320–323

Flinn PW, Hagstrum DW (2001) Augmentative releases of parasitoid wasps in stored wheat reduces insect fragments in flour. J Stored Prod Res 37:179–186

Flinn PW, Hagstrum DW (2002) Temperature-mediated functional response of *Theocolax elegans* (Hymenoptera: Pteromalidae) parasitizing *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat. J Stored Prod Res 38:185–190

Gagnepain C, Rasplus JY (1989) *Caryedon serratus* and its parasitoids in the savanna around Lamto, Ivory Coast. Entomophaga 34:559–567

Ghimire MN, Phillips TW (2010) Mass rearing of *Habrobracon hebetor* Say (Hymenoptera: Braconidae) on larvae of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae): effects of host density, parasitoid density, and rearing containers. J Stored Prod Res 46:214–220

Golizadeh A, Abedi Z (2016) Comparative performance of the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) on various wheat cultivars. J Stored Prod Res 69:159–165

Golob P (1997) Current status and future perspectives for inert dusts for control of stored product insects. J Stored Prod Res 33:69–79

Golob P, Kilminster A (1982) The biology and control of *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) infesting red kidney beans. J Stored Prod Res 18:95–101

Gusmão NM, de Oliveira JV, Navarro DMAF, Dutra KA, da Silva WA, Wanderley MJ (2013) Contact and fumigant toxicity and repellency of *Eucalyptus citriodora* Hook., *Eucalyptus staigeriana* F., *Cymbopogon winterianus* Jowitt and *Foeniculum vulgare* Mill. essential oils in the management of *Callosobruchus maculatus* (FABR.) (Coleoptera: Chrysomelidae, Bruchinae). J Stored Prod Res 54:41–47

Hadaway AB (1956) The biology of the dermestid beetles, *Trogoderma granarium* Everts and *Trogoderma versicolor* (Creutz.). Bull Entomol Res 46:781–796

Hafeez MAMA, Chapman G (1966) Effects of temperature and high relative humidity on the rate of development and mortality of *Latheticus oryzae* Waterhouse (Coleoptera, Tenebrionidae). J Stored Prod Res 1:235–242

Hagstrum D, Subramanyam B (2006) Fundamentals of stored-product entomology. Elsevier, Amsterdam

Hagstrum D, Subramanyam B (2009) Stored-product insect resource. Elsevier, Amsterdam

Hagstrum D, Klejdysz T, Subramanyam B, Nawrot J (2013) Atlas of stored-product insects and mites. Elsevier, Amsterdam

Hajek AE (2004) Natural enemies: an introduction to biological control. Cambridge University Press, Cambridge

Halstead DGH (1967) Biological studies on species of *Palorus* and *Coelopalorus* with comparative notes on *Tribolium* and *Latheticus* (Coleoptera: Tenebrionidae). J Stored Prod Res 2:273–313

Halstead DGH (1968a) Observations on the biology of *Murmidius ovalis* (Beck) (Coleoptera: Cerylonidae). J Stored Prod Res 4:13–21

Halstead DGH (1968b) Some observations on the biology of *Lophocateres pusillus* (Klug) (Coleoptera: Trogositidae). J Stored Prod Res 4:197–202

Hasan MM, Phillips TW (2010) Mass-rearing of the redlegged ham beetle, *Necrobia rufipes* De Geer (Coleoptera: Cleridae) for laboratory research. J Stored Prod Res 46:38–42
Hassan SA (1993) The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook. Pestic Sci 37:387–391
Háva J (2015) Three new species of *Orphinus Motschulsky*, 1858 from Thailand (Coleoptera: Dermestidae: Megatominae). Arquivos Entomolóxicos 13:225–229
Háva J, Herrmann A, Clark WH (2011) *Phradonoma nobile* (Reitter, 1881) (Coleoptera: Dermestidae), first record of the genus *Phradonoma Jacquelin* du Val, 1859 in the Nearctic region. Coleopt Bull 65:434–435
Hefner M, Allen B, Moses C (2018) Nevada pesticide applicator's certification workbook. University of Nevada Cooperative Extension, Las Vegas, NV
Hemp C, Dettner K (2003) Description of larvae of the genus *Formicomus laferte*, and data on the life cycles of *Omonadus floralis* (Linne) and *Notoxus monoceros* (Linne) (Coleoptera: Anthicidae). Coleopt Bull 57:361–369
Hertlein MB, Thompson GD, Subramanyam B, Athanassiou CG (2011) Spinosad: a new natural product for stored grain protection. J Stored Prod Res 47:131–146
Hinton HE, Stephens FL (1941) Notes on the biology and immature stages of *Cryptophagus acutangulus*, Gyll. (Col., Cryptophagidae). Bull Entomol Res 32:135–143
Hora AM (1934) On the biology of the mite, glycyphagus domesticus de geer. (tyroglyphidae, acarina). Ann Appl Biol 21:483–494
Horstmann K, Shaw MR (1984) The taxonomy and biology of *Diadegma chrysostictos* (Gmelin) and *Diadegma fabricianae* sp. n. (Hymenoptera: Ichneumonidae). Syst Entomol 9:329–337
Howe RW (1956) The biology of the two common storage species of *Oryzaephilus* (coleoptera, cucujidae). Ann Appl Biol 44:341–355
Howe RW (1973) Loss of viability of seed in storageattributable to infestations of insects and mites. Seed Sci Technol 1:562–586
Howe RW, Burges HD (1951) Studies on beetles of the family ptinidae.* VI.—the biology of *Ptinus fur* (L.) and *P. sexpunctatus* Panzer. Bull Entomol Res 42:499–511
Howe RW, Lefkovitch LP (1957) The distribution of the storage species of *Cryptolestes** (Col., Cucujidae). Bull Entomol Res 48:795–809
Huang Y, Ho SH (1998) Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. J Stored Prod Res 34:11–17
Huang F, Subramanyam B (2003) Effects of delayed mating on reproductive performance of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). J Stored Prod Res 39:53–63
Hubert J (2012) The pest importance of stored product mites (Acari: AcariDida). Nova Science Publishers Inc., New York, NY
Imamura T, Murata M, Miyanoshita A (2008) Biological aspects and predatory abilities of hemipterans attacking stored-product insects. Jpn Agr Res Q 42:1–6
Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608
Jackai LE (1995) Integrated pest management of borers of cowpea and beans. Int J Trop Insect Sci 16:237–250
Jackai LEN, Asante SK (2003) A case for the standardization of protocols used in screening cowpea, *Vigna unguiculata* for resistance to *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). J Stored Prod Res 39:251–263
Jackai LE, Singh SR (1983) Varietal resistance in the integrated pest management of cowpea pests. Int J Trop Insect Sci 4:199–204
Jacob TA (1988) The effect of temperature and humidity on the developmental period and mortality of *Typhaea stercorea* (L.) (Coleoptera: Mycetophagidae). J Stored Prod Res 24:221–224
Jacob TA (1996) The effect of constant temperature and humidity on the development, longevity and productivity of *Ahasverus advena* (Waltl.) (Coleoptera: Silvanidae). J Stored Prod Res 32:115–121

Jacob TA, Cox PD (1977) The influence of temperature and humidity on the life-cycle of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). J Stored Prod Res 13:107–118

Jacobson M (1989) Botanical pesticides, past, present, and future. In: Arnason JT, Philogene BJR, Morand P (eds) Insecticides of plant origin. American Chemical Society, Washington DC, pp 1–10

Jagadeesan R, Nayak MK, Dawson K, Byrne V, Collins PJ (2013) Dietary media for mass rearing of rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) and flat grain beetle, *Cryptolestes pusillus* (Schonherr) (Coleoptera: Cucujidae). J Stored Prod Res 55:68–72

Jarrahi A, Safavi SA (2016) Temperature-dependent functional response and host preference of *Habrobracon hebetor* between fungus-infected and uninfected *Ephestia kuehniella* larvae. J Stored Prod Res 67:41–48

Jemâa JMB, Tersim N, Toudert KT, Khouja ML (2012) Insecticidal activities of essential oils from leaves of Laurus nobilis L. from Tunisia, Algeria and Morocco, and comparative chemical composition. J Stored Prod Res 48:97–104

Kadej M, Guziak J, Marczak D (2017) A detailed updated description of the morphology of the larva of *Reesa vespulae* (Coleoptera: Dermestidae: Megatominae: Megatomini). Fla Entomol 100:286–292

Kambouzia J, Negahban M, Moharramipour S (2009) Fumigant toxicity of *Eucalyptus leucoxylon* against stored product insects. Am-Eurasian J Sustain Agric 3:229–233

Karimi-Pormehr MS, Borzoui E, Naseri B, Dastjerdi HR, Mansouri SM (2018) Two-sex life table analysis and digestive physiology of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) on different barley cultivars. J Stored Prod Res 75:64–71

Khattak SUK, Hamed M, Khatoon R, Mohammad T (1987) Relative susceptibility of different mungbean varieties to *Callosobruchus maculatus* F.(Coleoptera: Bruchidae). J Stored Prod Res 23:139–142

Kučerová Z, Horak P (2004) Arthropod infestation in samples of stored seeds in the Czech Republic. Czech J Genet Plant 40:11–16

Kumar S, Mohapatra D, Kotwaliwale N, Singh KK (2017) Vacuum hermetic fumigation: a review. J Stored Prod Res 71:47–56

Kuronic Z (1998) Diatomaceous earths, a group of natural insecticides. Review. J Stored Prod Res 34:87–97

Lazarević J, Radojković A, Kostić I, Krnjajić S, Mitrović J, Kostić MB, Novaković T, Branković Z, Branković G (2018) Insecticidal impact of alumina powders against *Acanthoscelides obtectus* (Say). J Stored Prod Res 77:45–54

Lee BH, Annis PC, Choi WS (2004) Fumigant toxicity of essential oils from the Myrtaceae family and 1, 8-cineole against 3 major stored-grain insects. J Stored Prod Res 40:553–564

Lee SH, Chang Y, Na JH, Han J (2017) Development of anti-insect multilayered films for brown rice packaging that prevent *Plodia interpunctella* infestation. J Stored Prod Res 72:153–160

Lefkovitch LP (1967) A laboratory study of *Stegobium paniceum* (L.) (Coleoptera: Anobiidae). J Stored Prod Res 3:235–249

Lieberman FV, Dicke F, Hills OA (1961) Some insect pests of important seed crops. In: U.S. Dept. Agr. Yearbook, pp 251–258

Lim J, Oh M, Lee J, Lee S (2007) *Cephalonomia gallicola* (Hymenoptera: Bethylidae), new to Korea, an ectoparasitoid of the cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae). J Asia Pac Entomol 10:335–338

Limonta L, Locatelli DP (2015) Susceptibility of eggs of *Idaea inquinata* (Scop.) (Lepidoptera, Geometridae) to high and low temperatures. J Stored Prod Res 60:1–4

Longstaff BC (1981) Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. Prot Ecol 3:83–130

Lopes LM, Sousa AH, Santos VB, Silva GN, Abreu AO (2018) Development rates of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in landrace cowpea varieties occurring in southwestern Amazonia. J Stored Prod Res 76:111–115

Lotfalizadeh H, Gharali B (2008) Pteromalidae (Hymenoptera: Chalcidoidea) of Iran: new records and a preliminary checklist. Entomofauna 6:93–120

Machekano H, Mvumi BM, Chinwada P, Richardson-Kageler SJ, Rwafa R (2017) Efficacy of diatomaceous earths and their low-dose combinations with spinosad or deltamethrin against three beetle pests of stored-maize. J Stored Prod Res 72:128–137

Maldonado SG, Marín-Jarillo A, Castellanos JZ, De Mejía EG, Acosta-Gallegosc JA (1996) Relationship between physical and chemical characteristics and susceptibility to *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae) and *Acanthoscelides obtectus* (Say) in common bean (*Phaseolus vulgaris* L.) varieties. J Stored Prod Res 32:53–58

Marouf A, Ebrahimi E (2018) First report of *Baryscapus bruchophagi* (Hym.: Eulophidae) from Iran. J Entomol Sci Iran 38:361–363

Matin AA, Hooper GHS (1974) Susceptibility of *Rhyzopertha dominica* to ionizing radiation. J Stored Prod Res 10:199–207

Mbata GN (1993) Evaluation of susceptibility of varieties of cowpea to *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic.) (Coleoptera: Bruchidae). J Stored Prod Res 29:207–213

Mbata GN, Phillips TW, Payton ME (2009) Effects of cowpea varietal susceptibility and low pressure on the mortality of life stages of Callosobruchus maculatus (Coleoptera: Bruchidae). J Stored Prod Res 45:232–235

Meena SR, Kant K, Mishra BK, Mehta RS, Dubey PN, Meena NK, Sinha P (2015) Influence of cultural practices on infestation of fennel seed wasp (*Systole albipennis* Walker). Int J Seed Spices 5:92–94

Meikle WG, Adda C, Azoma K, Borgemeister C, Degbey P, Djomamou B, Markham RH (1998) The effects of maize variety on the density of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in post-harvest stores in Benin Republic. J Stored Prod Res 34:45–58

Mertins JW (1980) Life history and behavior of *Laelius pedatus*, a gregarious bethylid ectoparasitoid of *Anthrenus verbasci*. Ann Entomol Soc Am 73:686–693

Mertins JW (1981) Life history and morphology of the odd beetle, *Thylodrias contractus*. Ann Entomol Soc Am 74:576–581

Mew TW, Hossain M (2008) Seed health improvement for pest management and crop production. In: Limited Proceedings of the Technical Sessions of the Final Workshop on the Rice Seed Health Improvement Project, Dhaka, Bangladesh

Mikhaiel AA (2011) Potential of some volatile oils in protecting packages of irradiated wheat flour against *Ephestia kuehniella* and *Tribolium castaneum*. J Stored Prod Res 47:357–364

Mohandass S, Arthur FH, Zhu KY, Throne JE (2007) Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. J Stored Prod Res 43:302–311

Moharramipour S, Negahban M (2014) Plant essential oils and pest management. In: Sahayaraj K (ed) Basic and applied aspects of biopesticides. Springer, New Delhi, pp 129–153

Moharramipour S, Taghizadeh A, Meshkatalsadat MH, Talebi AA, Fathipour Y (2008) Repellent and fumigant toxicity of essential oil from *Thymus persicus* against *Tribolium castaneum* and *Callosobruchus maculatus*. Commun Agric Appl Biol Sci 73:639–642

Murata M, Imamura T, Miyanoshita A (2007) Suppression of the stored-product insect *Tribolium confusum* by *Xylocoris flavipes* and *Amphibolus venator*. J Appl Entomol 131:559–563

Namin FR, Naseri B, Ghanbalani GN, Razmjou J (2018) Demographic studies of *Tribolium castaneum* (Coleoptera: Tenebrionidae) on various barley cultivars. J Stored Prod Res 79:60–65

Nansen C, Meikle WG (2002) The biology of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Integr Pest Manag Rev 7:91–104

Nayak MK, Collins PJ (2001) An improved method for mass rearing of three liposcelid psocids (Psocoptera: Liposcelidae) infesting stored commodities. J Stored Prod Res 37:323–328

Negahban M, Moharramipour S (2007) Fumigant toxicity of *Eucalyptus intertexta*, *Eucalyptus sargentii* and *Eucalyptus camaldulensis* against stored-product beetles. J Appl Entomol 131:256–261

Negahban M, Moharramipour S, Sefidkon F (2006) Chemical composition and insecticidal activity of *Artemisia scoparia* essential oil against three coleopteran stored-product insects. J Asia Pac Entomol 9:381–388

Negahban M, Moharramipour S, Sefidkon F (2007) Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. J Stored Prod Res 43:123–128

Nenaah GE (2011) Toxic and antifeedant activities of potato glycoalkaloids against *Trogoderma granarium* (Coleoptera: Dermestidae). J Stored Prod Res 47:185–190

Nenaah GE, Ibrahim SI, Al-Assiuty BA (2015) Chemical composition, insecticidal activity and persistence of three Asteraceae essential oils and their nanoemulsions against *Callosobruchus maculatus* (F.). J Stored Prod Res 61:9–16

Nhamucho E, Mugo S, Gohole L, Tefera T, Kinyua M, Mulima E (2017) Resistance of selected Mozambican local and improved maize genotypes to maize weevil, *Sitophilus zeamais* (Motschulsky). J Stored Prod Res 73:115–124

Nielsen PS (2003) Predation by *Blattisocius tarsalis* (Berlese) (Acari: Ascidae) on eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). J Stored Prod Res 39:395–400

Norhisham AR, Abood F, Rita M, Hakeem KR (2013) Effect of humidity on egg hatchability and reproductive biology of the bamboo borer (*Dinoderus minutus* Fabricius). Springerplus 2:1–6

Nwosu LC (2016) Chemical bases for maize grain resistance to infestation and damage by the maize weevil, *Sitophilus zeamais* Motschulsky. J Stored Prod Res 69:41–50

Ofuya TE, Credland PF (1995) Responses of three populations of the seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), to seed resistance in selected varieties of cowpea, *Vigna unguiculata* (L.) Walp. J Stored Prod Res 31:17–27

Okada K, Archer CR, Katsuki M, Suzaki Y, Sharma MD, House CM, Hosken DJ (2015) Polyandry and fitness in female horned flour beetles, *Gnatocerus cornutus*. Anim Behav 106:11–16

Oliveira CRF, Faroni LRDA, Guedes RNC, Pallini A (2003) Parasitism by the mite *Acarophenax lacunatus* on beetle pests of stored products. BioControl 48:503–513

Olsson POC, Ryne C, Wallén R, Anderbrant O, Löfstedt C (2006) Male-produced sex pheromone in *Tribolium confusum*: behaviour and investigation of pheromone production locations. J Stored Prod Res 42:173–182

Onagbola EO, Fadamiro HY (2008) Morphology and development of *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) on *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). BioControl 53:737

Opit GP, Throne JE (2014) Population growth and development of the psocid *Lepinotus reticulatus* at constant temperatures and relative humidities. J Econ Entomol 101:605–615

Oussou RD, Meikle WG, Markham RH (1998) Factors affecting larval survivorship and development rate of *Teretriosoma nigrescens* Lewis. Int J Trop Insect Sci 18:53–58

Pal S, Biswas S, Shukla G, Pala NA, Chakravatry S (2016) Insect pests of forest seeds and their management in natural and storage environment. In: Shukla G, Pala NA, Chakravarty S (eds) Forest seed science and management. New India Publishing Agency, New Delhi, pp 85–102

Papachristos DP, Stamopoulos DC (2002) Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J Stored Prod Res 38:117–128

Parajulee MN, Phillips TW, Hogg DB (1994) Functional response of *Lyctocoris campestris* (f.) adults: effects of predator sex, prey species, and experimental habitat. Biol Control 4:80–87

Pedersen JR (1984) Insects and pests that affect seed quality. In: Proceedings of the Short Course for Seedsmen, Mississippi State University, pp 61–71

Pekár S, Hubert J (2008) Assessing biological control of *Acarus siro* by *Cheyletus malaccensis* under laboratory conditions: effect of temperatures and prey density. J Stored Prod Res 44:335–340

Pereira AI, Pikart TG, Ramalho FS, Manickavasagam S, Serrão JE, Zanuncio JC (2013) *Antrocephalus mitys* (Hymenoptera: Chalcididae) in laboratory cultures of *Tenebrio molitor* (Coleoptera: Tenebrionidae), and possible role in biological control of *Ephestia cautella* (Lepidoptera: Pyralidae). Fla Entomol 96:634–638

Pfaffenberger GS (1985) Description, differentiation, and biology of the four larval instars of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Coleopt Bull 39:239–256

Phankaen Y, Manaprasertsak A, Pluempanupat W, Koul O, Kainoh Y, Bullangpoti V (2017) Toxicity and repellent action of *Coffea arabica* against *Tribolium castaneum* (Herbst) adults under laboratory conditions. J Stored Prod Res 71:112–118

Phillips TW, Throne JE (2010) Biorational approaches to managing stored-product insects. Annu Rev Entomol 55:375–397

Pitman AJ, Jones EG, Jones MA, Oevering P (2003) An overview of the biology of the wharf borer beetle (*Nacerdes melanura* L., Oedemeridae) a pest of wood in marine structures. Biofouling 19:239–248

Plarre R, Lieber K, Burkholder W, Phillips J (1999) Host and host instar preference of *Apanteles carpatus* (Say) (Hymenoptera: Braconidae) a possible parasitoid for biological control of clothes moths (Lepidoptera: Tineidae). J Stored Prod Res 35:197–213

Powell D (1938) The biology of *Cephalonomia tarsalis* (Ash.), a vespoid wasp (Bethylidae: Hymenoptera) parasitic on the sawtoothed grain beetle. Ann Entomol Soc Am 31:44–49

Rafiei-Karahroodi Z, Moharramipour S, Farazmand H, Karimzadeh-Esfahani J (2011) Insecticidal effect of six native medicinal plants essential oil on Indian meal moth, *Plodia interpunctella* Hübner (Lep.: Pyralidae). Mun Ent Zool 6:339–345

Rahman MM, Islam W, Ahmed KN (2009) Potentiality of *Plastanoxus westwoodi* (Kieffer) (Hymenoptera: Bethylidae) against flat grain beetle, *Cryptolestes pusillus* (Schoenherr) (Coleoptera: Cucujidae). J Biol Control 23:111–114

Raina AK (1971) Comparative resistance to three species of *Callosobruchus* in a strain of chickpea (Cicer arietinum L.). J Stored Prod Res 7:213–216

Ramputh A, Teshome A, Bergvinson DJ, Nozzolillo C, Arnason JT (1999) Soluble phenolic content as an indicator of sorghum grain resistance to *Sitophilus oryzae* (Coleoptera: Curculionidae). J Stored Prod Res 35:57–64

Rees DP (2004) Insects of stored products. Csiro Publishing, Clayton, VIC

Rees DP (2007) Insects of stored grain: a pocket reference. Csiro Publishing, Clayton, VIC

Rezaei M, Karimzadeh J, Shakarami J, Jafari S (2014) The influences of space and plant-host biomass on some biological key factors of *Cotesia vestalis* in mass-rearing conditions. J Entomol Zool Stud 2:31–34

Rezaei M, Karimzadeh J, Shakarami J (2018) Size of interacting resource-host-parasitoid populations influences mass rearing of *Cotesia vestalis*. J Entomol Res Soc 20:23–32

Rezaei M, Talebi AA, Tazerouni Z (2019) Functional response of parasitoids: its impact on biological control. In: Donnelly E (ed) Parasitoids; biology, behavior and ecology. Nova Science Publishers, New York, NY, pp 1–34

Riudavets J, Pons MJ, Messeguer J, Gabarra R (2018) Effect of CO_2 modified atmosphere packaging on aflatoxin production in maize infested with *Sitophilus zeamais*. J Stored Prod Res 77:89–91

Rosel A (1969) Oviposition, egg development and other features of the biology of five species of Lyctidae (Coleoptera). Aust J Entomol 8:145–152

Rumbos CI, Athanassiou CG (2012) Insecticidal effect of six entomopathogenic nematode strains against *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). J Stored Prod Res 50:21–26

Russo A, Cocuzza GE, Vasta MC (2004) Life tables of *Xylocoris flavipes* (Hemiptera: Anthocoridae) feeding on *Tribolium castaneum* (Coleoptera: Tenebrionidae). J Stored Prod Res 40:103–112

Ryoo MI, Hong YS, Yoo CK (1991) Relationship between temperature and development of *Lariophagus distinguendus* (Hymenoptera: Pteromalidae), an ectoparasitoid of *Sitophilus oryzae* (Coleoptera: Curculionidae). J Stored Prod Res 84:825–829

Sahaf BZ, Moharramipour S (2008) Fumigant toxicity of Carum copticum and *Vitex pseudo-negundo* essential oils against eggs, larvae and adults of *Callosobruchus maculatus*. J Pest Sci 81:213–220

Sahaf BZ, Moharramipour S, Meshkatalsadat MH (2007) Chemical constituents and fumigant toxicity of essential oil from *Carum copticum* against two stored product beetles. Insect Sci 14:213–218

Sánchez-Ramos I, Castañera P (2009) Chemical and physical methods for the control of the mite *Acarus farris* on Cabrales cheese. J Stored Prod Res 45:61–66

Sánchez-Ramos I, Álvarez-Alfageme F, Castañera P (2007) Development and survival of the cheese mites, *Acarus farris* and *Tyrophagus neiswanderi* (Acari: Acaridae), at constant temperatures and 90% relative humidity. J Stored Prod Res 43:64–72

Santos ACV, Fernandes CC, Lopes LM, Sousa AH (2015) Use of plant oils from the southwestern Amazon for the control of maize weevil. J Stored Prod Res 63:67–70

Saroukolai AT, Moharramipour S, Meshkatalsadat MH (2010) Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. J Pest Sci 83:3–8

Scheff DS, Subramanyam B, Arthur FH, Dogan H (2018) *Plodia interpunctella* and *Trogoderma variabile* larval penetration and invasion of untreated and methoprene-treated foil packaging. J Stored Prod Res 78:74–82

Schmale I, Wäckers FL, Cardona C, Dorn S (2003) Combining parasitoids and plant resistance for the control of the bruchid *Acanthoscelides obtectus* in stored beans. J Stored Prod Res 39:401–411

Sétamou M, Schulthess F, Bosque-Perez NA, Poehling HM, Borgemeister C (1999) Bionomics of *Mussidia nigrivenella* (Lepidoptera: Pyralidae) on three host plants. Bull Entomol Res 89:465–471

Shang X, Yang M, Zhang C, Cai L, Feng Y, Qiu T (2013) Effects of temperature on the growth and development of *Pyralis farinalis* (Lepidoptera: Pyralidae), one insect used for producing insect tea in China. Acta Entomol Sin 56:671–679

Shojaaddini M, Moharramipour S, Sahaf B (2008) Fumigant toxicity of essential oil from *Carum copticum* against Indian meal moth, *Plodia interpunctella*. J Plant Prot Res 48:411–419

Shu S, Koepnick WL, Mbata GN, Ramaswamy SB (1996) Sex pheromone production in *Callosobruchus maculatus* (Coleoptera: Bruchidae): electroantennographic and behavioral responses. J Stored Prod Res 32:21–30

Silva GN, Faroni LRA, Sousa AH, Freitas RS (2012) Bioactivity of Jatropha curcas L. to insect pests of stored products. J Stored Prod Res 48:111–113

Simmons P, Ellington GW (1925) The ham beetle, *Necrobia rufipes* De Geer. J Agric Res 9:845–863

Singh P (1982) The rearing of beneficial insects. Nz Entomol 7:304–310

Sokoloff A (1977) The biology of *Tribolium* with special emphasis on genetic aspects, vol 3. Clarendon Press, Oxford

Sommerman KM (1946) A revision of the genus *Lachesilla* north of Mexico (Corrodentia: Caeciliidae). Ann Entomol Soc Am 39:627–661

Sood S, Pajni HR (2006) Effect of honey feeding on longevity and fecundity of *Uscana mukerjii* (Mani) (Hymenoptera: Trichogrammatidae), an egg parasitoid of bruchids attacking stored products (Coleoptera: Bruchidae). J Stored Prod Res 42:438–444

Southgate BJ (1979) Biology of the Bruchidae. Annu Rev Entomol 24:449–473

Stamopoulos DC, Damos P, Karagianidou G (2007) Bioactivity of five monoterpenoid vapours to *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). J Stored Prod Res 43:571–577

Stejskal V, Aulicky R, Kucerova Z (2014) Pest control strategies and damage potential of seed-infesting pests in the Czech stores-a review. Plant Prot Sci 50:165–173

Strong F (1962) Laboratory studies of the biology of the alfalfa seed chalcid, *Bruchophagus roddi* Guss. (Hymenoptera: Eurytomidae). Hilgardia 32:229–249

Subramanyam B, Hagstrum DW (2000) Alternatives to pesticides in stored-product IPM. Springer Science & Business Media, New York, NY

Suthisut D, Fields PG, Chandrapatya A (2011) Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. J Stored Prod Res 47:222–230

Taha HA, Mahmoud HI, Hassan MI, Omar NR, Nasr HM (2010) Effect of different food types on the biology, fecundity and life table parameters of the stored grain mite *Gohieria fusca* (OUD) (Acari: Astigmata: Lapidophoridae). Egypt J Agric Res 88:133–142

Talukder FA, Howse PE (1995) Evaluation of *Aphanamixis polystachya* as a source of repellents, antifeedants, toxicants and protectants in storage against *Tribolium castaneum* (Herbst). J Stored Prod Res 31:55–61

Tazerouni Z, Rezaei M, Talebi AA (2019a) Cowpea: insect pest management. In: Gorawala P, Mandhatri S (eds) Agricultural research updates, vol 26. Nova Science Publishers, New York, NY, pp 1–48

Tazerouni Z, Talebi AA, Rezaei M (2019b) Functional response of parasitoids: its impact on biological control. In: Donnelly E (ed) Parasitoids; biology, behavior and ecology. Nova Science Publishers, New York, NY, pp 35–58

TeKrony DM (1976) Applicator training manual for: seed treatment pest control. UK cooperative extension service. University of Kentucky, College of Agriculture, Lexington, KY

Titouhi F, Amri M, Messaoud C, Haouel S, Youssfi S, Cherif A, Jemôa JMB (2017) Protective effects of three Artemisia essential oils against *Callosobruchus maculatus* and *Bruchus rufimanus* (Coleoptera: Chrysomelidae) and the extended side-effects on their natural enemies. J Stored Prod Res 72:11–20

Uçkan F, Sengül H, Sak O, Korkmaz M (2007) Effects of 5-aza-2′-deoxycytidine on biological parameters of larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae) and on its host *Achoria grisella* (Lepidoptera: Pyralidae). Ann Entomol Soc Am 100:265–269

Urrelo R, Wright VF, Mills RB, Horber EK (1990) An abbreviated procedure to determine the inherent resistance of maize to *Sitophilus zeamais* Motsch.(Coleoptera: Curculionidae). J Stored Prod Res 26:97–100

Veer V, Prasad R, Rao KM (1991) Taxonomic and biological notes on *Attagenus* and *Anthrenus* spp (Coleoptera: Dermestidae) found damaging stored woolen fabrics in India. J Stored Prod Res 27:185–198

Weber RG, Connell WA (1975) *Stelidota geminata* (Say): studies of its biology (Coleoptera: Nitidulidae). Ann Entomol Soc Am 68:649–653

Williams RN, Fickle DS, Kehat M, Blumberg D, Klein MG (1983) Bibliography of the genus *Carpophilus* Stephens (Coleoptera: Nitidulidae). Ohio Agricultural Research and Development Center, Wooster, OH

Wong-Corral FJ, Castañé C, Riudavets J (2013) Lethal effects of CO_2-modified atmospheres for the control of three Bruchidae species. J Stored Prod Res 55:62–67

Woodroffe GE (1951a) A Life-history Study of *Endrosis lactella* (Schiff.) (Lep. Oecophoridae). Bull Entomol Res 41:749–760

Woodroffe GE (1951b) A life-history study of the brown house moth, *Hofmannophila pseudospretella* (Staint.) (Lep., Oecophoridae). Bull Entomol Res 41:529–553

Xia B, Luo D, Zou Z, Zhu Z (2009) Effect of temperature on the life cycle of *Aleuroglyphus ovatus* (Acari: Acaridae) at four constant temperatures. J Stored Prod Res 45:190–194

Zakka U, Lale NES, Umeozor OC (2015) Efficacy of combining varietal resistance with harvest time and planting date for the management of *Sitophilus zeamais* Motschulsky infestation in stored maize. J Stored Prod Res 60:31–35

Zanetti NI, Visciarelli EC, Centeno ND (2016) Biological strategies of *Dermestes maculatus* DeGeer (Coleoptera: Dermestidae) at larval stages in different temperatures. Neotrop Entomol 45:652–657

Žd'árková E (1998) Biological control of storage mites by *Cheyletus eruditus*. Integr Pest Manag Rev 3:111–116

Zhai G, Wang H, Li S, Liu H, Shao J, Tao Y, Zou G (2016) Identification of genomic region associated with rice weevil resistance in sorghum [*Sorghum bicolor* (L.) Moench]. J Stored Prod Res 69:166–171

Ziaee M, Moharramipour S, Mohsenifar A (2014) Toxicity of *Carum copticum* essential oil-loaded nanogel against *Sitophilus granarius* and *Tribolium confusum*. J Stored Prod Res 138:763–771

Effect of Climate Change on Pollination, Fertilization and Seed Development in Agricultural Crops

12

T. Eevera, S. Venkatesan, P. Masilamani, and R. Umarani

Abstract

Change in condition of abiotic factors like temperature, atmospheric gas composition (particularly carbon dioxide) and radiation activity leads to pose serious threats to all the living entity of the earth. Particularly change of temperature conditions induces lot of changes in reproductive behaviour of angiosperms that resulted in loss of some useful plant resources. Even though the carbon dioxide addition to the earth favour for increasing biomass production and accumulation in crop plants, that one alters the temperature conditions of the earth. Change of all the above said factors started to influence the existing living entities to undergo some evolutionary changes in order to survive and withstand the adverse conditions. In this chapter, the influence of elevated temperature, increased carbon dioxide concentration and UV-B radiation activity on pollination, fertilization and seed development in some important crops like groundnut, pulses and cereals were discussed based on available literature.

Keywords

Seed development · Elevated carbon dioxide · Elevated temperature · UV-B irradiation · Climate change · Flowering plants

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12.1 Introduction

Influence of climate on successful flower formation and sexual mode of transforming ovule into a seed and ovary into a fruit is critical in most of the agriculture and horticulture crops. Prevalence of high temperature normally causes abnormality in flower development and resulted in poor seed and fruit development in tomato (Abdul-Baki 1991), pepper (Erickson and Markhart 2002), bean, cowpea (Craufurd et al. 1998), pea, avocado and cotton (Reddy et al. 1992).

Above all, extreme temperature variance or lower level of irradiance causes 'blindness' in roses and thus leads to prevention of flower formation in the early stage itself (Hubbell 1934). Time of exposure to various temperatures was playing a vital role with reference to floral bud development across many numbers of species.

Changes in day and night temperature from 25/20 °C to 30/25 °C were found seriously affect the flower development in the case of *Phalaenopsis amabilis* Blume (Chen et al. 1994). Flower development in cowpea was affected by high night temperature of 24 °C, but not affected by the high day temperature of above 33 °C (Ahmed and Hall 1993).

In contrast, day temperature of 35 °C and night temperature of 27 °C do not have any influence on cotton flower and seed development, but increased diurnal temperature of 40/32 °C affected 90% of the boll development in cotton (Reddy et al. 1992). Exposure of broccoli (*Brassica oleracea* var. *italica* L.) to the temperature of 35 °C for 7 days was seriously disrupting inflorescence development, when the inflorescences were 5 mm in diameter.

In another experiment, when the inflorescences were 5–10 mm or larger in diameter, it was not affected by the above said temperature treatment (Bjorkman and Pearson 1998). Plants were exposed to high temperature during reproductive stage, which severely affect the reproductive structure development by the way of reducing photosynthetic activity. Due to reduction of photosynthetic activity, the corresponding assimilate formation was very much reduced and resulted in abortion of reproductive structures in more number of agriculture and horticulture crops (Dinar and Rudich 1985b; Aloni et al. 1991).

Larcher (1995) observed that thylakoid membranes were affected by temperature variation and thus lead to reduction of photosynthetic activity. The above recorded observation may be used as an indicator to assess the influence of heat stress on crop growth and development. Similar kind of plant cell organ like chloroplast biogenesis affected by temperature variation was recorded in barley crop exposed to the temperature of 32 °C (Smillie et al. 1978). Abortion of reproductive structure in *Rosa L.* sp. is also responsible for reduction of photosynthesis under abnormal temperature prevailing conditions (Nell and Rasmussen 1979).

Ahmed and Hall (1993) during their work observed that exposure of cowpea to beyond the critical temperature during flower initiation time, abnormal temperature suppress the flower development but did not have any influence on the process of floral initiation. Further, Mutters et al. (1989b) added that flower occurrence and development were affected when they did this experiment under long-day

conditions. But they did not observed similar kind of influence on floral development under short-day condition.

12.2 Effect of Climate Change on Pollination and Fertilization

Pollination is the process of reaching of pollen from anther to the female reproductive organ of a flower. The pollen originates in the pollen sac, which is completely covered by multilayered anther wall. As the first step of the pollination, the anther sacs need to be opened and thus enable the release of pollen grains.

Plant reproduction may get affected when the plant is experiencing higher level of stress created by prevailing increased temperature at the time of gametogenesis. This causes immediate and long-term effects on reproductive functions of the plants. Increased temperature not only affects the opening of anther sac but also quantity and quality aspects of pollen. With reference to quantity parameters, it will reduce the quantum of pollen available for pollination. Regarding the quality parameters, this stress affects anatomical features of pollen, which leads to occurrence of anomalies in pollen wall development and other internal features of pollen. It also alters the chemical composition and viability potential of pollen grains as a whole.

Monterroso and Wien (1990) during their study found the sensitivity of beans to higher temperature particularly during flower development. Further the above stress resulted in reduction of number of pods per plant and number of seeds per pod. The lowest number of productive pod development was recorded when the flower buds were exposed to high temperature of 32/27 °C for 6–12 hours before opening of the buds (anthesis) (Gross and Kigel 1994). The reason for reduced number of productive pod production is the high temperature causes severe reduction in number of pollen production and further produced pollen viability also severely get affected. It was also found to affect anther dehiscence and pollen dispersal at correct time. Entry of tube portion of the pollen into stigmatic column also got reduced and impaired female performance.

In 2001, Porch and Jahn conducted study with sensitive as well as heat-tolerant genotypes of beans to know how the high temperature stress influences the occurrence of microsporogenesis-related event. As an outcome of the above study, they found that heat stress not favoured the occurrence of anther dehiscence and also thickness of endothelial wall was reduced very much. Above all, in the case of heat-sensitive genotypes of beans, interlobular septa was completely degenerated. Similar study was also conducted by Suzuki et al. (2001) in beans. He recorded occurrence of pollen sterility event when he has grown the bean plants under beyond optimum temperature level. Formation of sterile pollen in the above case was associated with occurrence of tapetum layer structural abnormality and degeneration of endoplasmic reticulum of tapetal cells. All these structural abnormalities that lead to degeneration of tapetum took place at faster rate under high temperature condition when compared to the plant grown under optimum conditions.

Because of premature collapse of tapetum cells, nutrient mobilization to the pollen gets affected, and further it also affects the translocation of proline

(an important stress tolerant-related amino acid produced only in the plant during stress conditions) from wall of anther to pollen. Prevention of proline transfer and occurrence of structural abnormality of tapetum cells leads to production of sterile or non-viable pollen under heat-stressed environmental conditions (Mutters et al. 1989a; Ahmed et al. 1992; Hesse and Hess 1994).

Suguru Sato et al. (2002) conducted an experiment to find out the time of thermosensitivity of tomato flowers and its associated physiological process during flowering stage of the crop growth by exposing the tomato flowers to high temperature conditions of 28/22 °C or 32/26 °C day/night temperature regimes. During reproductive stage plants were shifted to opposite temperature condition for about 0–15 days before or 0–24 h after anthesis. From the above study, they found that tomato plants maintained under 28/22 °C initially showed decreased fruit set per plant due to exposure of plants to moderate temperature stress before anthesis. But in the case of plants maintained under $28/22^{0}$C and then exposed to moderate temperature stress after anthesis did not have any significant influence on regular fruit yield.

In the case of tomato plant grown at the condition of 32/26 °C, fruit set was prevented completely. But fruit set was increased when the tomato plant grown under same condition but provided with stress relief of 3–24 h after anthesis. Tomato flowers were very sensitive to the temperature of 32/26 °C during 7–15 days before anthesis.

The sensitivity of tomato flower to high temperature was due to impairment of the following essential organ/parts like pollen, endothecium and stomium development. Above all, in tomato, meiosis occurs both in micro and megaspore mother cell 8–9 days before opening of the flowers. Hence, any stress during meiosis stage of sexual organ development that ultimately affect the fruit and seed yield (Iwahori 1965).

He further added that, when ovules are exposed to the temperature of 40 °C within 18 h of pollination, ovules gets aborted. The abortion of the ovules will inhibit the growth and development of pollen tube, and ultimately there won't be any embryo and endosperm development. Peet et al. (1998) conducted a study with male sterile tomato plants, and he found that the process of pollen development (microsporogenesis) was very much affected by the elevated temperature stress, when compared to female organ development and post-anthesis process.

Morrison (1991) reported that elevated temperature treatment given to the *Brassica napus* plants during pre-anthesis stage of flower development ultimately reduce viability/fertility of the pollen grains. Similarly enriched amount of CO_2 in the atmosphere also causes problem to crop plants particularly the flower and sexual organ development process like that of elevated temperature. Vara Prasad et al. (2002) reported that kidney beans were grown under elevated CO_2; the susceptibility of pollen to high temperature was increased by 1–2 °C. The possible reason for the above may be due to the CO_2-enriched environment normally increasing the temperature of tissue. Further, they recorded the leaf temperature of the plant grown at elevated CO_2 level was 1.5 °C more than that of the plants grown under normal CO_2 condition, across the 28–40 °C ranges of midday chamber air temperature.

Pan (1996) observed that temperature of bulk foliage of soya was 1–2 °C greater at enriched CO_2 levels. This phenomenon occurs because enriched or at higher level of CO_2 causes closure of stomata partially and thereby increases leaf resistance to water vapour efflux, resulting in decreased transpiration rate. Decreased transpiration causes warming of leaves slightly due to less latent heat are lost.

Foliar temperature was increased about 1 °C with doubling of CO_2 concentration owing to decreased leaf conductance in the energy balance simulations experiment performed with soil plant atmosphere relationship (Allen 1990).

12.3 Effect of Elevated Temperature on Pollen Development

The pollen produced in the plants cultivated under cool weather conditions provisioned with resources from the sporophyte. Particularly, those resources were synthesized during germination of pollen grains and in turn used for the pollen tube growth. Those metabolites are energy products like carbohydrate (starch), lipids and phytate (Stephenson et al. 1994).

In almost all the angiosperms, temperature greatly influences the quantity of pollen production and also affects the biochemical constituents stored in the pollen (Bertin 1998). In the case of pepper, fruit formation was very much affected by the temperature stress. The possible reason for the above one was impairment of pollen function when we grow the pepper plant under high temperature, and further it also in a lesser extent affects the maternal part of the flower (Han et al. 1996; Peet et al. 1998).

Aloni et al. (1991) reported that in the case of pepper during flower development, the assimilate allocation for the flower was very much affected by the temperature when compared to the plant grown under stress-free conditions. The tapetum layer available in the anther is initially responsible for growth and nourishment of the pollen. The tapetum layer provides food to the pollen mainly in the form of sugars (Pacini 1996). The developing pollen absorbs the given sugar through the apoplast, and that may be utilized immediately or put it in reserve in the amyloplast in the form of starch.

Pacini and Viegi (1995) observed that during pollen development in the case of tomato, starch is kept as a reserve source of energy in amyloplasts and not in fully developed pollen grain. Polowick and Sawhney (1993) have reported that starch synthesis normally takes place during the initial stages of pollen development, but during maturity stages, starch synthesis cannot happen.

In rice, instead of accumulation of starch in pollen during its development, soluble sugars get accumulated when the plants were grown under heat-stressed conditions. The above diversion in routine biochemical accumulation pathway leads to inhibition of activity of important enzyme like invertase and starch synthase normally took place in anthers, whereas ADP-glucose pyrophosphorylase and sucrose synthase activity is not affected by the heat stress in the anther. All the above anomalous biochemical process leads to occurrence of structural injury, and

desiccation of pollen will be the final result in the case of heat-stressed plants (Saini 1997).

Dorion et al. (1996) study the effect of water stress on wheat plant pollen production function. They reported that stressed wheat plant was not able to metabolize the sucrose into hexose. The above inability was the key factor with reference to malfunctioning of the pollen grains under water-stressed conditions. From this study, they concluded that pollen germination behavior, onset of reproductive phase, duration of reproductive phase and the quality and quantity of both male and female organ formation in plants are mainly regulated by the abiotic factors.

12.4 Effect of Climate Change on Pollen Germination

Among the different abiotic factors at presented projected, the factors like carbon dioxide concentration, heat and ultraviolet-B radiation were expected to change in the ensuing period. Carbon dioxide concentration is expected to be increased and reach the level from 560 and 970 mmol mol^{-1} during the later part of the twenty-first century (Sailaja koti et al. 2005). Near equator and midlatitude area receives the UV-B radiation of more than 12 kJ m^{-2} day^{-1} on a given day. Remaining area UV-B radiation ranges between 2 and 12 kJ m^{-2} day^{-1}.

If we compare the present UV-B radiation with the 1970s radiation, the percentage increase ranges between 6% and 14% in the earth surface. The above increase was mainly attributed by the chlorofluorocarbons liberated to the atmosphere by the usage of different artificial cooling-related machines for the past few decades (UNEP 2002). Interaction among the various abiotic stress factors in the open atmosphere exacerbated the rate and increased the impact of individual factors on both terrestrial and aquatic ecosystems.

The elevated CO_2 concentration favourably increased the crop yield. But the other abiotic stress factors like elevated temperature (Wheeler et al. 2000 al.) and UV-B radiation (Teramura et al. 1990; Gwynn-Jones et al. 1997; Sullivan 1997; Tosserams et al. 2001; Zhao et al. 2003) cause serious problem to the crop plants by the way of impairing their reproductive structure and its function.

Response of different genotypes to elevated temperature and UV-B radiation stress was found to vary. In most of the cases, the above said two abiotic stresses inhibit the pollen germination, tube growth and related process (Huan et al. 2000; Kakani et al. 2003). This resulted in total crop failure, and some of the valuable genotypes are not able to regenerate further due to non-availability or inability of the plant to produce seeds.

In soybean, the increased concentration of carbon dioxide and elevated temperature favour for the increased flower production (Nakamoto et al. 2001; Zheng et al. 2002), where a higher amount of UV-B radiation severely affected flower production and morphology of produced cotton flowers. Flower size of cotton was reduced very much with fewer anthers (Kakani et al. 2003). Carbon dioxide concentration of 720 lmol mol^{-1} increased the pollen production by 61% in the case of ragweed plants (Wayne et al. 2002), and also this much level of carbon dioxide not causes any

problem to groundnut plant pollen viability (Prasad et al. 2003). In contrast to above, elevated temperature (36.8 and 40.8 °C) causes problem to pollen viability in groundnut plant. Wang et al. (2004) studied the combined effect of high temperature (36.8 and 40.8 °C) and heavy dose of UV-B irradiation (900–1500 lW cm^{-2}) in tall fescue. As an outcome of the above study, they reported that pollen viability was very much reduced.

However, in the case of *Ipomoea purpurea*, UV-B irradiation and high temperature stress were not able to cause any problem to pollen viability and fertilization success. The possible reason for the above one is mainly governed by the flavanoids available in the *Ipomoea purpurea* plants. In general, the type of flavanoids available in the *Ipomoea purpurea* blocks the UV-B radiation and also regulates the plant to withstand high temperature stress (Coberly and Rausher 2003). In soybean some of the authors observed that the interaction among the elevated temperature, CO_2 and UV-B irradiation also gave some positive impact on flower production and its fertility-related process (Sailaja koti et al. 2005).

Smaller flowers with smaller flower component part such as shorter standard petal and staminal column lengths were observed for the soya plant grown in treatment condition where enhanced temperature or elevated UV-B was involved either alone or in combination. The flowers produced under these treatments also had reduced pollen production even under elevated CO_2 condition.

Prasad et al. (1999a, b) reported that increased temperature (day temperature of more than 33 °C) during reproductive stage especially during micro- and megasporogenesis time reduced the viability of pollen and also reduced the quantity of pollen produced in more number of flowering plants.

12.5 Effect of Climate Change on Seed and Fruit Set

Currently groundnut is grown as an important grain legume for the purpose of fulfilling oil and edible protein requirement of the human population. Almost entire quantity of groundnut is produced under tropical and semi-arid tropical regions of the earth. All the above regions are characterized by prevalence of high temperature and uncertainty in receipt of rainfall. Prasad et al. (1999a, b) observed that prevailing day temperature under tropical regions is always more than 34 °C; this resulted in decreased pod formation and severe yield reduction in groundnut. Decreased pod set was associated with reduction in pollen production and reduced pollen tube growth; all these complication leads to poor fertilization of flowers (Prasad et al. 1999a, 2000a, 2001).

The increased daytime temperature ranges between 26–30 and 34–36 °C cause serious problem in pollination and fertilization of groundnut and resulted in reduction of subterranean pegs and pods, size of the kernel and kernel yield by 30–50% (Cox 1979; Ketring 1984; Ong 1984). Prasad et al. (2000b) conducted an experiment to know the impact of daytime soil and air temperature of 28 °C and 38 °C, respectively, on groundnut pod yield. The above condition was ensured from start of flowering to maturity, and they found that 50% reduction in pod yield under above

said conditions. The above experimental results were proved by the Vara Prasad et al. (2003) by conducting similar type of experiment in groundnut.

Viability of pollen, kernel development, number of kernels per pod, kernel size and harvest index were all decreased by temperature of more than 32/22 °C under both ambient and elevated CO_2 in groundnut. Further same author pointed that the reproductive process of ground nut, soya and dry bean is more sensitive to exhibition by super-optimal temperature than vegetative processes. Their experimental observation showed that the ideal temperature for getting better seed yield was similar at both ambient and elevated CO_2 conditions.

Under elevated CO_2 and temperature conditions, the yield reduction impact caused by the elevated temperature was equalized by the elevated CO_2 concentration. There was no yield difference if we compare the groundnut crop yield under normal temperature conditions, i.e. 32/22 °C coupled with ambient CO_2 and seed yield obtained from plants grown at temperature of 36.4/26.4 °C under elevated CO_2. Elevated temperatures individually created problem to the pollen fertility, when it was in combination with elevated CO_2 conditions not causes any problem to pollen viability and pod production. Similar to that of groundnut, Aloni et al. (2001) observed positive influence of elevated CO_2 and temperature in bell pepper (*Capsicum annuum* L.) yield. In the above case, elevated CO_2 concentration increased the pollen production and viability even under elevated temperature conditions. But in the case of dry beans (Prasad et al. 2002) and rice (Matsui et al. 1997), elevated CO_2 caused problem to pollination and fertilization and resulted in yield reduction in both the crop.

Similar to that of groundnut, Aloni et al. (2001) observed positive influence of elevated CO_2 and temperature in Bell pepper (*Capsicum annum* L.) yield. In the above case, elevated CO_2 concentration increased the pollen production and viability even under elevated temperature conditions. But in the case of dry beans (Prasad et al. 2002) and rice (Matsui et al. 1997), elevated CO_2 caused problem to pollination and fertilization, and resulted in yield reduction in both the crop.

Occurrence of early degeneration of tapetum layer, poor accumulation of carbohydrate in the developing pollen grains and loss of pollen viability were associated with high temperature stress. All the above said problems were not recorded when the plants were grown under elevated CO_2 conditions. Particularly, elevated CO_2 concentration not affected the phenology of the plants, days of flower production and days to maturity, etc.

In groundnut the days to first flowering were recorded 3 days earlier in the plant grown under the temperature of 40/30 °C than 32/22 °C. But pod formation and kernel filling at above-mentioned higher temperature conditions were found to be delayed by 10 days both under normal and elevated CO_2 conditions. Wheeler et al. (1997) exposed the groundnut plant to a high daytime temperature (between 30 and 45 °C) for a short period of 6 days during flowering stage. As an outcome of this study, they found that seed filling in groundnut was delayed by 7 days even though the exposure time was short period.

In another experiment conducted by Craufurd et al. (2002) recorded that initiation of pod filling was delayed by 5–9 days when they have grown the groundnut plants under 38/22 °C conditions.

Like that of groundnut, the other crops like soybean, rice, cowpea, etc. also the flowering and seed formation were very much affected under elevated conditions. Pan (1996) and Thomas et al. (2003) recorded delayed seed formation in soybean at 40/30 °C than 32/22 °C.

Jolliffe and Ehret (1985) conducted an experiment with soybean under elevated carbon dioxide conditions, and they recorded the following observations: (1) elevated CO_2 favour for maximizing the vegetative growth in terms of more branching and (2) more number of flower production. All the above two events happened mainly because of shift in plant tissue temperature condition, i.e. elevated CO_2 conditions decreased the upper limit of temperature by 2 °C for seed formation when compared to seed formation under ambient CO_2 levels.

Growth rate of individual soybean seed was not affected by elevated carbon dioxide conditions ranging from 3330 to 990 μmol CO_2 mol^{-1} according to Allen et al. (1991).

In rice, vital temperature for induction of sterility in spikelet was decreased by 1 °C at increased concentration of CO_2 when compared to ambient conditions (Matsui et al. 1997).

12.6 Conclusion

Thus, change of climate is coupled with increased temperature; economic yield potential of elevated temperature stress-sensitive crops will be reduced even though the crop is experiencing beneficial effect of CO_2 enrichment in the atmosphere.

Research has shown that heat-tolerant cultivars of cowpea are more reactive to enriched CO_2 conditions with respect to economic yield under both elevated and medium heat conditions. Ahmed et al. (1993) suggest that high temperature tolerance is the main criterion in breeding cultivars that can adjust to the upcoming climate condition changes. Exposure to increased concentration of CO_2 (700 μmol^{-1}) increased photosynthesis and thus resulted in increased economic yield of kidney bean as typically recorded in other crops like rice (Baker and Allen 1993), and soybean (Allen and Boote 2000) peanut

In most, but not all, C3 crops, the CO_2 level of 370 μmol^{-1} is a main limiting factor for the photosynthesis, growth and productivity (Bowes 1993). Photosyntheate productions are direct results of the activity of rubisco (ribulose bisphosphate carboxylase-oxygenase) enzyme, which is strongly influenced by the increased CO_2 concentration and resulted in saturation of rubisco in C3 species; therefore, an increased availability of CO_2 up-to 370 μmol^{-1} results in greater leaf photosynthetic rates (Bowes 1993) and enhances biomass accumulation.

References

Abdul-Baki AA (1991) Tolerance of tomato cultivars and selected germplasm to heat stress. J Am Soc Hortic Sci 116:1113–1116

Ahmed FE, Hall AE (1993) Heat injury during early floral bud development in cowpea. Crop Sci 33:764–767

Ahmed FE, Hall AE, Demason DA (1992) Heat injury during floral development in cowpea (*Vigna unguiculata*, Fabaceae). Am J Bot 79:784–791

Allen LH (1990) Plant responses to rising carbon dioxide and potential interactions with air pollutants. J Environ Qual 19:14–34

Allen LH, Boote KJ (2000) Crop ecosystem responses to climate change: soybean. In: Reddy KR, Hodges HF (eds) Climate change and Global crop productivity. CABI, Wallingford, pp 133–160

Allen LH, Bisbal EC, Boote KJ, Jones PH (1991) Soybean dry matter allocation under subambient and superambient levels of carbon dioxide. Agron J 83:875–883

Aloni B, Pashkar T, Karni L (1991) Partitioning of [14C] sucrose and acid invertase activity in reproductive organs of pepper plants in relation to their abscission under heat stress. Ann Bot 67:371–377

Aloni B, Peet M, Pharr M (2001) The effect of high temperature and high atmospheric CO2 on carbohydrate changes in bell pepper (*Capsicum annum*) pollen in relation to its germination. Physiol Plant 112:505–512

Baker JT, Allen LH (1993) Effects of Co2 and temperature on rice: summary of five growing seasons. Agric For Meteorol 48:575–582

Bertin RI (1998) Paternity in plants. In: Doust JL, Doust LL (eds) Plant reproductive ecology: patterns and strategies. Oxford University Press, Oxford, pp 30–59

Bjorkman T, Pearson KJ (1998) High temperature arrest of inflorescence development in broccoli (*Brassica oleracea* var. *italica* L.). J Exp Bot 49:101–106

Bowes G (1993) Facing the inevitable: plant and increasing atmospheric Co2. Annu Rev Plant Physiol Plant Mol Biol 44:309–332

Chen WS, Liu HY, Liu ZH, Yang L, Chen WH (1994) Gibberellin and temperature influence carbohydrate content and flowering in *Phalaenopsis*. Physiol Plant 90:391–395

Coberly LC, Rausher MD (2003) Analysis of a chalcone synthase mutant in *Ipomoea purpurea* reveals a novel function for flavonoids: amelioration of heat stress. Mol Ecol 12:1113–1124

Cox FR (1979) Effect of temperature treatment on peanut vegetative and fruit growth. Peanut Sci 6:14–17

Craufurd PQ, Bojang M, Wheeler TR, Summerfield RJ (1998) Heat tolerance in cowpea: effect of timing and duration of heat stress. Ann Appl Biol 133:257–267

Craufurd PQ, Prasad PVV, Summerfield RJ (2002) Dry matter production and harvest index at high temperature in peanut. Crop Sci 42:146–151

Dinar M, Rudich J (1985b) Effect of heat stress on assimilate partitioning in tomato. Ann Bot 56:239–248

Dorion S, Lalonde S, Saini HS (1996) Induction of male sterility in wheat by meiotic-stage under water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. Plant Physiol 111:137–145

Erickson AN, Markhart AH (2002) Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant Cell Environ 25:123–130

Gross Y, Kigel J (1994) Differential sensitivity to high temperature of stages in the reproduction development of common beans (*Phaseolus vulgaris* L.). Field Crop Res 36:201–212

Gwynn-Jones D, Lee JA, Callaghan TV (1997) Effects of enhanced UV-B radiation and elevated carbon dioxide concentrations on a sub-arctic forest health system. Plant Ecol 128:242–249

Han XB, Li RQ, Wang JB, Miao C (1996) Effect of heat stress on pollen development and pollen viability of pepper. Acta Hortic Sin 23:359–364

Hesse M, Hess M (1994) Resent trends in tapetum research – a cytological and methodological review. Plant Syst Evol 7:127–145
Huan F, LiZhe A, LingLing T, ZongDong H, XunLing W (2000) Effect of enhanced ultraviolet-B radiation on pollen germination and tube growth of 19 taxa in vitro. Environ Exp Bot 43:45–53
Hubbell DS (1934) A morphological study of blind and flowering rose shoots, with special reference to flower-bud differentiation. J Agric Res 48:91–95
Iwahori S (1965) High temperature injuries in tomato IV. Development of normal flower buds and Morphological abnormalities of flower buds treated with high temperature. J Jpn Soc For Horti Sci 34:33–41
Jolliffe PA, Ehret DL (1985) Growth of bean plants at elevated carbon dioxide concentrations. Can J Bot 63:2021–2025
Kakani VG, Reddy KR, Zhao D, Mohammed AR (2003) Effects of ultraviolet-B radiation on cotton (Gossypium hirsutum L.) morphology and anatomy. Ann Bot 91:817–826
Ketring DL (1984) Temperature effects on vegetative and reproductive development of peanut. Crop Sci 24:877–882
Larcher W (1995) Physiological plant ecology. Springer, Berlin
Matsui T, Namuco OS, Ziska LH (1997) Effect of high temperature and CO2 concentration on spikelet sterility in indica rice. Field Crop Res 51:213–219
Monterroso VA, Wien HC (1990) Flower and pod abscission due to heat stress in beans. J Am Soc Hortic Sci 115:631–634
Morrison MJ (1991) Heat stress during reproduction in summer rape. Can J Bot 71:521–529
Mutters RG, Ferrerira LGR, Hall AE (1989a) Proline content of the anthers and pollen of heat tolerant and heat sensitive cowpea subjected to different temperatures. Crop Sci 29:1497–1500
Mutters RG, Hall AE, Patel PN (1989b) Photoperiod and light quality effects on cowpea floral development at high temperatures. Crop Sci 29:1501–1505
Nakamoto H, Zheng S, Furuya T, Tanaka K, Yamazaki A, Fukuyama M (2001) Effects of long-term exposure to atmospheric carbon dioxide enrichment on flowering and podding in soybean. J Fac Agric Kyushu Univ 46:23–29
Nell TA, Rasmussen HP (1979) Blindness in roses: effects of high intensity light and blind shoot prediction severity. J Am Soc Hortic Sci 104:21–25
Ong CK (1984) The influence of temperature and water deficit on the partitioning of dry matter in groundnut (*Arachis hypogaea* L.). J Exp Bot 35:746–755
Pacini E (1996) Types and meaning of pollen carbohydrate reserves. Sex Plant Reprod 9:362–366
Pacini E, Viegi L (1995) Total polysaccharide content of developing pollen in two angiosperm species. Grana 34:237–241
Pan D (1996) Soybean responses to elevated temperature and doubled CO2. Dissertation, University of Florida, Gainesville, Florida, USA, 227 pp. Dissertation Abstracts International 57
Peet MM, Sato S, Gardner RG (1998) Comparing heat stress effects on male fertile and male sterile tomatoes. Plant Cell Environ 21:221–231
Polowick PL, Sawhney VK (1993) An ultrastructural study of pollen development in tomato (*Lycopersicon esculentum*) II. Pollen maturation. Can J Bot 71:1048–1055
Porch TG, Jahn M (2001) Effect of high temperature stress on microsporogenesis in heat sensitive and heat tolerant genotypes of Phaseolus vulgaris. Plant Cell Environ 24:723–731
Prasad PVV, Craufurd PQ, Summerfield RJ (1999a) Fruit number in relation to pollen production and viability in groundnut exposed to short episodes of heat stress. Ann Bot 84:381–386
Prasad PVV, Craufurd PQ, Summerfield RJ (1999b) Sensitivity of peanut to timing of heat stress during reproductive development. Crop Sci 39:1352–1357
Prasad PVV, Craufurd PQ, Summerfield RJ (2000a) Effects of short episodes of heat stress on flower production and fruit set of groundnut (*Arachis hypogaea* L.). J Exp Bot 51:777–784
Prasad PVV, Craufurd PQ, Summerfield RJ (2000b) Effect of high air and soil temperature on dry matter production, pod yield and yield components of groundnut. Plant Soil 222:231–239
Prasad PVV, Boote KJ, Allen LH (2002) Effects of elevated temperature and carbon dioxide on seed-set and yield of kidney bean (Phaseolus vulgaris L.). Glob Chang Biol 8:710–721

Prasad PVV, Boote KJ, Allen H, Thomas JMG (2003) Superoptimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. Glob Chang Biol 9:1775–1787

Reddy KR, Hodges HF, Reddy VR (1992) Temperature effects on cotton fruit retention. Agron J 84:26–30

Reddy KR, Hodges HF, McKinion JM (1997) A comparison of scenarios for the effect of global climate change on cotton growth and yield. Aust J Plant Physiol 24:707–713

Sailaja Koti K, Raja Reddy VR, Reddy VG, Kakani DZ (2005) Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. J Exp Bot 56:725–736

Saini HS (1997) Effects of water stress on male gametophyte development in plants. Sex Plant Reprod 10:67–73

Sato S, Mary M, Judith F (2002) Determining critical pre and post anthesis periods and physiological processes in *Lycopersicon esculentum* exposed to moderately elevated temperatures. J Exp Bot 53:1187–1195

Smillie RM, Critchley C, Bain JM, Nott R (1978) Effect of growth temperature on chloroplast structure and activity in barley. Plant Physiol 62:191–196

Stephenson AG, Erickson C, Lau TC, Quesada MR, Winsor JA (1994) Effects of growing conditions on the male gametophyte. In: Stephenson AG, Kao TH (eds) Pollen pistil interactions and pollen tube growth. American Society of Plant Physiologist, Rockville, MD, pp 220–229

Sullivan JH (1997) Effects of UV-B radiation and atmospheric CO2 on photosynthesis and growth: implications for terrestrial ecosystems. Plant Ecol 128:195–206

Suzuki K, Takeda H, Tsukaguchi T, Egawa Y (2001) Ultrastructural study on degeneration of tapetum in anther of snap bean (Phaseolus vulgaris L.) under heat stress. Sex Plant Reprod 13:293–299

Teramura AH, Sullivan JH, Ziska LH (1990) Interaction of elevated ultraviolet-B radiation and CO2 on productivity and photosynthetic characteristics in wheat. Plant Physiol 94:470–475

Thomas JMG, Boote KJ, Allen LH (2003) Elevated temperature and carbon dioxide effects of soybean seed composition and transcript abundance. Crop Sci 43:1548–1557

Tosserams M, Visser A, Groen M, Kalis G, Magendans E, Rozema J (2001) Combined effects of CO2 concentration and enhanced UV-B radiation on faba bean. Plant Ecol 154:197–210

UNEP (2002) Executive summary. Final report of UNEP/WMO scientific assessment panel of the Montreal protocol on substances that deplete the ozone layer. UNEP, Nairobi

Vara Prasad PV, Boote KJ, Hartwell Allen JR, Jean MG (2002) Effect of elevated temperature and carbon dioxide on seed set and yield of Kidney Bean. Glob Chang Biol 8:710–721

Vara Prasad PV, Kenneth J, Boote L, Hartwell Allen JR, Jean MG (2003) Super-optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. Glob Chang Biol 9:1775–1787

Wang ZY, Ge YX, Scott M (2004) Viability and longevity of pollen from transgenic and non-transgenic tall fescue (*Festuca arundinacea*) (Poaceae) plants. Am J Bot 91:523–530

Wheeler TR, Chatzialioglou A, Craufurd PQ (1997) Dry matter partitioning in peanut exposed to high temperature stress. Crop Sci 37:1507–1513

Wheeler TR, Craufurd PQ, Ellis RH, Porter JR, Prasad PVV (2000) Temperature variability and the yield of annual crops. Agric Ecosyst Environ 82:159–167

Zhao D, Reddy KR, Kakani VG, Read JJ, Sullivan JH (2003) Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environmental conditions. Plant Cell Environ 26:771–782

Zheng S, Nakamoto H, Yoshikawa K, Furuya T, Fukuyama M (2002) Influences of high night temperature on flowering and pod setting in soybean. Plant Prod Sci 5:215–218

Seed Dormancy

13

Archana Siraree and Varucha Misra

Abstract

Seed dormancy is a failure of viable, intact, and mature seeds to germinate under favorable environmental conditions which may last from few days to several months depending on their habitat and growth conditions. It is also apparent that dormancy is more common in undomesticated species as compared to the species which have been domesticated to a large scale. Extensive breeding followed by the selection for desirable traits of early germination and rapid plant establishment has played a major role in eliminating dormancy that prevailed in the seeds of their wild ancestors. However, the fact that presence of dormancy up to a certain extent is always desirable to avoid great damage to seed quality due to pre-harvest sprouting or vivipary necessitated the deliberate selection of this character in cultivated species. Classification system of dormancy in seed is primarily based on the embryo and seed coat factors which divided dormancy into five broad groups, *viz*., morphological dormancy, physiological dormancy, morpho-physiological dormancy, physical dormancy, and combinational dormancy. Abscisic acid (ABA) also plays a significant role in the mechanism of dormancy and is considered as the most important germination inhibitor. Also, the accumulation of different phenolic compounds, *viz*., phenolic acids, flavonoids, tannins, etc., in the seed covering structures strongly inhibits the germination of some cereals like wheat, barley, sorghum, etc. Temperature and light also contribute in regulating seed dormancy. Although gibberellic acid is not directly involved in the control of dormancy, it promotes and maintains the germination in seeds, and its ratio to abscisic acid has always been a critical

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parameter in determining the state of dormancy. Although natural breakdown of seed dormancy may occur in some seed species, different chemical and mechanical seed treatments are also required for the successful termination of seed dormancy.

Keywords

Abscisic acid · Dormancy · Gibberellic acid · Mechanism · Morphological · Physical · Seed

13.1 Introduction

Seed dormancy is defined as a condition in which viable, intact, and mature seeds fail to germinate immediately after harvest even when they are provided favorable environmental conditions or conditions ideal for seed germination (Hilhorst 1995; Baskin and Baskin 2004), whereas non-dormant seeds are known to be in a quiescent stage when they are not able to germinate due to lack of favorable environmental condition (Harper 1957, 1977). In a more advanced way, it can be said that dormant seed remains incapable to germinate for a definite period of time even when all the physical environmental factors responsible for seed germination come together (Baskin and Baskin 2004). The resting period of seed prior to germination is often called as after-ripening period, and the term after-ripening is used to describe the changes which occur in the seed during this period (Joshi and Singh 2004). Habitat and growth conditions of different crop species are the main factors responsible for varying degrees of dormancy period which may differ from a few days to several months. It is evident that occurrence of seed dormancy is best utilized by the undomesticated plant species which decreases their vulnerability towards adverse natural conditions by arresting their out-season germination and avoiding competition with other species, thereby ensuring their continued survival (Bewley and Black 1994). On the other hand, the concept of dormancy does not appeal much in the case of agricultural crops where fast, uniform germination and rapid growth are required and therefore is considered undesirable. However, the presence of dormancy to a certain extent in crop plants is crucial particularly during the seed development stage which otherwise can give rise to a condition referred to as pre-harvest sprouting (PHS) or vivipary defined as a pre-mature germination of seed while still on the ear/pods of the parent plant and causes extensive damage to seed quality and also possesses a great problem during post-harvest management and industrial use, e.g., wheat, maize, rice, and barley (Bewley and Black 1994; Bewley 1997; Shu et al. 2015). In wheat, it is reported that pre-harvest sprouting lowers the seed value, vigor, and milling and baking quality as well as affects the grain yield. Domestication at large scale and extensive breeding followed by selection for desirable traits of early germination and rapid plant establishment are the major factors responsible for the elimination of dormancy mechanism which existed in the seeds of their wild ancestors (Kilian et al. 2009). It is possible to control the pre-harvest sprouting in

wheat seed by the several characters of spike, but dormancy has a significant role to play. Therefore, deliberate selection is practiced to achieve a certain level of seed dormancy so that problems of pre-mature germination of seeds can be addressed. The ability of plant spp. in nature to adapt themselves in changing environmental conditions decides their survival by way of escaping conditions which are considered as detrimental and unfavorable for their growth. Since various metabolic and physiological activities which occur in organisms come to a halt during the period of dormancy, the dependence of organisms on their environment is significantly reduced, and they become more tolerant to adverse environmental conditions. Unusual structures, organs, and tissues are formed in the dormant seed and buds, as a result of which they develop tolerance against unfavorable environments. Dormancy not only is confined to seeds but also involves different plant organs such as stem tubers, bulbs, corms, and rhizomes. Other than plant species, it is also exhibited by micro-organisms and animals (Joshi and Singh 2004).

13.2 Classification System for Seed Dormancy

Several classifications of seed dormancy are available in literature. Crocker (1916) has classified dormancy into seven types based on immature embryo, seed coat impermeability to water, mechanical resistance of seed coat, less seed coat permeability to gases, metabolic blocks within the embryo, and combination of all these as well as secondary dormancy in which dormancy is induced by imbibing seeds under unfavorable environment. Innate, induced, and enforced are the types of dormancy given by Harper (1957) and Roberts (1972). In Nikolaeva's (1969, 1977) classification, dormancy has been divided into three major groups, i.e., exogenous, endogenous, and combined dormancy, and subsequent sub-groups on the basis of the factors accountable for causing dormancy. Baskin and Baskin (1998, 2004) have further modified the above classification in order to make it more comprehensive and divided the seed dormancy into five broad groups, namely, morphological dormancy, physiological dormancy, morpho-physiological dormancy, physical dormancy, and combinational dormancy.

Physiological dormancy (PD): Deep, intermediate, and non-deep are the three levels associated with physiological dormancy. Deep physiological dormancy is characterized by the emergence of abnormal seedlings from the excised embryos of the dormant seeds with no role of gibberellic acid (GA) in promoting germination. Cold stratification of 3–4 months is required for seeds in order to start germination. In the case of intermediate level of PD, excised embryos on germination give rise to normal seedlings, also in some species germination can be promoted by GA, and for breaking dormancy in such seeds, cold stratification is required for a period of 2–3 months. Deep and intermediate PD is found in *Acer platanoides* and *Acer pseudoplatanus*, respectively (Finch-Savage et al. 1998). Non-deep physiological dormancy is exhibited by the majority of seeds and further divided into five types (1, 2, 3, 4, and 5) based on their differential pattern in physiological responses to temperature (Baskin and Baskin 2004). Most seeds fall under the category of type

1 and type 2. It is documented that as seed progresses from dormant to non-dormant stage, the temperature, suitable for seed germination, increases gradually from low to higher in type 1 and from high to lower in type 2. Embryos which are excised from those seeds which are physiologically dormant with non-deep level produce normal seedlings, their germination is promoted by GA, and dormancy in such seeds can be terminated by scarification, after ripening in dry storage and cold or warm stratification depending on the spp. Type 1 non-deep PD has been reported in *A. thaliana* (Finch-Savage and Leubner-Metzger 2006).

Morphological dormancy (MD): This stage of dormancy is found in seeds with immature or underdeveloped but differentiated (i.e., into cotyledons, hypocotyls-radicle) embryo. As these embryos are physiologically non-dormant, no pre-treatment is required in such seeds to break their dormancy. With the time, seed grows to their full size and germinate. Morphological dormancy is present in *Apium graveolens* (celery) of the family Apiaceae (Jacobsen and Pressman 1979).

Morpho-physiological dormancy (MPD): Similar to MD, this type of dormancy is also associated with seeds having underdeveloped or immature embryos, but unlike MD, these embryos are physiologically dormant (Baskin and Baskin 2004) so that seeds with MPD need to be treated in order to break the dormancy. Embryos of such seeds take more time to grow as compared to seeds exhibiting morphological dormancy. There are eight levels of MPD on the basis of pre-treatments required to break seed dormancy and to start the process of germination. Examples are *Trollius* of Ranunculaceae (Hepher and Roberts 1985) and *Fraxinus excelsior* of Oleaceae family (Finch-Savage and Clay 1997).

Physical dormancy: Sometimes seeds fail to germinate due to the presence of water-impermeable layers of palisade cells in the seed or fruit coat. Such type of dormancy is known as physical dormancy and can be broken by chemical or mechanical scarification so that water can be absorbed by the seeds to initiate the process of germination. In some taxa of Fabaceae family, it has been reported that seeds may also overcome physical dormancy by heat treatment as it disrupts the seed coat regions but not strophiole (Morrison et al. 1998). *Melilotus* and *Trigonella* are the examples of physical dormancy.

Combinational dormancy: It is a combination of both physical and physiological dormancy in which seeds are not able to germinate due to water-impermeable seed or fruit coat together with physiologically dormant embryo. Combinational dormancy is found in *Geranium* and *Trifolium*.

13.3 Biological Significance of Dormancy

Repetitive exposure of plant and animal species to favorable and unfavorable weather conditions in their natural habitats has made them adapted to their environments through the process called natural selection. Presence of dormancy can help the organisms to bypass the detrimental effect of adverse weather conditions and thereby can increase their chances of survival. Organisms in dormant state encounter complete cessation of physiological activities such as growth, while

photosynthesis, respiration, and water movement may be prohibited. The state of dormancy makes the organisms less dependent on their environments, as a result of which they become more tolerant to unfavorable environments (Joshi and Singh 2004). Some specialized organs, structures, and tissues are formed within the seeds and buds and enhance their tolerance level to adverse conditions. Dormant seeds do not germinate immediately after harvest even if there are favorable environmental conditions as these conditions might prevail only for a short span of time and may not be favorable for growth of that species. Germination occurs only on the occurrence of favorable weather conditions (Yan and Chen 2020). If a crop species requires some specific environment in order to complete its life cycle, it is essential that seeds of such species remain dormant till the onset of favorable environments for their survival. An interesting example has been reported in wild oat (*Avena fatua* L.) in which all the seeds obtained from one-time harvest do not germinate at the same time and only some seeds germinate, while others remain dormant (Joshi and Singh 2004). Such a dormancy pattern in crop species may escape them from the possibility of their complete destruction in case the adverse conditions come before the completion of their life cycle. During autumns and winters, the seeds of wild oat remain dormant, and germination occurs only in the following spring. There is no dormancy mechanism present in the seeds of cultivated oat; therefore, plants cannot escape the adverse environmental conditions and are killed by the successive cold weather. Moisture stress in deserts greatly affects plant survival and confined the growth period to a short period of time depending on the duration of moisture availability. This is the time during which new dormant seeds or regeneration buds are formed. Similar to seed dormancy, dormant shoot buds or regeneration buds are equally important for enhancing plant survival. Regeneration buds sustain adverse environmental conditions even better as they remain attached to the mother plant, thereby ensuring more stored food and water supply. It is also evident that seed dormancy is much less in domesticated species as compared to wild species. This lack of dormancy in seeds often causes their pre-mature germination within ears or pods when rain occurs just before the harvest and deteriorates the quality of crop produce. Seed dormancy can help farmers to avoid post-harvest losses to a great extent as they get sufficient time to store their crop produce safely after harvesting and threshing. Seed germination cannot be determined just after harvesting in dormant seeds which is essential for the purpose of seed certification; therefore, effective dormancy-breaking treatments are also required to deal with such situations (Joshi and Singh 2004). The concept of dormancy gives the clear vision to understand the reason of survival of individuals or populations under natural conditions and also protects the crop from deterioration caused by pre-harvest sprouting of seeds.

13.4 Factors Affecting Dormancy

Embryo factors in combination with seed coat factors exhibit different control mechanisms in seed dormancy. When dormancy is caused by dormant embryo or immature embryo, it is called as endogenous dormancy or embryo dormancy (Nasreen et al. 2002) because factors responsible for dormancy are present within the embryo. In certain cases, seed coats are very hard and do not allow embryo to grow properly or restrict the entry of water or gases into the seeds due to which germination does not take place and seeds remain dormant. This is termed as exogenous dormancy or seed coat-imposed dormancy (Nasreen et al. 2002) or coat-enhanced dormancy (Kucera et al. 2005), as the dormancy-causing factors reside within the seed coat but outside the embryo. Failure of mature excised embryo of seed to germinate on wet substratum under favorable conditions determines the presence of embryo dormancy which can be confirmed when excised embryo begins to germinate on the same medium and conditions after dormancy-breaking treatment. Germination of some cereal grains at harvest is prevented due to the combined effect of seed coat- and embryo-imposed dormancy with major contribution of seed coat. Glumes are the main factors responsible for causing seed dormancy in barley (Lenoir et al. 1983; Corbineau and Côme 1996). Similarly, these structures together with testa and pericarp induce dormancy in oat (Corbineau et al. 1986). In wheat and sorghum, the embryo is enclosed by the endosperm, testa, and pericarp and contributes to dormancy in the absence of hulls (Steinbach et al. 1995). Due to the covering structures of seed, the embryo may not get enough oxygen supply for its germination and therefore remains ungerminated. Suppression of dormancy is encountered when structures such as glumes, testa, and endosperm are detached from the grains, and this reduction of dormancy occurs at different range of temperatures (Côme et al. 1984; Simpson 1990; Benech-Arnold et al. 2003). Increase of dormancy and tolerance of wheat varieties toward pre-harvest sprouting are due to the fact that embryo factor also contributes to seed coat-imposed dormancy (Flintham 2000). In some cases, presence of cotyledons inhibits the germination of mature embryo. Removal of one cotyledon in *Euonymus europaeus* is required for its germination (Bulard 1960a, b), whereas in *Fraxinus excelsior* both the cotyledons are needed to be removed (Bulard 1960a, b). Barley cultivars may overcome dormancy by removing their scutellum which is particularly a modified cotyledon in the embryo (Grahl 1970). Incomplete embryo development is another factor responsible for dormancy in the members of the families Magnoliaceae, Araliaceae, Palmaceae, etc. (Joshi and Singh 2004). In some seeds, embryos are non-dormant but remain underdeveloped and start growing when favorable conditions like temperature, moisture, and light are provided to them. Such seeds germinate when the embryo matures completely without any treatment, but there is a need of dormancy-breaking conditions such as high summer and/or low winter temperatures for the growth of immature and dormant embryo during or before its growth (Joshi and Singh 2004). Sometimes dormancy is caused by the inhibitors which are present within the embryo, and leaching of such inhibitors out of the embryo is essential for seed germination (Jackson and Blundell 1963, 1965), e.g.,

Xanthium, *Fraxinus*, etc. (Joshi and Singh 2004). Induction of seed dormancy is also controlled by the abscisic acid (ABA) which is considered as the most important germination inhibitor and present in the fruits and seeds of avocado, coconut, maize (Milborrow 1967) and peach (Life and Crane 1966). Negative relationship has been found between ABA and germination of seeds of *Rosa arvensis* (wild rose) (Cornforth et al. 1966). Exhibition of dormancy in barley and oat grains also indicates the presence of ABA in their embryo (Jacobsen et al. 2002; Leymarie et al. 2008). It has also been reported that cotyledon growth and greening were prevented when treated with the solution of ABA (Durand 1975). Presence of this inhibitor in the pericarp and testa of the seeds, has made their removal essential for the commencement of germination (Jackson and Blundell 1963, 1965). Inhibition of germination has also been reported in rose seeds (Milborrow 1967) and hazel seeds (Bradbeer 1968) on application of synthetic ABA. Contrary to this, it has also been documented that ABA promotes dormancy only when it is produced in the embryo of seed. Induction of dormancy is not possible if its synthesis occurs in maternal tissues or it is applied externally (Nambara and Marion-Poll 2003); however, it is possible that ABA if transported to the embryo may induce seed dormancy (Kanno et al. 2010). In many species radical protrusion does not occur due to the physical barrier created by seed covering layers (Kucera et al. 2005). Characteristics of seed coat or testa are responsible for causing dormancy in non-endospermic seeds and also in *Arabidopsis* seeds where only a single cell layer of endosperm is present (Debeaujon and Koornneef 2000; Debeaujon et al. 2000). Seed dormancy in radish (Schopfer and Plachy 1993) and *Arabidopsis* (Debeaujon and Koornneef 2000, Debeaujon et al. 2000) is caused by testa which inhibits the radicle emergence (Schopfer and Plachy 1993), but radicle protrusion remains unaffected in the case of rape (Schopfer and Plachy 1984) and pea (Petruzzelli et al. 2000). Since the endospermic seeds are covered with both testa and endosperm, dormancy exhibited in such seeds is considered as a combined effect of these two. Endosperm limits the germination of lettuce, tomato, tobacco, and coffee. It covers the radicle tip which interferes in the process of radicle protrusion (Hilhorst 1995; Leubner-Metzger 2003). Grain structure along with chemical composition of seed coats reportedly plays a major role in causing seed dormancy. The presence of lemma and palea which together known as glumes are the characteristic feature of barley, oat, rice and some varieties of sorghum which restricts the uptake of water and oxygen to the embryo. Presence of such structures may also allow the entry of water-soluble chemicals to the grain (Rodríguez et al. 2015). Different phenolic compounds, such as phenolic acids, flavonoids, tannins, etc., are accumulated in the structures covering the grains (Tian et al. 2004; Weidner et al. 1993, 2002). Pro-anthocyanidins and anthocyanins are the major flavonoids, pro-anthocyanidins impart red color (Oki et al. 2002), and the latter is responsible for purple and black pigmentation in rice grains (Reddy et al. 1995). Accumulation of such compounds on the seed surface strongly inhibited the germination of some cereals, e.g., wheat, barley, and sorghum (Krogmeier and Bremner 1989). These compounds which are water soluble can make their route from the seed coat to embryo during the process of imbibition and make the seed dormant, e.g., wheat (Rathjen et al. 2009). Dormancy period is also

regulated by the temperature. Seeds of many species come out of dormancy after being exposed to low temperature for a definite period of time, e.g., Rosaceae, Oleaceae, Aceraceae, etc. Generally low temperature is required to overcome seed dormancy, but germination in the seeds of *Cyperus rotundus* (commonly known as nut grass) occurs when they are exposed to 40 °C for 3–6 weeks (Joshi and Singh 2004). Temperate cereals such as wheat, barley, rye, oat, tropical sorghum, rice, and millets express dormancy, whereas maize lacks dormancy at the time of maturity (Simpson 1990). Seeds of temperate cereals exhibit dormancy at high temperature, i.e., >15–20 °C (Corbineau and Côme 1996; Benech-Arnold et al. 2006), while seeds of tropical cereals remained dormant at lower temperature and attain higher germination at high temperature (Simpson 1990; Benech-Arnold et al. 2003). Condition such as low temperature at grain development stage favored dormancy in wheat (Nakamura et al. 2011) and also in barley (Rodriguez et al. 2001). Dormancy of seeds is maintained when dry seeds are stored at temperature of −18 °C (Lenoir et al. 1983). The water stress favored the dormancy in less dormant wheat at the time of grain maturity irrespective of the available temperature (Biddulph et al. 2005).

High temperature and hypoxia are the factors responsible for initiating secondary dormancy in most of the cereal crops which already possess primary dormancy up to some level (Simpson 1990). The examples are wheat (Grahl 1965), barley (Leymarie et al. 2008; Hoang et al. 2013), and oat (Corbineau et al. 1993). It is evident that incubation of barley and oat seeds for 3–8 h at 30 °C is required for developing secondary dormancy (Corbineau et al. 1993; Leymarie et al. 2008). Imposing secondary dormancy in seeds is also possible at low temperatures, i.e., 10–15 °C, together with unfavorable germination conditions such as <10% of O_2 tension or blue light during imbibition (Hoang et al. 2013, 2014). Light also regulates the dormancy. *Betula* spp. (Betulaceae family), *Lepidium virginicum* (also known as pepper grass, Brassicaceae), *Nicotiana tabacum* (cultivated tobacco, Solanaceae) and *Lactuca sativa* (lettuce, Asteraceae family) are some examples in which light is responsible for promoting dormancy, whereas seeds of *Phacelia tanacetifolia* (blue tansy or purple tansy) germinate only in darkness (Serrato-Valenti et al. 1998), also in *Nemophila insignis*, light has been associated with the termination of dormancy (Joshi and Singh 2004). White or blue light also inhibits the germination of wheat and barley grains (Rodríguez et al. 2015).

13.5 Importance of Gibberellic Acid and Abscisic Acid in Seed Dormancy

The role of gibberellic acid in seed dormancy is basically known for promoting seed germination. It also plays an important part in altering the state of dormancy in seeds (Finkelstein et al. 2008). The ratio of GA to ABA has always been a critical parameter in determining the state of dormancy. Significant increase in germination due to GA has been reported in papaya, whereas in *Arabidopsis* seeds, a higher amount of ABA neutralized the growth-enhancing property of GA (Derkx and Karssen 1993). The presence of GA in seeds also helps in stimulating the synthesis

of hydrolytic enzymes particularly amylases which are responsible for embryonic growth by way of producing amino acids and reducing sugars (Khan 1971; Metivier 1979; Mayer and Poljakoff-Mayber 1989). Degree of dormancy in rice seeds may be depicted clearly by their alpha amylase activity (Viera et al. 2002). Not only the natural occurrence of GA in seeds but its exogenous application has also been found effective in breaking dormancy (Furutani and Nagao 1987; Tseng 1992; Andreoli and Khan 1993; Paz and Vázquez-Yanes 1998; Salomão and Mundim 2000; Bhattacharya and Khuspe 2001). Exogenous application of GA helps in counter balancing the effect of ABA on dormant seeds (Viera et al. 2002). The application of GA at 200 ppm for a period of 24 h showed increased germination percentage in papaya seeds. Also 500 and 1000 ppm of GA were effective in dormancy breaking of papaya seeds (Tseng 1992). Soaking of dormant rice seeds in a solution of GA (60 mg/L for a period of 36 h) was sufficient enough to break their dormancy (Viera et al. 2002). The following table shows the effect of GA application on various dormant seeds (Table 13.1).

The role of ABA in context with seed dormancy is well established in a number of studies (Koornneef et al. 2002; Finch-Savage and Leubner-Metzger 2006; Finkelstein et al. 2008; Wang et al. 1994). This hormone regulates the seed dormancy in cereal crops such as barley, sorghum, and wheat (Walker-Simmons 1987; Jacobsen et al. 2002). De novo synthesis of ABA is needed for embryo- or endosperm-induced dormancy (Nambara and Marion-Poll 2003). It has been reported that seeds may sustain dormancy even in the absence of ABA due to late maturation and dessication stage of seed development (Kermode 2005), for example, seeds of *Helianthus annuus* (sunflower), barley, beech-nut, *Lactuca sativa* (lettuce), etc. (Wang et al. 1995; Bianco et al. 1997; Yoshioka et al. 1998). However, ABA and GA together play an important role in regulating seed dormancy (Karssen and Lacka 1986; Karssen 1995). According to the regulation model of dormancy and germination with respect to GA and ABA, the synthesis of former and its signalling influences the initiation of dormant state, while the latter influences the transition from dormant to germination. The former process is known as catabolism of GA, while the latter one is known as ABA catabolism (Fig. 13.1). ABA content is high in mature dormant seeds, while in non-dormant seeds, it is present in reduced level. Also, during imbibition, a dormant seed keeps on synthesizing ABA, but this trend is not followed by the non-dormant seeds. Dormancy can be terminated in the seeds of *Myrica esculenta*, *M. pensylvanicum*, and *M. adenophora* by application of GA as it neutralizes the ABA effect (Hamilton and Carpenter 1977; Bhatt et al. 2000; Chien et al. 2000), and also helps in promoting embryonic growth by weakening the surrounding tissues within the embryo (Bewley 1997).

13.6 Role of ABA in Seed Dormancy Termination

In general, ABA level and its effect on the embryo are reduced during seed development (especially in later stages) and dessication (Kermode et al. 1989; Xu and Bewley 1991; Kermode 1990, 1995; Bewley 1997; Schmidtz 2000; Schmitz

Table 13.1 Effect of gibberellic acid application on various dormant seeds

	Name of the plant	Scientific name	Family	Conc/ amount of GA	Duration	Response to dormant seeds	Reference
1	Ouricury palm or licuri palm	*Syagrus coronata*	Arecaceae	0.3 mM	24 h	Higher emergence	Medeiros et al. (2015)
2	Creeping sage	*Salvia sonomensis* Greene	Lamiaceae	500 ppm	4 h	Favored germination	Nord et al. (1971)
3	Lentil genotypes	*Lens culinaris*	Fabaceae	0 µM/L	[a]	Positive germination	Shohani and Mehrabi (2014)
				2 µM/L			
				4 µM/L			
				6 µM/L			
4	Eucalyptus seedling	Eucalyptus	Myrtaceae	50 mg/ml	[a]	Positive effect on growth and germination	Bachelard (1967)
5	Loquat	*Eriobotrya japonica*	Rosaceae	150	12, 24, 36 h	Germination	Al-Hawezy (2013)
				200	12, 24, 36 h	Germination	
				250	12, 24, 36 h	Highest germination rate than the above concentration	
6	Thymus	*Thymus satureioides* L	Lamiaceae	50 ppm AG3	[a]	Increase germination 27%	Chetouani et al. (2017)
7	Lavender	*Lavandula dentata*	Lamiaceae	1000 ppm	[a]	67% enhanced germination	
8	Persian shallot	*Allium hirtifolium* Boiss	Amaryllidaceae	[a]	[a]	No response in seed germination	Dashti et al. (2012)
9	Black cumin	*Bunium persicum*	Apiaceae	100 µL	[a]	Germination	Emamipoor and Maziah (2014)
10	Mazzard cherry seeds	*Prunus avium*	Rosaceae	500 ppm	After 120 days of stratification	79.74% germination	Cetinbas and Koyuncu (2006)

[a]Data not available

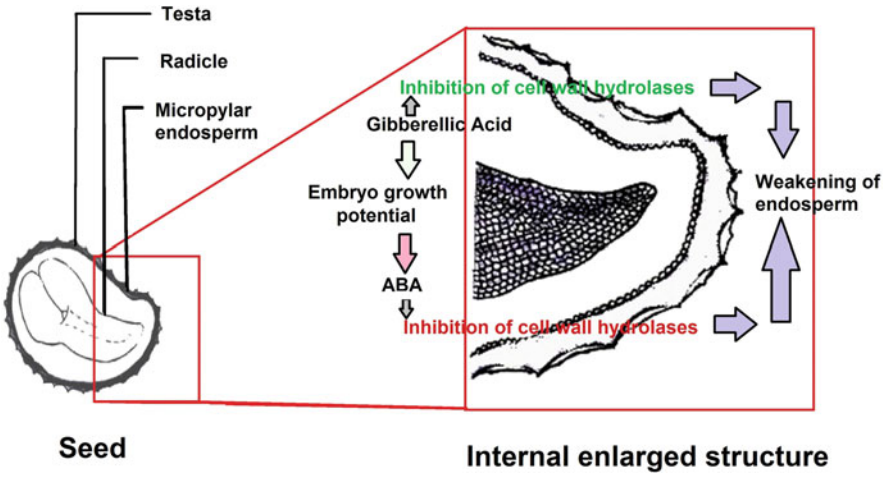

Fig. 13.1 Mechanism of ABA and GA in seed dormancy. Presence of gibberellic acid in seeds causes weakening of the cells enclosing the embryo with induction of cell wall hydrolases leading to breakage in the endosperm and initiation of germination while vice-versa in the case of presence of ABA in seeds

et al. 2001, 2002); therefore, ABA biosynthesis is necessary for imbibed seeds in order to sustain dormancy. Furthermore, dormancy breaking in the seeds of several plants, such as barley, yellow cedar, beech-nut, Douglas fir, etc., has been found to be connected with increased ABA catabolism (Le Page-Degivry et al. 1997; Schmidtz 2000; Schmitz et al. 2002; Corbineau et al. 2002; Jacobsen et al. 2002). The decline in ABA level is started during the dormancy termination of seed and continues as the germination progresses. For example, not much effect on ABA was seen in dry barley grains when in after-ripening stage, but the level declined rapidly after 12 h when germination begins and that level was maintained up to 30 h. ABA content is reduced in imbibed dormant seeds through the process of oxidation and conjugation (Zaharia et al. 2005) by adopting several pathways (Zaharia et al. 2005; Cutler and Krochko 1999; Zeevaart 1999) as depicted in the following flowchart (Fig. 13.2). Embryonic capacity to metabolize ABA is altered in the seeds of yellow cedar plant in order to overcome dormancy (Schmitz et al. 2002). Similar mechanism has been found to be involved in dormancy breaking of barley and *Arabidopsis* seeds where the product of 8-hydroxylation of ABA has been observed (Jacobsen et al. 2002; Kushiro et al. 2004).

Dormancy breaking by way of chemical means has been tested in several seeds. The application of carotenoid and fluridone (inhibitor of ABA synthesis) along with GA has been found very effective in breaking dormancy in yellow cedar seeds (Schmitz et al. 2001). Fluridone in combination with GA was found more effective as compared to when applied solely. Imbibed dormant seeds of *Arabidopsis* on germination showed rapid decrease of ABA after 12 h of their treatment with

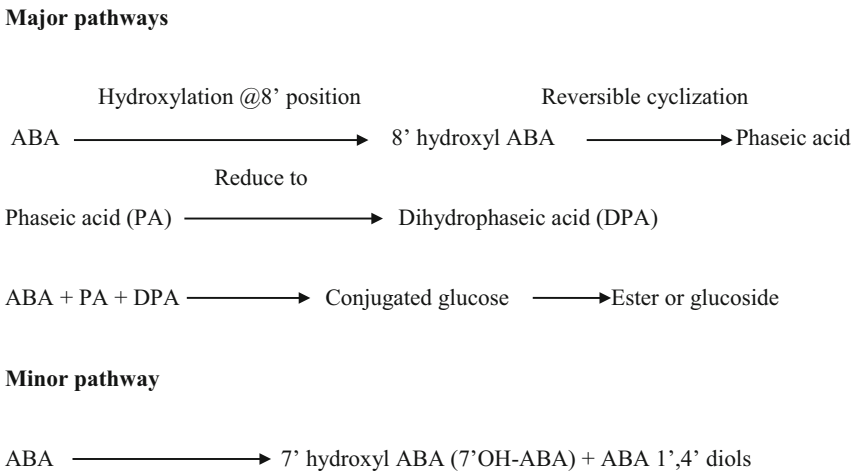

Fig. 13.2 Major and minor pathway of declination of ABA in seeds showing transfer from dormant to germination state

fluridone (Ali-Rachedi et al. 2004). Application of smoke on several dormant seeds has also found effective in terminating seed dormancy of several plants (Flematti et al. 2004; Gubler et al. 2005). It is known that butenolide, a component of smoke, has the potential to terminate dormancy and promote germination in seeds (Flematti et al. 2004). It has also been reported that smoke-associated compounds help in changing the hormonal levels of seeds, for instance, in the seeds of *Nicotiana attenuata*, use of smoke water declined the ABA level by eight fold when compared with the control (Schwachtje and Baldwin 2004). Nitric oxide is also used to break dormancy (Bethke et al. 2004). Promotive factors of nitric oxide interfere with exogenous ABA, as a result of which ABA content declines due to the presence of nitric oxide radical (Fig. 13.3). Seeds of many species on their exposure to either white or red light overcome dormancy, e.g., lettuce seeds started germinating when exposed to red light at 660 nm or to white light (Joshi and Singh 2004). Germination had also occurred in *Pinus* seeds after their exposure to white or red light due to declined ABA level in seeds, but there was no decline in ABA content when exposed to far-red light and red light simultaneously (Tillberg 1992). The ABA content in the embryo of yellow cedar seeds was less (about twofold than in dormant seed's embryo) during their progression from dormant to non-dormant stage, but in mega-gametophyte, there was no such reduction in ABA content (Schmidtz 2000; Schmitz et al. 2002). It has also been reported that ABA content in seeds had declined four folds after getting chilling treatment for about 7 weeks. The rate of decrease in ABA content depends on the duration of chilling treatment (Corbineau et al. 2002). GA and ABA both play crucial roles in controlling the seed dormancy in *Nicotiana plumbaginifolia* (Grappin et al. 2000). Production of ABA in dormant seeds has been controlled by catabolic gene, CYP707A2, of ABA which has been found to be regulated by exogenous nitrate (Matakiadis et al. 2009). Seed dormancy

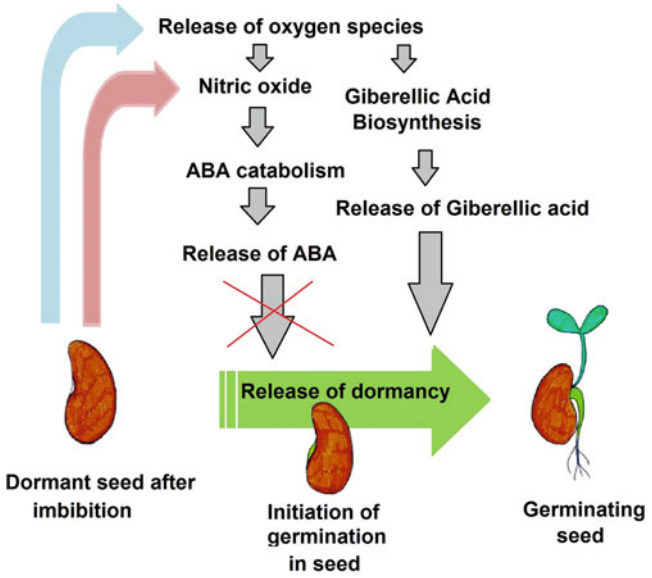

Fig. 13.3 Association of ABA and GA in sustaining and breaking seed dormancy

is governed by several genes present in seeds, and at times these genes are linked to a particular chromosome region (Li and Foley 1997). Several factors, like the environmental conditions, production of ABA, etc., govern the controlling power of dormancy in seeds. These factors have been described earlier in the chapter. However, a recent study has shown that dormancy can be controlled by the expression levels of delay of germination 1 gene (DOG 1). The higher the production of DOG 1 protein in plants, the more is the period of lasting seed dormancy. If this protein is not formed in seeds, then there will be no dormancy condition. In the seeds of *Arabidopsis*, this is a chief trait locus on quantitative basis in which dormancy can be controlled and its negative regulation helps in the germination of seeds (Fedak et al. 2016).

13.7 Imposing Seed Dormancy

Seed dormancy can be imposed by the following ways:

Seed coatings/testa: Hardening of seed coat in seeds is one of the ways to impose dormant conditions as well as maintain the former condition in seeds. In leguminous plants, the seed coat is impermeable to water and gases due to the hardness attained by the combination of structural as well as chemical properties (Gonclaves et al. 2011).

Use of inhibitors: Psoralen has been recognized for its germination-inhibiting property in *Psoralea subacaulis* of Leguminosae family (Baskin et al. 1967).

Germination of sugarbeet is reduced when coated with polymer film. This film not only restricts the oxygen supply to embryo but also prevents germination inhibitors to be leached out (Duan and Burris 1996).

13.8 Advantages and Disadvantages of Seed Dormancy

Advantages

- Seed dormancy makes sure that the seed germination will occur only when conditions are favorable for its growth.
- Seed dormancy ensures suitable propagation of seeds to spread in far-off places.
- Seed dormancy helps in the storage of seeds for longer duration.
- Presence of dormancy can help the organisms to by-pass the detrimental effect of adverse weather conditions and thereby can increase their chances of survival.
- Seed dormancy inhibits pre-harvest sprouting or viviparity in seeds.
- Dormancy in seeds is also one way to save the seeds of different plant species before being extinct which may be due to occurrence of certain calamity.
- Also it helps in creating seed banks in soil.

Disadvantages

- In dormant seeds germination does not occur immediately after harvesting.
- It causes problems in maintaining the plant population.
- After maturity of seeds, sowing of seeds is not recommended due to the dormancy period.
- It poses problems in seed germination tests.
- In the case of weeds particularly the noxious ones, occurrence of dormancy makes seeds dormant for several years, and so their eradication becomes difficult.

13.9 Methods of Breaking Dormancy

There are two ways in which seed dormancy can be broken: (1) natural breakdown of seed dormancy and (2) dormancy breaking by different treatments.

13.9.1 Natural Breakdown of Seed Dormancy

Seeds overcome dormancy naturally by a number of ways. Some seeds are dormant due to the presence of hard seed coats which restricts the gaseous exchange and water supply to the embryo; such hard seed coats are weakened up by the direct action of microbes on their physical as well as chemical properties, e.g., damaging effect of *Rhizoctonia* spp. on the seed coats of *Albizia julibrissin*. Seed coats of *Acacia*, *Crotalaria*, *Gossypium*, and many plants of Papilionaceae family have been reported to be cracked by the fire and heat in the natural vegetation. Inhibitors present on the seed may be lost, or the seed coats may become more permeable by

the action of digestive enzymes present in the digestive tracts of animals, as a result of which seeds may overcome dormancy. The effect of digestive enzymes of birds' digestive tract on seed germination was demonstrated by feeding seeds to caged birds, and improved germination was found in seeds passed through birds' digestive tract (Joshi and Singh 2004). Natural breakdown of dormancy may also occur due to leaching of inhibitors present in the hard seed coats, weakening of seed coats, or rupturing of the hard covering by mechanical means unknowingly; exposure to heat, cold, and light by inactivation/oxidation of growth inhibitors; formation of growth hormones which helps in neutralizing the effect of growth inhibitors; and production of low levels of gibberellins, cytokinins, and ethylene which help in breaking the seed dormancy and attainment of full maturity by the embryo in due course of time when it gets adequate moisture, temperature, and aeration.

13.9.2 Dormancy Breaking by Different Treatments

Several methods have been developed in order to break dormancy in seed which either breaks the seed coat or make them soft in order to increase their permeability for water and gaseous exchange, which are the main factors affecting seed germination.

1. **Freeze-thaw scarification:** A new method developed for breaking the seed dormancy wherein the seeds were stored in a freezer for a time period at −80 °C followed by immediate treatment with hot water at 90 °C/5 s (Tiryanki and Topu 2014). Dormant seeds of *Lupinus albus* L. and *Trifolium pratense* L. showed higher germination percentage by this method of breaking seed dormancy.
2. **Hot water treatment** (for dissolving of surface inhibitors present on seed coat)**:** This is basically known as the hot water scarification method wherein the dormancy of hard seeds is even known to be overcome by soaking seeds in hot water for 120–124 h, having a temperature of 77–100 °C, and the volume of the water 4–5 times more than the seed. After the seeds are exposed to this treatment, they should be planted immediately. Soaking seeds in hot water also helps in breaking the hard seed coat; however, the duration of soaking may vary from one seed to another. Several studies have shown the effective role of hot water treatments in dormancy breaking of tropical and sub-tropical seeds (Doussi and Thanos 1994; Prasad and Nautiyal 1996; Doran et al. 1983). Besides its role in dormancy breaking, it was also found effective against the pathogens residing on seed coats (Hoersten and Luecke 2001).
3. **Exposure of seeds to different types of light**
   (a) **White light:** This method is used to break dormancy in seeds which are sensitive to light (e.g., tobacco seeds, tomato seeds, *Betula* and *Digitalis* seeds). Only those seeds overcome dormancy in the presence of white light which have imbibed the moisture of 30–40%.
   (b) **Red and far-red light:** Exposure of light-sensitive seeds toward low-intensity light for a period of 1–2 min is adequate enough for seeds

to overcome dormancy. The most effective wavelength for germination of seeds is the red portion of white light possessing a wavelength of 660 nm. It is interesting that germination in seeds does not occur when they are exposed to far-red region of light (730 nm). Seeds when first exposed to red light followed by far-red light remain ungerminated, but germination occurs when they are first exposed to far-red light in place of red light.

4. Exposure of seeds to darkness: Photoblastic seeds are known to overcome dormancy by keeping them in dark place.
5. Treatment of seeds with fat solvents for dissolving surface inhibitors present on the seed coat.
6. Exposure of seeds to hydraulic pressure (up to 2000 kg) for a period of 5–20 min helps in weakening off the hard covering of seed coat.
7. Rupturing of hard seed coats by mechanical means (referred to as scarification) which involves breaking of hard seed coat so that water can enter into the seed. Rubbing of the seed coat with a rough sandpaper, scratching the hard coat with the help of a sharp knife, and keeping the seeds in a processing machine whose blades are covered with tape to reduce its sharpness are the ways to modify seed coat properties, or even dormancy. Seeds with hard coats could be broken by nut cracker (Delouche 1964). These methods come under mechanical scarification. Sandpaper, file, and hammer are the best tools to be used for mechanical scarification of seeds in order to terminate their dormancy (Fig. 13.4) (Baskin and Baskin 1998; Hoffma et al. 1989; Page et al. 1966; Roth et al. 1987). In hybrids of *Althaea*, piercing, chipping, or filing the seed coat helps in enhancing the germination (ISTA 2011). Hundred percent germination has also been reported in mechanically scarified seeds of *Iliamna corei* (Baskin and Baskin

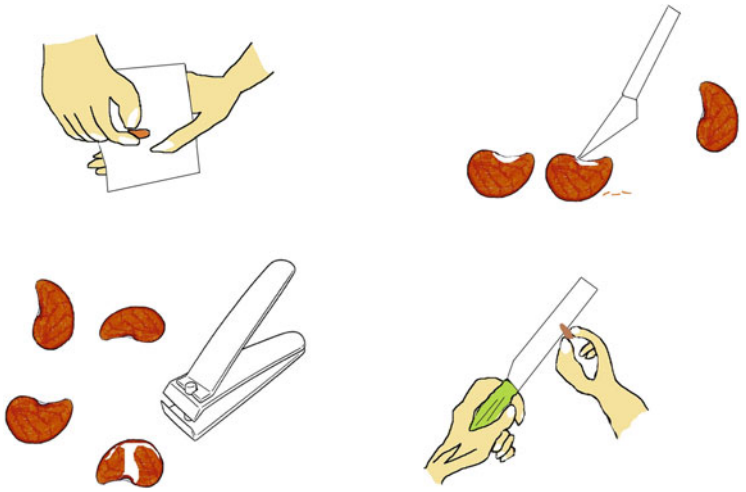

Fig. 13.4 Different mechanical ways of scarification

1997). Proper care should be taken during the scarification of seeds in order to avoid damage to the embryonic axis.

8. **Acid scarification:** Seed dormancy can be broken by acid stratification in which seeds are soaked in concentrated sulfuric acid for 10 min to 1 h which makes the seed coat permeable. It may vary from species to species that for how long seeds are required to be treated with acid (Delouche 1964). An increase in germination up to 40% was seen in *S. grossulariifolia* seeds after dipping it in sulfuric acid against its control (Page et al. 1966). Likewise in the case of seeds of Sphaeralcea, 77% improvement (against 5% in control) in germination was observed in *S. coccinea*, while 69% and 62% improvement in germination were seen in *S. grossulariifolia* (against 14% and 32% in control) when the seeds were immersed in a solution of 18 M sulfuric acid for 10 min (Roth et al. 1987). However, in one species, *S. munroana*, this method was not found effective in breaking the dormancy. In *Allium hirtifolium* Boiss., sulfuric acid scarification showed no response in seed germination. But the combination of sulfuric acid scarification followed by cold stratification after 60 days showed the highest germination by breaking the dormancy (Dashti et al. 2012).
9. **Rupturing of seed coats by other ways of scarification:** Warm moist scarification and high temperature scarification can also be used. In warm moist scarification, seeds are kept in non-sterile, moist, warm medium for several months, as a result of which seeds may overcome dormancy as their seed coats become soft through the activity of micro-organisms. Dormancy which is present in seeds due to hard seed coat can also be broken with the help of high temperature scarification.
10. **Embryo treatments:** Stratification, high temperature treatment, and chemical treatments are also used to eradicate those factors which are present within the embryo and make the seeds dormant. Stratification is a process in which seeds are first incubated at 0–15 °C over a moist substratum prior to their transfer to a temperature optimum for germination, e.g., *Prunus cerasus* (cherry), *Brassica campestris* (mustard), etc., 2–6 months of incubation at 5–10 °C has been found effective in breaking dormancy in the species of family Rosaceae (Joshi and Singh 2004). In high temperature treatment, incubation temperature of 40–50 °C for few hours to 1–5 days has been found useful for seeds of some species to overcome dormancy. Moisture content of seeds undergoing high temperature treatment should be less than 15%, e.g., *Oryza sativa* (Joshi and Singh 2004). Seeds of some species may overcome dormancy when treated with different chemicals such as growth regulators or other chemicals. GA_3 (usually 100 ppm) and kinetin (usually 10–15 ppm) are the most commonly used growth regulators for the purpose of breaking seed dormancy. Seeds are also treated with potassium nitrate (e.g., *Avena sativa*, *Hordeum vulgare*, and *Lycopersicon*) and thiourea (lettuce, gladiolus, etc.) for breaking dormancy (Joshi and Singh 2004; Agrawal and Dadlani 1995). Organic solvents are also being used in breaking dormancy like diethyl dioxide, thidiazuron and forchlorfenuron, benzylaminopurine, etc. 67% germination has been reported in the dormant seeds of *S. grossulariifolia* when soaked in diethyl dioxide solution for 4 h (Page

et al. 1966), whereas only 3 h of soaking in dimethyl dioxide was found effective for breaking dormancy in the case of *S. coccinea*, *S. munroana*, and *S. grossulariifolia* (Roth et al. 1987), but this chemical is hazardous (Mallinckrodt Baker 2008a, b). Germination also occurred in dormant seeds of *Bunium persicum* after their treatment with thidiazuron and forchlorfenuron, benzylaminopurine, and gibberellic acid (Emamipoor and Maziah 2014). It has also been suggested that the highest germination may be achieved when seeds are treated with 7500 ppm concentration of potassium nitrate after 120 days of stratification (Cetinbas and Koyuncu 2006). In potato, sprouting of the tuber was enhanced by use of growth promoters at low levels such as thiourea, rindite, carbon disulfide, and bromoethane. In *Pyrus betulaefolia* and *Pyrus calleryana*, treatment of seeds with 6-benzylaminopurine, indole-3-acetic acid, and GA showed partial breakage in dormancy by initiating the germination (Bao and Zhang 2010).

13.10 Conclusion

The concept of dormancy gives us the clear vision to understand the reason of survival of individuals or populations under natural conditions and also protects the crop from deterioration caused by pre-harvest sprouting or vivipary. A comprehensive classification of seed dormancy based on the embryo and seed coat factors clearly explains the morphological, physiological, morpho-physiological, physical, and combinational dormancy. The role of ABA and GA in seed dormancy is very crucial as the former is considered as the most important germination inhibitor, while GA has its major role in promoting and maintaining seed germination. Incapability of dormant seeds to germinate immediately after harvesting may cause hindrance in the process of seed certification and therefore require the effective seed dormancy-breaking treatments other than its natural breakdown.

References

Agrawal PK, Dadlani M (1995) Techniques in seed science and technology, 2nd edn. South Asian Publishers; International Book Company, New Delhi; Absecon Highlands, NJ, pp 109–113

Al-Hawezy SMN (2013) The role of the different concentrations of GA3 on Seed Germination and Seedling Growth of Loquat (Eriobotrya japonica L.). IOSR J Agric Veterin Sci 4(5):03–06

Ali-Rachedi S, Bouinot D, Wagner M-H, Bonnet M, Sotta B, Grappin P, Jullien M (2004) Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. Planta 219:479–488

Andreoli C, Khan AA (1993) Improving papaya seedling emergence by matriconditioning and gibberellin treatment. HortScience 28:708–709

Bachelard EP (1967) Effects of gibberellic acid, kinetin and light on germination of dormant seeds of some Eucalypt species. Aust J Bot 15:393

Baker M (2008a) Sulfuric acid. MSDS No. S8236
Baker M (2008b) 1,4-Dioxane. MSDS No. D7552
Bao JP, Zhang SL (2010) Effects of seed coat, chemicals and hormones on breaking dormancy in pear root stock seeds (*Pyrus betulaefolia* Bge. and *Pyrus calleryana* Dcne). Seed Sci Technol 38 (2):348–357
Baskin JM, Baskin CC (1997) Methods of breaking seed dormancy in endangered species Iliamna corei (Sherff) (Malvaceae), with special attention to heating. Nat Areas J 17:313–323
Baskin CC, Baskin JM (1998) Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, CA
Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16. https://doi.org/10.1079/SSR2003150
Baskin JM, Ludlow CJ, Harris TM, Wolf FT (1967) Psoralen, an inhibitor in the seeds of Psoralea subacaulis (Leguminosae). Phytochemistry 6(9):1209–1213
Benech-Arnold RL, Enciso S, Sanchez RA, Rodriguez MV (2003) On the hormonal nature of the stimulatory effect of high incubation temperatures on germination of dormant sorghum (S. bicolor) caryopses. New Phytol 160:371–377
Benech-Arnold RL, Gualano N, Leymarie J, Côme D, Corbineau F (2006) Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryo of dormant barley grains. J Exp Bot 57:1423–1430
Bethke PC, Gubler F, Jacobsen JV, Jones RL (2004) Dormancy of *Arabidopsis* seeds and barley grains can be broken by nitric oxide. Planta 219:847–855
Bewley JD (1997) Seed germination and dormancy. Plant Cell 9:1055–1066
Bewley JD, Black M (1994) Seeds: physiology of development and germination. Plenum Press, New York, NY
Bhatt ID, Rawal RS, Dhar U (2000) Improvement in seed germination of Myrica esculenta Buch.-Ham. ex D. Don: a high value tree species of Kumaun Himalaya, India. Seed Sci Technol 28:597–605
Bhattacharya J, Khuspe SS (2001) In vitro and in vivo germination of papaya (Carica papaya L.) seeds. Sci Hortic 91:39–49
Bianco J, Garello G, Le Page-Degivry MT (1997) *De novo* ABA synthesis and expression of seed dormancy in a gymnosperm: *Pseudotsuga menziesii*. J Plant Growth Regul 21:115–119
Biddulph TB, Mares DJ, Plummer JA, Setter TL (2005) Drought and high temperature increases preharvest sprouting tolerance in a genotype without grain dormancy. Euphytica 143:277–283
Bradbeer JW (1968) Studies in seed dormancy. IV. The role of endogenous inhibitors and gibberellin in the dormancy and germination of *Corylus avellana* L. seeds. Planta 78:266–276
Bulard C (1960a) Physiology and biochemistry of seeds. J.D. Bewley. C R Acad Sci 250:2922–2924
Bulard C (1960b) Physiology and biochemistry of seeds. J.D. Bewley. Phyton 20:115–125
Cetinbas M, Koyuncu F (2006) Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea. Hortic Sci 33:119–123
Chetouani M, Mzabri I, Aamar A, Boukroute A, Kouddane N, Berrichi A (2017) Effect of gibberellic acid (AG3) on the germination of seeds of *Thymus satureioides* L and *Lavandula dentate*. J Mater Environ Sci 8(3):942–948
Chien CT, Chen YC, Chen SY, Hong KY (2000) Dormancy-releasing strategies for Myrica seeds. Taiwan J For Sci 15:473–481. In Chinese with summary, tables, figures and literature cited in English
Côme D, Lenoir C, Corbineau F (1984) La dormance des céréales et son elimination. Seed Sci Technol 12:629–640
Corbineau F, Côme D (1996) Barley seed dormancy. Bios 261:113–119
Corbineau F, Lecat S, Côme D (1986) Dormancy of three cultivars of oat seeds (*Avena sativa* L.). Seed Sci Technol 14:725–735
Corbineau F, Black M, Côme D (1993) Induction of thermodormancy in *Avena sativa* seeds. Seed Sci Res 3:111–117

Corbineau F, Bianco J, Garello G, Côme D (2002) Breakage of *Pseudotsuga menziesii* seed dormancy by cold treatment as related to changes in seed ABA sensitivity and ABA levels. Physiol Plant 114:313–319

Cornforth JW, Milborrow BV, Ryback G (1966) Identification and estimation of (+)-abscisin II ("dormin") in plant extracts by spectropolarimetry. Nature 210:627–628

Crocker W (1916) Mechanics of dormancy in seeds. Am J Bot 3:99–120

Cutler AJ, Krochko JE (1999) Formation and breakdown of ABA. Trends Plant Sci 4:472–478

Dashti F, Ghahremani-Majd H, Esna-Ashari M (2012) Overcoming seed dormancy of mooseer (*Allium hirtifolium*) through cold stratification, gibberellic acid and acid scarification. J For Res 23:707–710

Debeaujon I, Koornneef M (2000) Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiol 122:415–424

Debeaujon I, Leon-Kloosterziel KM, Koornneef M (2000) Influence of the testa on seed dormancy, germination and longevity in *Arabidopsis*. Plant Physiol 122:403–414

Delouche JC (1964) Seed dormancy. Seed Technology Laboratory, Starkville, MS

Derkx MPM, Karssen CM (1993) Effects of light and temperature on seed dormancy and gibberellin-stimulated germination in *Arabidopsis thaliana*: studies with gibberellins deficient and -insensitive mutants. Physiol Plant 89(2):360–368

Doran I, Tumbull JW, Boland DJ, Gunn BN (1983) Hand book on seeds of dry. Acacia Division of Forest Research, CSIRO, Australia Food and Agriculture Organization of the United Nation, Canberra ACT

Doussi MA, Thanos CA (1994) Post-fire regeneration of hard seeded plants: ecophysiology of seed germination. In: Viegas DX (ed) Proceeding of the 2nd conference on forest fire research, Coimbra, Portugal, November, 1994. D 25, vol II, pp 1035–1044

Duan X, Burris JS (1996) Film coating impairs leaching of germination inhibitors in sugar beet seed. Crop Sci 32(2):515–520

Durand M (1975) Physiology and biochemistry of seeds. J.D. Bewley. Physiol Veg 13:603–610

Emamipoor Y, Maziah M (2014) An efficient method in breaking of dormancy from *Bunium persicum* (boiss) Fedtsch seeds: a valuable herb of Middle East and central Asia. Asian Pac J Trop Biomed 4(8):642–649

Fedak H, Palusinskaa M, Krzyczmonika K, Brzezniaka L, Yatusevicha R, Pietrasa Z, Kaczanowskia S, Swiezewskia S (2016) Control of seed dormancy in *Arabidopsis* by a cis-acting non-coding antisense transcript. PNAS 113:E7846–E7855

Finch-Savage WE, Clay HA (1997) The influence of embryo restraint during dormancy loss and germination of *Fraxinus excelsior* seeds. In: Ellis RH, Black M, Murdoch AJ, Hong TD (eds) Basic and applied aspects of seed biology. Kluwer Academic Publishers, Dordrecht, pp 245–253

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171(3):501–523

Finch-Savage WE, Bergervoet JHW, Bino RJ, Clay HA, Groot SPC (1998) Nuclear replication activity during seed-dormancy breakage and germination in the three tree species: Norway maple (*Acer platanoides* L.), sycamore (*Acer pseudoplatanus* L.) and cherry (*Prunus avium* L.). Ann Bot 81:519–526

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol 596(1):387–415

Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD (2004) A compound from smoke that promotes seed germination. Science 305:977

Flintham JE (2000) Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. Seed Sci Res 10(1):43–50

Furutani SC, Nagao MA (1987) Influence of temperature, KNO_3, GA_3 and seed drying on emergence of papaya seedlings. Sci Hortic 32:67–72

Gonclaves EP, Franklim SJS, Silva SS, Tavares DS, Viana IS, Cardoso BCC (2011) Dormancy breaking in *Ormosia arborea* Seeds. Int J Agron 2011:524709. 5 p
Grahl A (1965) Induction of secondary dormancy in wheat by means of high temperature and the effect of the humidity of the seed bed. Proc Int Seed Test Assoc 30:787–801
Grahl A (1970) Physiology and biochemistry of seeds. Proc Int Seed Test Assoc 36:427–438
Grappin P, Bouinot D, Sotta B, Miginiac E, Julien M (2000) Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. Planta 210(2):279–285
Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol 8:183–187
Hamilton DF, Carpenter PL (1977) Seed germination of *Myrica pensylvanicum* L. HortScience 12:565–566
Harper JL (1957) The ecological significance of dormancy and its importance in weed control. Proc Int Cong Crop Protect 4:415–420
Harper JL (1977) Population biology of plants. Academic Press, London
Hepher A, Roberts JA (1985) The control of seed germination in *Trollius ledebourii*: the breaking of dormancy. Planta 166:314–320
Hilhorst HWM (1995) A critical update on seed dormancy. I. Primary dormancy. Seed Sci Res 5:61–73
Hoang HH, Bailly C, Corbineau F, Leymarie J (2013) Induction of secondary dormancy by hypoxia in barley grains and its hormonal regulation. J Exp Bot 64:2017–2025. https://doi.org/10.1093/jxb/ert062
Hoang HH, Sechet J, Bailly C, Leymarie J, Corbineau F (2014) Inhibition of germination of dormant barley (*Hordeum vulgare* L.) grains by blue light as related to oxygen and hormonal regulation. Plant Cell Environ 37:1393–1403. https://doi.org/10.1111/pce.12239
Hoersten DV, Luecke W (2001) Thermal treatment of seeds-comparison of different methods for eradicating seedborne fungi. ASABE 12:21–26
Hoffma MT, Cowling RM, Douie C, Pierce SM (1989) Seed predation and germination of *Acacia erioloba* in the Kuiseb River Valley, Namib Desert. S Afr J Bot 55:103–106
ISTA (2011) International rules for seed testing. International Seed Testing Assoc, Bassersdorf
Jackson GAD, Blundell JB (1963) Germination in *Rosa*. J Hortic Sei 38:310–320
Jackson GAD, Blundell JB (1965) Germination of *Rosa arvensis*. Nature 205:518–519
Jacobsen JV, Pressman E (1979) A structural study of germination in celery (*Apium graveolens* L.) seed with emphasis on endosperm breakdown. Planta 144:241–248
Jacobsen JV, Pearce DW, Poole AT, Pharis RP, Mander LN (2002) Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. Physiol Plant 115:428–444
Joshi AK, Singh BD (2004) Dormancy. In: Seed science and technology. Kalyani Publishers, New Delhi, pp 43–45
Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, Kamiya Y, Seo M (2010) Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. Plant Cell Physiol 51:1988–2001. https://doi.org/10.1093/pcp/pcq158
Karssen CM (1995) Hormonal regulation of seed development, dormancy and germination studied by genetic control. In: Kigel J, Galili G (eds) Seed development and germination. Marcel Dekker Inc, New York, NY, pp 333–350
Karssen CM, Lacka E (1986) A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid deficient mutants of *Arabidopsis thaliana*. In: Bopp M (ed) Plant growth substances. Springer, Berlin, pp 315–323
Kermode AR (1990) Regulatory mechanisms involved in the transition from seed development to germination. Crit Rev Plant Sci 9:155–195

Kermode AR (1995) Regulatory mechanisms in the transition from seed development to germination: interactions between the embryo and the seed environment. In: Kigel J, Galili G (eds) Seed development and germination. Marcel Dekker Inc, New York, NY, pp 273–332
Kermode AR (2005) Role of abscisic acid in seed dormancy. J Plant Growth Regul 24:319–344
Kermode AR, Dumbroff EB, Bewley JD (1989) The role of maturation drying in the transition from development to germination VII. Effects of partial and complete desiccation on abscisic acid levels and sensitivity in *Ricinus communis* L. seeds. J Exp Bot 40(303):313
Khan AA (1971) Cytokinins: permissive role and seed germination. Science 171(3974):853–859
Kilian B, Özkan H, Pozzi C, Salamini F (2009) Domestication of the *Triticeae* in the fertile crescent. In: Feuillet C, Muehlbauer GJ (eds) Genetics and genomics of the Triticeae. Plant genetics and genomics: crops and models, 7th edn. Springer Science+Business Media, LLC, New York, NY, pp 81–119
Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. Curr Opin Plant Biol 5:33–36
Krogmeier MJ, Bremner JM (1989) Effects of phenolic acids on seed germination and seedling growth in soil. Biol Fertil Soils 8:116–122
Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15:281–307. https://doi.org/10.1079/SSR2005218
Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656
Le Page-Degivry MT, Garello G, Barthe P (1997) Changes in abscisic acid biosynthesis and catabolism during dormancy breaking in *Fagus sylvatica* embryo. J Plant Growth Regul 16:57–61
Lenoir C, Corbineau F, Côme D (1983) Rôle des glumelles dans la dormance des semences d'orge. Physiol Veg 21:633–643
Leubner-Metzger G (2003) Functions and regulation of β-1,3-glucanases during seed germination, dormancy release and after-ripening. Seed Sci Res 13:17–34
Leymarie J, Robayo-Romero ME, Gendreau E, Benech-Arnold RL, Corbineau F (2008) Involvement of ABA in induction of secondary dormancy in barley (*Hordeum vulgare* L.) seeds. Plant Cell Physiol 49:1830–1838
Li B, Foley ME (1997) Genetic and molecular control of seed dormancy. Plant X Metrics 2(10):384–389
Life WN, Crane JC (1966) Dormancy regulation in Peach seeds. Science 153:541–542
Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou JP, Kamiya Y, Nambara E, Troung HN (2009) The *Arabidopsis* abscisic acid catabolic gene CYP707A2 plays a key role in nitrate control of seed dormancy. Plant Physiol 149:949–960
Mayer AM, Poljakoff-Mayber A (1989) The germination of seeds, 4th edn. Pergamon Press, Toronto, ON, p 270
Medeiros MJ, Oliveira MT, Willadino L, Santos MG (2015) Overcoming seed dormancy using gibberellic acid and the performance of young *Syagrus coronata* plants under severe drought stress and recovery. Plant Physiol Biochem 97:278–286
Metivier JR (1979) Dormência e germinação. In: Ferri MG (ed) Fisiologia vegetal. EDUSP, São Paulo, pp 343–392
Milborrow BV (1967) The identification of (+)-Abscisin II [(+)-Dormin] in plants and measurement of its concentrations. Planta 76:93–113
Morrison DA, McClay K, Porter C, Rish S (1998) The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes. Ann Bot 82:35–40
Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, Matsumoto T, Utsugi S, Ogawa T, Handa H, Ishida H, Mori M (2011) A wheat homolog of MOTHER OF FT AND TFL1 acts in the regulation of germination. Plant Cell 23(9):3215–3229
Nambara E, Marion-Poll A (2003) ABA action and interactions in seeds. Trends Plant Sci 8:213–217

Nasreen S, Yousaf M, Mohmand AS, Ashraf Mailk M (2002) Study of seed dormancy mechanisms; causes and control. Asian J Plant Sci 1(2):210–212
Nikolaeva MG (1969) Physiology of deep dormancy in seeds. Izdatel'stvo 'Nauka', Leningrad. (Translated from Russian by Z. Shapiro, National Science Foundation, Washington, DC.)
Nikolaeva MG (1977) Factors controlling the seed dormancy pattern. In: Khan AA (ed) The physiology and biochemistry of seed dormancy and germination. North-Holland, Amsterdam, pp 51–74
Nord EC, Gunter LE, Graham SA Jr (1971) Gibberellic acid breaks dormancy and hastens germination of creeping sage. Res. Note PSW-RN-259. U.S. Department of Agriculture, Forest Service, Pacific Southwest Forest and Range Experiment Station, Berkeley, CA, pp 1–6
Oki T, Masuda M, Kobayashi M, Nishiba Y, Furuta S, Suda I, Sato T (2002) Polymeric procyanidins as radical-scavenging components in red-hulled rice. J Agric Food Chem 50:7524–7529
Page RJ, Goodwin DL, West NE (1966) Germination requirements of scarlet globemallow. J Range Manag 19:145–146
Paz L, Vázquez-Yanes C (1998) Comparative seed ecophysiology of wild and cultivated Carica papaya trees from a tropical rain forest region in Mexico. Tree Physiol 18:277–280
Petruzzelli L, Coraggio I, Leubner-Metzger G (2000) Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclo-propane-1-carboxylic acid oxidase. Planta 211(1):144–149
Prasad P, Nautiyal AR (1996) Physiology of germination in Bauhinia: involvement of seed coat in inhibition of germination in *B. racemosa* Lin. seeds. Seed Sci Technol 24:305–308
Rathjen JR, Strounina EV, Mares DJ (2009) Water movement into dormant and non-dormant wheat (*Triticum aestivum* L.) grains. J Exp Bot 60:1619–1631
Reddy VS, Dash S, Reddy AR (1995) Anthocyanin pathway in rice (*Oryza sativa* L.): identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanidins in pericarp. Theor Appl Genet 91:301–312
Roberts EH (1972) Dormancy: a factor affecting seed survival in soils. In: Roberts EH (ed) Viability of seeds. Chapman Hall, London, pp 320–359
Rodriguez MV, González Martín J, Insausti P, Margineda JM, Benech-Arnold RL (2001) Predicting preharvest sprouting susceptibility in barley: a model based on temperature during grain filling. Agron J 93:1071–1079
Rodríguez MV, Barrero JM, Corbineau F, Gubler F, Benech-Arnold RL (2015) Dormancy in cereals (not too much, not so little): about the mechanisms behind this trait. Seed Sci Res 25 (2):99–119
Roth TE, Holechek JL, Hussain MY (1987) Germination response of 3 globemallow species to chemical treatment. J Range Manag 40:173–175
Salomão AN, Mundim RC (2000) Germination of papaya seed in response to desiccation, exposure to sub-zero temperatures and gibberellic acid. HortScience 35:904–906
Schmidtz L (2000) Guide to handling of tropical and subtropical forest seed. Danida Forest Seed Centre, Hoersholm, pp 263–303
Schmitz N, Xia J-H, Kermode AR (2001) Dormancy of yellow-cedar seeds is terminated by gibberellic acid in combination with fluridone or with osmotic priming and moist chilling. Seed Sci Technol 29:331–346
Schmitz N, Abrams SR, Kermode AR (2002) Changes in ABA turnover and sensitivity that accompany dormancy termination of yellow-cedar (*Chamaecyparis nootkatensis*) seeds. J Exp Bot 53:89–101
Schopfer P, Plachy C (1984) Control of seed germination by abscisic acid. II. Effect on embryo water uptake in *Brassica napus* L. Plant Physiol 76:155–160
Schopfer P, Plachy C (1993) Photoinhibition of radish (*Raphanus sativus* L) seed germination: control of growth potential by cell wall yielding in the embryo. Plant Cell Environ 16 (2):223–229

Schwachtje J, Baldwin IT (2004) Smoke exposure alters endogenous gibberellin and abscisic acid pools and gibberellin sensitivity while eliciting germination in the post-fire annual, *Nicotiana attenuata* seed. Seed Sci Res 14:51–60

Serrato-Valenti S, Mariotti MG, Cornara L, Corallo A (1998) A histological and structural study of *Phacelia tanacetifolia* endosperm in developing, mature, and germinating seed. Int J Plant Sci 159(5):753–761

Shohani F, Mehrabi AA (2014) Effect of gibberellic acid on seed germination and early growth of lentil seedlings under salinity stress. Middle-East J Sci Res 19(7):995–1000

Shu K, Meng YJ, Shuai HW, Liu WG, Du JB, Liu J, Yang WY (2015) Dormancy and germination: how does the crop seed decide? Plant Biol 17:1104–1112. https://doi.org/10.1111/plb.12356

Simpson GM (1990) Seed dormancy in grasses. Cambridge University Press, Cambridge

Steinbach HS, Benech-Arnold RL, Kristof G, Sanchez RA, Marcucci-Poltri S (1995) Physiological basis of pre-harvest sprouting resistance in *Sorghum bicolor* (L.) Moench. ABA levels and sensitivity in developing embryos of sprouting resistant and susceptible varieties. J Exp Bot 45:701–709

Tian S, Nakamura K, Kayara H (2004) Analysis of phenolic compounds in white rice, brown rice and germinated brown rice. J Agric Food Chem 52:4808–4813

Tillberg E (1992) Effect of light on abscisic acid content in photosensitive Scots pine (*Pinus sylvestris* L.) seed. J Plant Growth Regul 11:147–152

Tiryanki I, Topu M (2014) A novel method to overcome coat imposed seed dormancy in *Lupinus albus* L and *Trifolium pratense* L. J Bot 2014:647469. 6 p

Tseng MT (1992) Effects of sarcotesta removal, gibberellic acid and drying treatments on the germination of papaya seeds. J Agric Assoc China 158:46–54

Viera AR, Gracas MD, Vieira CG, Fraga AC, Oliveira JA, Santos CDD (2002) Action of gibberellic acid (GA_3) on dormancy and activity of α-amylase in rice seeds. Revista Brasileira de Sementes 24(2):43–48

Walker-Simmons MK (1987) ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol 84:61–66

Wang M, Bakhuizen R, Heimovaara-Dijkstra S, Van Zeijl MJ, De Vries MA, Van Beckum JM, Sinjorgo KMC (1994) The role of ABA and GA in barley grain dormancy: a comparative study between embryo and aleurone dormancy. Russ J Plant Physiol 41:577–584

Wang M, Heimovaara-Dijkstra S, Van Duijn B (1995) Modulation of germination of embryos isolated from dormant and non-dormant barley grains by manipulation of endogenous abscisic acid. Planta 195:586–592

Weidner S, Paprocka J, Kamieniecki B, Zadernowski R (1993) The role of phenolic acids in dormancy of barley caryopses. In: Walker-Simmons WK, Ried JL (eds) Preharvest sprouting in cereals 1992. American Association of Cereal Chemists, St Paul, MN, pp 200–211

Weidner S, Krupa U, Amarowicz R, Karamac M, Abe S (2002) Phenolic compounds in embryos of triticale caryopses at different stages of development and maturation in normal environment and after dehydration treatment. Euphytica 126:115–122

Xu N, Bewley JD (1991) Sensitivity to abscisic acid and osmoticum changes during embryogenesis of alfalfa (*Medicago sativa*). J Exp Bot 42:821–826

Yan A, Chen Z (2020) The Control of Seed Dormancy and Germination by Temperature, Light and Nitrate. The Botanical Review, pp 1–37

Yoshioka T, Endo T, Satoh S (1998) Restoration of seed germination at supraoptimal temperature by fluridone, an inhibitor of abscisic acid biosynthesis. Plant Cell Physiol 39:307–312

Zaharia LI, Walker-Simmon MK, Rodríguez CN, Abram SR (2005) Chemistry of abscisic acid and abscisic acid catabolites and analogs. J Plant Growth Regul 24:274

Zeevaart JAD (1999) Abscisic acid metabolism and its regulation. In: Hooykaas PJJ, Hall KR, Libbenga KR (eds) Biochemistry and molecular biology of plant hormones. Elsevier Science BV, Amsterdam, pp 189–207

Seed-Borne Mycoflora of Edible Oilseed Crops of India

14

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Abstract

Seeds play an important role in the dissemination of pathogens externally and internally, which affect the crop either at early or at late stages of growth. The importance of seed-borne fungi is well recognized. Seeds of oilseed crops are heavily affected both quantitatively and qualitatively by seed mycoflora. The storage fungi tremendously affect the quality of the seeds during storage by depleting the reserve food material stored in the seed and reduce the seedling vigour by producing hydrolytic enzymes, which adversely affect the chemical constituents of the seed. The storage fungi also affect the seed viability adversely and decrease the germination percentage of infected seeds. Likewise storage mycoflora may result in the embryo or the whole seed discolouration. The presence of seed-borne mycoflora in oilseeds results in colour changes and decreased oil content; they produce a foul smell and lead to hydrolytic rancidity. Invasion by seed mycoflora also brings about biochemical changes, evident by increase in respiration process. In our country, very little attention has been paid towards seed fungal flora of oilseed crops. So, in the present chapter, cumulative report regarding predominant seed mycoflora of eight major edible oilseed crops, viz. groundnut, mustard, soybean, sunflower, sesame, castor, safflower and linseed, has been summarized and the changes caused by them during the storage are assessed.

Keywords

Biochemical degradation · Deterioration · Mycoflora · Oilseeds · Storage

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14.1 Introduction

Oilseeds are raised in almost all the parts of the country (Ghosh et al. 2018). India is one of the world's leading producers of oilseeds with average yield production of about 29 million tons per annum following the USA, China and Brazil (Singh et al. 2017; Thapa et al. 2019). Besides being the fourth largest oilseed producer, India also ranks second in importing and third in consuming edible oil around the world and thereby plays a global role in this arena (Singh et al. 2017). Oilseed sector contributes significantly in Indian economy (DOR 2013). The prime source of edible oil is comprised of nine oils from the annual oilseed crops (DRMR 2015). Occupying approximately 19% of the global region with just about 2.7% of the worldwide production, these oilseed crops are the second essential determinant next to cereal crops in Indian agricultural economy (Reddy and Immanuelraj 2017). The main contributors amongst all Indian oilseed crops are groundnut, soybean, rapeseed and mustard. Under rainfed conditions the oilseed crops are grown on poor and fertile land (Singh et al. 2013, 2014).

Healthy and pathogen-free seeds play significant role in healthy crop production. To catch the right season, seeds are stored for a considerable period of time. It is estimated that mould or fungal development affects 25% of the world's crop. Seeds are considered to be responsible for dissemination of plant diseases since they bear a number of pathogens which get associated either in the field or in post-harvest storage conditions (Begum et al. 2013).

Seed is the basic and most critical input for substantial agriculture (Bhajbhuje 2014). Deterioration of seed can be characterized as loss of seed vigour due to the increased seed exposure to external challenges and reduced the viability of seed to survive (Jyoti and Malik 2013). Manoharachary and Kunwar (2006) and Kapoor et al. (2011) described deterioration as catabolic process which involves cytological, physiological, biochemical and physical changes in seeds. Infections triggered by pathogens (fungal, bacterial or viral) during germination are classified as seed-borne diseases, resulting in inflammation on the seed surface or inside leading to crop loss (Verma and Saxena 2012). According to Christensen (1957), deterioration occurs more rapidly in stored grains due to the invasion of microorganisms, and losses caused by them are referred to as biodeterioration. It was also reported that seed-borne mycoflora are responsible for the reduction of seed quality (Neergaard 1979) and cause decreased plant growth and crop productivity (Williams and McDonald 1983; Kubiak and Korbas 1999; Dawson and Bateman 2001). The seed-borne microorganisms associated with seeds externally or internally may cause different infections, such as seed rot, seed necrosis, reduction or elimination of germination ability as well as seedling damage resulting in disease production through systemic infection at later stages of plant growth (Bateman and Kwasna 1999; Asif et al. 2001; Ijaz et al. 2001; Khanzada et al. 2002).

Fungi also have gained great economic importance, not only infecting the seeds during crop production but also generating numerous mycotoxins (Ismaiel and Papenbrock 2015). Various reports show that storage moulds induce seed nutritional changes. Due to storage fungi, biochemical changes in groundnut (Ward and Diener

1961; Singh et al. 1974) and other oilseeds (Sharma 1977) have been found to cause damage or discolouration of kernels, which ultimately affect fat and reduce sugar content.

After harvesting, seeds of oilseed crops are stored under various storage conditions, and if these conditions are not appropriate, then several pathogens such as viruses, bacteria, fungi and nematodes get associated with these seeds. Amongst these pathogens, fungi play a governing role in reducing the quality and viability of seed. Fungi cause numerous abnormalities to seeds such as seed discolouration, damaged seeds, shrunken seeds, undersized seeds, rotten seeds and decreased germination ability. Fungal species play an important role in infection, altering seed quality and viability of seeds during storage (Christensen and Kaufmann 1969). These seeds are not suitable for human use and are also rejected at an industrial level. Eventually, this impacts the country's yield and economy. Fungi which grow on stored seeds may decrease the germination percentage along with reduced carbohydrate, protein and total oil content, induce higher moisture content and free fatty acid content and increase other biochemical changes which adversely affect seed quality. The tropical environmental conditions of high temperature and relative humidity, together with unscientific storage conditions, are harmful for the conservation of cereal grains, oilseeds, etc., resulting in the complete loss of seed value.

Agar plate method (Muskett 1948) and blotter method (De Tempe 1953) recommended by the International Seed Testing Association (Anonymous 1966) were used for fungal isolation. An agar plate is a Petri plate containing a medium of growth (typically agar plus nutrients) used to cultivate microorganisms. The blotter system is one of the methods for incubation in which the seeds are placed on well water-soaked filter papers and typically incubated at $25 \pm 2\ ^{\circ}C$ for 7 days under 12 h alternating periods of light and darkness. After incubation, fungi produced on each seed are analysed and classified under various stereomicroscope magnifications. For isolation of fungi, the sterilized and unsterilized seeds are used. Mercuric chloride ($HgCl_2$) solution (0.1%) was used for surface sterilization (Ramakrishna et al. 1991). Surface sterilization is done to get rid of rapidly growing species there by allowing the slowly growing internal seed-borne microflora to express themselves (Ramesh et al. 2013).

Agar Plate Technique: Externally the seeds are sterilized by 0.1% mercuric chloride solution to 1–2 min and then washed with sterilized distilled water (Habib et al. 2007). The isolated fungi were identified using light microscope after slides were stained with lactophenol (Anonymous 1994; Henselov and Hudecov 2001; Gwary et al. 2006).

Blotter Test: The blotter test (Limonard 1966; Lantos et al. 2002) is used to isolate the fungal pathogens associated with the seeds while in storage.

Khattak et al. (1993) accounted the percentage of mycoflora isolated by agar plate technique was higher than the blotter method in soybean. Solanke et al. (1997) also stated that agar plate technique yields high mycoflora than the blotter method. Irrespective of the method of isolation and variety used, unsterilized

seed gave more number of mycoflora than the sterilized seed (Ahammed et al. 2006). These results are in agreement with the findings of Sundaresh and Hiremath (1978) in soybean seed mycoflora. The blotter method has shown better results than the agar plate technique (Rasheed et al. 2004; Srivastava et al. 2011; Dawar et al. 2014; Rao et al. 2015; Kakad et al. 2019). Jovicevic (1980) also accounted that the most practical method for routine seed health analysis was the filter paper method. Dawar and Ghaffar (1991) on sunflower seed and Khan et al. (1988) on rice seed also observed similar results.

Identification of Fungi: The growth characters and percentage of infection are recorded after incubation. Potato Dextrose Agar (PDA) is prepared and fungi are inoculate on the sterile media for the isolation of pure culture from those fungi and it is incubated it for 7 days. By using light microscope, the fungi were detected based upon their colour, morphology of spore and mycelium growth (Begum et al. 2004; Chuku et al. 2007; Al-Sheikh 2009). The seed mycoflora of major oilseed crops are identified by using the reference of Thom and Raper (1945), Raper and Thom (1949), Booth (1971), Ellis (1971) and Barnett and Hunter (1972).

14.2 Groundnut

Groundnut (*Arachis hypogaea* L.) is an essential oilseed crop of India which contains oil content up to an extent of 48–51% (Adithya et al. 2017). In fact, India is the leading producer of groundnut around the world (Ghosh et al. 2018). About 82% of the groundnut produced in India is used for the production of edible oil, 12% as seed and 5% as feed (Begum et al. 2013). Peanut is attacked by a variety of seed-borne and soil-borne fungal pathogens as well as other pathogens, under field condition as well as during storage causing economic losses. *Aspergillus flavus* and *Aspergillus niger* were the predominant mycoflora of groundnut. Mukherjee et al. (1992) also detected that *Aspergillus flavus* and *Aspergillus niger* were the predominant storage mycoflora of groundnut seed. Dawar and Ghaffar (1991) also produced similar studies on sunflower. *Aspergillus*, *Penicillium* and *Rhizopus* species were also recorded on groundnut seed (Lumpungu et al. 1989), which decreased the germination percentage of seeds and damaged the seeds during storage (Christensen 1973). The causal organisms for damping off of groundnut seedling are *Fusarium solani* and *Fusarium oxysporum* (Reddy and Rao 1980). Isolated fungus *Aspergillus flavus* is a major producer of mycotoxins which are hepatocarcinogenic and produces four metabolites of aflatoxin B1, B2, G1 and G2 (Goldblatt 1969). Therefore, to improve the storage environment, it is necessary to reduce fungal growth and mycotoxin development in groundnut seeds.

Three important seed-borne fungal pathogens, viz. *Aspergillus niger*, *A. flavus* and *Macrophomina phaseolina* were dominantly associated with groundnut seeds (Mukherjee et al. 1992; Oladipupo 2011). The above results of the present investigation are in accordance with the reports of other researchers (Mukherjee et al. 1992; Chavan 2011; Oladipupo 2011), who identified *Aspergillus* spp., *Penicillium*,

Fusarium, *Rhizoctonia* and *Alternaria* as storage mycoflora on groundnut seed, whereas Rasheed et al. (2004) reported that *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani*, *Aspergillus niger* and *A. flavus* were frequent in groundnut and seed coat was severely infected by mycoflora followed by cotyledon and axis. Because of *Fusarium equiseti* and *Rhizopus stolonifer*, there was a significant groundnut sugar reduction, while *Curvularia lunata*, *Fusarium equiseti* and *Penicillium digitatum* have been shown to be responsible for the greatest reduction in fat content (Kakde and Chavan 2011). The highest percentage association was with *Aspergillus flavus* followed by *Aspergillus niger*, and it was followed by *Fusarium oxysporum* (Kakad et al. 2019).

14.3 Mustard

Mustard (*Brassica juncea* (Linn.) Czern. and Coss.) is India's major oilseed crop. It is reaped in March and April and is stored in traditional warehouses during the hot summer and monsoon. Fungi are the main agents amongst the microorganisms which deteriorate quality of seeds in storage (Srivastava et al. 1981). Seed mycoflora of mustard under storage conditions has been studied by many workers from various geographical locations (Lodhi and Naeem 1955; Mishra and Kanaujia 1973; Upadhyay and Singh 1978; Ghotekar and Hedawoo 2010; Siddiqui 2013; Ghugal and Thakre 2014).

Aspergillus flavus and *Aspergillus parasiticus* are toxigenic strain causing the aflatoxin contamination of mustard (Diener et al. 1987). Seed storage at high humidity and temperature also results in microfloral infection and contamination with mycotoxins. Maintenance of seed quality during storage has been an issue in warm and humid conditions that allow fungi to invade seed. The storage structure used greatly influences the microfloral infection and contamination of aflatoxin (Ranjan et al. 1992). Mycoflora genera such as *Alternaria*, *Fusarium*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Chaetomium* and *Curvularia* were mainly observed to be associated with mustard seeds (Saleh et al. 2003).

Depending upon storage duration and detection method used, the percent incidence of various fungi varied (Mumtaz and Fatima 2017). Modified PDA approach was found to be successful in finding the occurrence of mustard seed-borne mycoflora accompanied by standard blotter and deep-freezing blotter process. Fungal genera, viz. *Alternaria alternata*, *Alternaria brassicae*, *Alternaria brassicicola*, *Aspergillus flavus*, *Helminthosporium brassicae*, *Fusarium oxysporum*, *Rhizoctonia bataticola*, *Rhizoctonia solani*, *Penicillium citrinum*, *Pythium* spp., *Curvularia*, *Cladosporium*, *Chaetomium* and *Rhizopus* were found to be associated with Indian mustard seeds (*Brassica juncea* L.) during storage period (Qumberani 2001; Mumtaz and Fatima 2017).

Fourteen genera have been detected with varying levels of incidence as both external and internal seed-borne mycoflora of *Brassica campestris* L. by blotter paper and agar plate techniques; these included *Alternaria alternata*, *Alternaria solani*, *Alternaria brassicicola*, *Aspergillus amstelodami*, *Aspergillus flavus*,

Aspergillus fumigatus, *Aspergillus niger*, *Aspergillus terreus*, *Chaetomium globosum*, *Cladosporium fulvum*, *Curvularia ovoidea*, *C. lunata*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Helminthosporium tetramera*, *Mucor pusillus*, *Paecilomyces variotii*, *Penicillium oxalicum*, *P. pallidum*, *Phytophthora infestans*, *Pythium aphanidermatum*, *Rhizopus stolonifer* and *Trichothecium roseum* (Bhajbhuje 2014). It was also reported that out of all above-mentioned mycoflora, the incidence of *Alternaria brassicae* on mustard seeds is the highest followed by *Alternaria brassicicola* and *Rhizoctonia bataticola*.

The percentage of seed germination and vigour index reduced with an increasing storage period (Mangena and Mokwala 2019). Species of *Aspergillus* and *Penicillium* also produce some carcinogenic mycotoxins, viz. aflatoxins, zearalenone and citrinin (Mumtaz and Fatima 2017). Same findings are also supported by Qumberani (2001) who proved that *Aspergillus flavus* was isolated as the most predominant fungus from mustard seeds and produces aflatoxins.

14.4 Soybean

Soybean is an essential oilseed and pulse crop (Ramesh et al. 2013) cultivated commercially and plays a major role in the Indian economy (Bhatnagar and Karmakumar 1995). Soy is a basic food ingredient of traditional Asian cuisine used for thousands of years (Rizzo and Baroni 2018), and soybean [(*Glycine max* L.) Merrill] the "golden bean" is the essential oilseed crop next to groundnut and mustard renowned for its high protein content (42–45%), oil content (22%) and starch content (21%). It is an excellent source of vitamin B complex, thiamine and riboflavin (Rao et al. 2015). In addition soybean is rich in major nutrients; about 40% of dry matter is protein and 20% is fat (Caldwell 1973). It is one of the most important sources of oil and protein in the world, and it is commonly used in both human and animal diets (Onwueme and Sinha 1991; Ariyo 1995). Soybean protein is abundant in essential amino acids such as lysine (5%) in which most of the cereals are deficient (Rao et al. 2015). The use of a range of protein sources could be beneficial for escaping a monochromatic nutrition in vegetarians, extensively based on soy (Kumar et al. 2017).

Occurrence of different diseases and pest in field results in the reduced yield and productivity in India. The vulnerability of soybean to a significant number of diseases induced by fungus, bacteria, viruses and nematodes is one of the main restrictions in the effort to increase production. In India, almost 40 fungal pathogens have been detected in soybean crop, but only a few of them are of economic importance (Sarabhoy and Agarwal 1983). The seeds of soybean proved that mainly four genera of mycoflora are related internally with soybean, i.e. *Alternaria alternata*, *Fusarium oxysporum*, *Macrophomina phaseolina* and *Aspergillus niger* (Chaudhary 2001). It was also reported that the number of internally seed-borne mycoflora was less than that of external seed-borne mycoflora (Chaudhary 2001). The seeds of soybean revealed the significant reduction in sugar due to *Alternaria dianthicola*, *Curvularia pallescens* and *Fusarium equiseti*, while *Penicillium*

digitatum, *Penicillium chrysogenum* and *Fusarium oxysporum* significantly dropped the fat content in soybean (Kakde and Chavan 2011). Ramesh et al. (2013) also observed that pathogenic seed mycoflora frequently isolated from soybean were *Macrophomina phaseolina*, *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Phoma* spp. and *Sclerotinia sclerotiorum*, while less often isolated fungi were *F. solani*, *Fusarium moniliforme*, *Rhizopus* spp., *Botrytis cinerea* and *Cercospora kikuchii*. Some fungal species, viz. *Alternaria alternata*, *Cladosporium cladosporioides*, *Diaporthe longicolla*, *Fusarium chlamydosporum*, *Fusarium equiseti*, *Fusarium proliferatum*, *Penicillium citrinum* and *Phoma* sp. were also reported from sprouted soybean seeds. Most of the microflora species isolated from soybean seeds were linked with production of mycotoxin, especially *Fusarium* spp., *Penicillium citrinum*, *Aspergillus flavus* and *Alternaria alternata*. Major microfloral species such as *Macrophomina phaseolina*, *Colletotrichum dematium*, *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer*, *Curvularia*, *Alternaria alternata*, *Cladosporium cladosporioides* and *Fusarium oxysporum* were isolated from stored seeds of soybean (Rao et al. 2015; Aoudou et al. 2017). Out of all isolated seed mycoflora, *Macrophomina phaseolina* was predominantly present, while the occurrence of *Cladosporium cladosporioides* was reportedly least (Rao et al. 2015). In addition, fungal infection and regular presence suggested a potential risk for recurrent mycotoxin exposure of soybean seeds and sprouts, which could result in significant economic losses for soybean farmers and the sprout industry (Escamilla et al. 2019).

14.5 Sunflower

In 1969, sunflower (*Helianthus annuus* L.) was first planted as an oilseed plant in India. The oil obtained from sunflower seed contains about 45–50%. The largest suppliers of sunflower seeds in the world market are the European Union, Russia, Ukraine, Argentina, the United States, China, India and Turkey. Sunflower oil is healthy because of light and odourless characters with ample amount of vitamin E especially recommended for heart patients. Around 25% is protein content, and for animal feed preparation, sunflower meal is used as a protein source. The oil is used for cooking purposes, vanaspati ghee preparation and soap and cosmetics production.

The seeds of sunflower were exploited to study the incidence of mycoflora which mainly gave the appearance of *Alternaria alternata*, *A. helianthi*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Curvularia lunata*, *Fusarium moniliforme*, *F. solani*, *Drechslera tetramera*, *Cladosporium* spp., *Penicillium citrinum*, *Macrophomina phaseolina*, *Mucor mucedo* and *Rhizopus nigricans* (Vijayalakshmi and Rao 1985; Kaur et al. 1990; Reddy 1993; Afzal et al. 2010). It was also observed by many scientists the sunflower seeds are extremely infected with the mycoflora which are competent to infest the plants at various stages of development subsequently during harvesting and storage (Vaidehi 2002; Morar et al. 2004). Nahar et al. (2005) reported that predominantly *Absidia corymbifera*, *Alternaria alternata*, *Aspergillus*

flavus, *A. niger*, *A. terreus*, *Chaetomium bostrychodes*, *C. globosum*, *Emericella nidulans*, *Fusarium pallidoroseum*, *F. solani*, *Macrophomina phaseolina*, *Penicillium* spp., *Rhizoctonia solani* and *Rhizopus stolonifer* were isolated from sunflower during storage. *Fusarium equiseti*, *Fusarium oxysporum* and *Curvularia lunata* showed extremum decrement in the content of sugar of sunflower seeds, while it is interesting to note that fat content of sunflower seeds gets increased due to the presence of *Rhizopus stolonifer* (Kakde and Chavan 2011).

14.6 Sesame

The sesame (*Sesamum indicum* L.) seed is a good source of edible oil having oil content specifically varying from 46% to 52%. Leading sesame-producing countries are India, China, Sudan, Mexico, Turkey, Burma and Pakistan. Besides using it for cooking purposes, sesame oil is also used for anointing the body, for preparing perfumed oils and for medicinal purposes. Sesame cake is a rich source of protein, carbohydrates and mineral nutrients, such as calcium and phosphorus. It is also a valuable and nutritious feed for milch cattle (Kakde et al. 2012).

Sesame seed is being infested by various seed-borne mycoflora (Noble and Richardson 1968). Seed-borne mycoflora, namely, *Alternaria dianthicola*, *A. sesami*, *A. sesamicola*, *A. tenuis*, *A. longissima*, *A. brassicicola*, *A. redicina*, *Aspergillus flavus*, *A. niger*, *A. ustus*, *A. alba*, *A. viridis*, *Macrophomina phaseolina*, *Curvularia lunata*, *Fusarium moniliforme*, *Penicillium rubrum*, *P. citrinum*, *Rhizopus nigricans*, *Cephalosporium* sp. and *Drechslera* sp. were detected on sesame during storage (Sinclair 1975; Seung-Hun et al. 1982; Kumar et al. 1984; Jonsyn 1988; Altaf et al. 2004; Enikuomehin 2010; Suleiman and Omafe 2013). In sesame seeds, sugar significantly declined due to the presence of *Macrophomina phaseolina* and *Fusarium oxysporum* under storage condition, while *Penicillium chrysogenum* and *Curvularia lunata* were recovered to be responsible for the maximal drop-off in the crude fibre content of sesame seed (Kakde and Chavan 2011). *Curvularia pallescens*, *Alternaria dianthicola* and *Macrophomina phaseolina* reduced fat content of sesame (Kakde and Chavan 2011).

14.7 Castor

Castor (*Ricinus communis* L.) is a species of flowering plant in the family Euphorbiaceae (Schery 1972; Oyewole et al. 2010). Castor is native to the Ethiopian region of tropical Africa and now has become naturalized in tropical as well as temperate regions throughout the world (Anjani 2012). Castor bean is an industrial oilseed crop of the world (Chukunda et al. 2015). The oil extracted from castor seeds is already achieving attention globally having more than 700 uses ranging from medicines and cosmetics, manufacturing biodiesel, plastics and lubricants, etc. (Cosmetic Ingredient Review Expert Panel 2007). Castor bean contains 50–55%

of non-edible oil and 26–30% protein due to its nature of chemical composition. Its oil is used in more than 300 compounds (Mirza 2009).

Aspergillus spp. were the commonest fungi found on the stored castor seeds, while species belonging to the *Cephaliophora*, *Penicillium* and *Syncephalastrum* genera were less common (Negedu et al. 2012). Other fungal species isolated and identified from the castor bean seeds are *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium* sp., *Fusarium oxysporum*, *Rhizopus stolonifer*, *Curvularia lunata* and *Botrytis cinerea* (Chukunda et al. 2015).

14.8 Safflower

Safflower (*Carthamus tinctorius* L.) seeds are edible and are eaten after roasting. Traditionally, the crop was cultivated for its seeds and used for colouring and flavouring foods, in medicines and in making red (carthamin) and yellow dyes, particularly before the existence of cheaper aniline dyes. Since the last 50 years, the plant has been grown basically for the vegetable oil extracted from its seeds having oil content which varies from 24% to 36%. The cold-pressed oil is golden yellow and is used for culinary purposes or for making soap. The oil obtained by dry hot distillation is black and sticky and is used only for greasing well ropes and leather goods exposed to water. The cake, especially from decorticated seed, is used as a cattle feed and that from undecorticated seed is used as a manure (Zohary et al. 2012).

Safflower seeds are mainly infected by different species of *Aspergillus* during storage conditions. Some other genera of fungi, viz. *Alternaria*, *Curvularia*, *Fusarium*, *Rhizopus*, *Aspergillus*, *Chaetomium*, *Helminthosporium*, *Bipolaris*, *Phoma* and *Colletotrichum* were also isolated from stored safflower seeds (Thakur et al. 2003; Ismail et al. 2004). There is significant drop-off in sugar content of safflower due to the existence of *Fusarium equiseti*, *Fusarium oxysporum* and *Alternaria dianthicola* during storage while fat content in safflower is significantly reduced due to *Fusarium oxysporum* and *Fusarium equiseti* (Kakde and Chavan 2011). Fibre content of safflower seeds was increased due to the presence of *Penicillium digitatum*, *Penicillium chrysogenum* and *Macrophomina phaseolina* (Kakde and Chavan 2011).

14.9 Linseed

Linseed (*Linum usitatissimum* L.) also known as common flax is an oilseed crop cultivated in cooler regions of the world to produce linen and seeds. In India, mainly it grows in Uttar Pradesh, Bihar, Maharashtra and Punjab (Gupta 2002). Linseed oil is a colourless to yellowish oil extracted from the dried and ripened flax seeds (Jones 2003). Linseed oil as a vast source of α-linolenic acid (an omega-3 fatty acid) is in demand as a nutritional supplement (Vereshchagin and Novitskaya 1965).

Seed-borne mycoflora in oilseed may decrease the oil content, cause a change in its colour, induce an unpleasant odour and lead to hydrolytic rancidity (Wilson 1947;

Goodman and Christensen 1952; Ward and Diener 1961; Vidhyasekaran and Govindaswamy 1968). Mycoflora isolated from seed surface of linseed includes species of *Aspergillus*, *Alternaria*, *Fusarium*, *Curvularia*, *Chaetomium*, *Rhizopus*, *Macrophomina* and *Penicillium* out of which maximum association of *Fusarium* species was also observed (Gupta 2002). Some other seed mycoflora such as *Alternaria lini*, *Rhizoctonia bataticola*, *Fusarium lini*, *Curvularia lunata*, *Nigrospora* sp., *Penicillium digitatum*, *Aspergillus niger*, *Cercospora* spp., *Trichothecium* spp., *Colletotrichum lini* and *Stachybotrys* spp. were detected from linseed seeds by detection methods, namely, agar plate method, blotter method and deep freeze method (Meena 2005).

Several commonly occurring seed-borne mycoflora which are known to cause reduction in yield as well as in oil content of major oilseed crops reported from different countries including India are summarized below (Table 14.1).

14.10 Environmental Factors

Temperature and relative humidity in the form of moisture are the most essential physical/environmental factors affecting the seed quality during storage. Safe pertinent content of both the factors can hamper the fungal growth and the hydrolysis of lipid; accordingly the quality of seeds or grains could be maintained for longer period (Dharmaputra et al. 2009). Low temperature and humidity results in belated seed deterioration and thereby leads to extended viability period (Jyoti and Malik 2013) because it is obvious that higher moisture content of the seeds provides conditions conducive for the mycoflora, thereby intensifying the seed deterioration and finally leading to frequent decline in germinability. Relatively warmer temperature of the environment also quickens the deterioration phenomenon (Toole 1939; Harrington 1967). This was not so prominent in the oilseed crops having lower oil content comparatively in cultivars having high oil content, thereby resulting in somewhat lower fungus activity (Mondal et al. 1981). According to Papavizas and Christensen (1958), stored seeds having moisture content up to 18% may be stored safely for as long as 19 months at temperature of 5 °C. Qasem and Christensen (1958) revealed that low temperature was as efficient as low moisture percentage in averting damage caused by the stored seed mycoflora. Some fungi especially species of *Aspergillus* and *Penicillium* enhance the moisture content of stored seeds so that seeds can be deteriorated very rapidly (Srivastava and Sinha 2013). The growth of various fungi in stored seeds is accompanied by large amount of heat. Under warm and wet storage situations, oilseed crops often become infested by storage mycoflora, and out of all oilseed crops, soybean is categorized as “poor storer” as it loses viability drastically under hot and humid conditions (Sharma 1977; Mondal et al. 1981; Nandi et al. 1982; Kakde et al. 2012).

Table 14.1 List of dominant seed mycoflora isolated from oilseed crops

S. No.	Oilseed crop	Dominant mycoflora	Reference
1.	Groundnut	*Aspergillus niger*, *A. flavus*, *Macrophomina phaseolina*, *Penicillium digitatum*, *Fusarium solani*, *F. oxysporum*, *F. equiseti*, *Rhizoctonia solani*, *Alternaria* spp., *Curvularia lunata* and *Rhizopus stolonifer*	Mukherjee et al. (1992), Rasheed et al. (2004), Chavan (2011), Oladipupo (2011), Kakad et al. (2019)
2.	Mustard	*Alternaria alternata*, *A. solani*, *A. brassicicola*, *A. brassicae*, *Aspergillus amstelodami*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Chaetomium globosum*, *Cladosporium fulvum*, *Curvularia ovoidea*, *C. lunata*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Helminthosporium tetramera*, *H. brassicae*, *Paecilomyces variotii*, *Penicillium oxalicum*, *P. pallidum*, *Phytophthora infestans*, *Pythium aphanidermatum*, *Mucor pusillus*, *Rhizopus stolonifer*, *Rhizoctonia bataticola* and *Trichothecium roseum*	Qumberani (2001), Bhajbhuje (2014), Mumtaz and Fatima (2017)
3.	Soybean	*Macrophomina phaseolina*, *Alternaria alternata*, *A. dianthicola*, *Fusarium oxysporum*, *F. equiseti*, *F. solani*, *F. moniliforme*, *F. chlamydosporum*, *F. proliferatum*, *Curvularia pallescens*, *Aspergillus niger*, *A. flavus*, *Penicillium digitatum*, *P. citrinum*, *P. chrysogenum*, *Fusarium oxysporum*, *Phoma* spp., *Sclerotinia sclerotiorum*, *Rhizopus* spp., *Botrytis cinerea*, *Cercospora kikuchii*, *Cladosporium cladosporioides*, *Diaporthe longicolla*, *Colletotrichum dematium* and *Rhizopus stolonifer*	Chaudhary (2001), Kakde and Chavan (2011), Ramesh et al. (2013), Rao et al. (2015), Aoudou et al. (2017)
4.	Sunflower	*Alternaria alternata*, *Alternaria helianthi*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *A. terreus*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium solani*, *F. equiseti*, *F. oxysporum*,	Vijayalakshmi and Rao (1985), Kaur et al. (1990), Reddy (1993), Nahar et al. (2005), Afzal et al. (2010), Kakde and Chavan (2011)

(continued)

Table 14.1 (continued)

S. No.	Oilseed crop	Dominant mycoflora	Reference
		F. pallidoroseum, *Drechslera tetramera*, *Cladosporium* spp., *Penicillium* spp., *Macrophomina phaseolina*, *Mucor mucedo*, *Rhizopus nigricans*, *R. stolonifer*, *Absidia corymbifera*, *Chaetomium bostrychodes*, *C. globosum*, *Emericella nidulans*, *Macrophomina phaseolina* and *Rhizoctonia solani*	
5.	Sesame	*Alternaria dianthicola*, *Alternaria sesami*, *Alternaria sesamicola*, *Alternaria tenuis*, *Alternaria longissima*, *Alternaria brassicicola*, *A. redicina*, *Aspergillus flavus*, *A. niger*, *A. ustus*, *A. alba*, *A. viridis*, *Macrophomina phaseolina*, *Curvularia lunata*, *Curvularia pallescens*, *Fusarium moniliforme*, *Penicillium rubrum*, *Penicillium citrinum*, *Rhizopus nigricans*, *Cephalosporium* sp. and *Drechslera* sp.	Sinclair (1975), Seung-Hun et al. (1982), Kumar et al. (1984), Jonsyn (1988), Altaf et al. (2004), Enikuomehin (2010), Kakde and Chavan (2011), Suleiman and Omafe (2013)
6.	Castor	*Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium* sp., *Fusarium oxysporum*, *Rhizopus stolonifer*, *Curvularia lunata*, *Botrytis cinerea*, *Cephaliophora* and *Syncephalastrum*	Negedu et al. (2012), Chukunda et al. (2015)
7.	Safflower	*Alternaria*, *Curvularia*, *Fusarium*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Macrophomina*, *Chaetomium*, *Helminthosporium*, *Bipolaris*, *Phoma* and *Colletotrichum*	Thakur et al. (2003), Ismail et al. (2004), Kakde and Chavan (2011)
8.	Linseed	*Aspergillus*, *Alternaria*, *Fusarium*, *Curvularia*, *Chaetomium*, *Rhizopus*, *Macrophomina*, *Rhizoctonia* and *Penicillium*	Gupta (2002), Meena (2005)

14.11 Conclusion

Seed constitutes basic agricultural productivity. Seed vigour may reduce due to seed-borne mycoflora causing disastrous diseases and weaken the plant at the initial stage of growth. Seed-borne mycoflora are comparatively challenging to manage as the

fungal hyphae get established and become dormant. The oilseed crops are attacked by numerous seed mycoflora, and these pathogens may affect the crop resulting in a reduction of the seed quantity and quality. Several commonly occurring seed-borne mycoflora, viz. *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp., *Fusarium* sp., *Helminthosporium* sp., *Mucor* sp., *Rhizopus* sp., *Rhizoctonia* sp., *Colletotrichum* sp., *Macrophomina phaseolina*, *Penicillium* sp., *Botrytis* sp., etc., are known to cause reduction in yield as well as in oil content of the oilseed crops and have been considered as potentially destructive on many oilseed crops reported from different countries including India. Out of all above-mentioned mycoflora, *Alternaria* sp. and *Aspergillus* spp. are the most destructive pathogens of oilseeds during storage.

Seed-borne pathogens are involved in seed deterioration under storage conditions and may spread diseases generation to generation and cause extensive yield loss. Hence availability of pathogen-free, healthy seed is the need of the hour to overcome the food demand of growing mouth on the globe. Data from various research works mentioned in this chapter revealed that the seeds of oilseed crops are more prone to fungal attack during storage and carried greater count of fungal propagules on seed surface which leads to seed spoilage. The deeply seated fungal pathogens in the embryonic or endospermic tissues of seed may transmit to the next generation and proliferate their population causing multifold losses in productivity. Only disease-free and non-deteriorated seeds respond better to all inputs; thus, having seeds that can be stored under ambient temperature and relative humidity at very less expense without quality declination for periods of subsequent season is of massive benefit for farmers. The farmers are recommended to adopt improved or modified scientific technologies of storage to discourage proliferation of seed-borne mycoflora.

References

Adithya G, Rajeshwari B, Keshavulu K, Sudini H, Swathi Y (2017) Mycoflora associated with groundnut seeds collected from selected groundnut growing districts of Telangana State, India. Int J Curr Microbiol App Sci 6(7):4335–4342

Afzal R, Mughal SM, Munir M, Sultana K, Qureshi R, Arshad M, Laghari AK (2010) Mycoflora associated with seeds of different sunflower cultivars and its management. Pak J Bot 42 (1):435–445

Ahammed SK, Anandam RJ, Babu GP, Munikrishnaiah M, Gopal K (2006) Studies on seed mycoflora of soybean and its effect on seed and seedling quality characters. Legum Res 29 (3):186–190

Altaf N, Khan SA, Ahmed M, Asghar R, Ahmed RA, Shaheen S, Zafar M, Saqib M (2004) Seed borne mycoflora of sesame (Sesamum indicum L.) and their effect on germination and seedling. Pak J Biol Sci 7:243–245

Al-Sheikh H (2009) Date-Palm fruit spoilage and seed-borne fungi of Saudi Arabia. Res J Microbiol 4:208–213

Anjani K (2012) Castor genetic resources, a primary gene pool for exploitation. Ind Crop Prod 35:1–14

Anonymous (1966) International rules for seed testing. Proc Int Seed Test Assoc 31:1–152

Anonymous (1994) The microbiology of water 1994 part 1: drinking water. Report on Public Health and Medical Subjects, No. 71. Methods for the Examination of Waters and Associated Materials. HMSO, London

Aoudou Y, Louis Childeric EE, Aurelie NN (2017) Essential oil of *Ocimum gratissimum* as seed treatment against seed-borne fungi of soybean (*Glycine max* L.). J Agric Res 3(4):13–29
Ariyo OJ (1995) Correlations sand path–coefficient analysis of components of seed yield in soybeans. Afr Crop Sci J 3(1):29–33
Asif MJ, Mak C, Othman RY (2001) Characterization of indigenous Musa species based on flow cytometric analysis of ploidy and nuclear DNA content. Caryologia 54:161–168
Barnett HL, Hunter BB (1972) Illustrated genera of imperfect fungi, 1st edn. Burgess Publishing Co., Minneapolis, MN
Bateman GL, Kwasna H (1999) Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Appl Soil Ecol 13:271–282
Begum N, Alvi KZ, Haque MI, Raja MU, Chohan S (2004) Evaluation of mycoflora associated with pea seeds and some control measures. Plant Pathol J 3:48–51
Begum A, Sandhya S, Ali SS, Vinod KR, Reddy S, Banji D (2013) An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). Acta Sci Pol Technol Aliment 12(1):61–74
Bhajbhuje MN (2014) Biodiversity of seed mycoflora in storage of *Brassica campestris* L. Int J Life Sci 2(4):289–303
Bhatnagar PS, Karmakumar PG (1995) Achievements and prospects of breeding researches on soybean (*Glycine max*) in India. Indian J Agric Sci 65:1–9
Booth C (1971) The genus *Fusarium*. Commonwealth Mycological Institute, Kew. ISBN-10: 851980465, 237 p
Caldwell BE (1973) Soybeans: improvement, production and uses. American Society of Agronomy, Madison, WI, pp 1–10. 17–22
Chaudhary SS (2001) Studies on seed mycoflora of soybean (*Glycine max* (L.) Merrill) and their effects on seed quality parameters. Dissertation, Mahatma Phule Krishi Vidyapeeth, Rahuri
Chavan AM (2011) Nutritional changes in oilseeds due to *Aspergillus* spp. J Exp Sci 2:29–31
Christensen CM (1957) Deterioration of stored grain by fungi. Bot Rev 23:108–134
Christensen CM (1973) Loss of viability in storage: Microflora. Seed Sci Technol 1:547–562
Christensen CM, Kaufmann HH (1969) Grain storage: the role of fungi in quality loss. University of Minnesota Press, Minneapolis, MN
Chuku EC, Ogbalu OK, Osakwe JA (2007) Fungi of coconut (*Cocos nucifera* L.) - their deteriorative ability, quality stability and the role of the fungus-eating insects. J Appl Sci 7:3106–3110
Chukunda FA, Emri UN, Ukoima HN (2015) Studies of seed-borne mycoflora of castor bean (*Ricinus communis* L.). Acad J Microbiol Res 3(1):006–009
Cosmetic Ingredient Review Expert Panel (2007) Final report on the safety assessment of *Ricinus communis* (castor) seed oil. Int J Toxicol 26(3):31–37
Dawar S, Ghaffar A (1991) Detection of the seed borne mycoflora of sunflower. Pak J Bot 23 (2):173–178
Dawar S, Khalid S, Tariq M (2014) Seed borne mycoflora of castor bean (*Ricinus communis* L.) from Pakistan. Pak J Bot 46(4):1485–1488
Dawson WAJM, Bateman GL (2001) Fungal communities on roots of wheat and barley and effects of seed treatments containing fluquinconazole applied to control take-all. Plant Pathol 50:5–82
De Tempe J (1953) The blotter method of seed health testing. Proc Int Seed Test Assoc 21:133–151
Dharmaputra OS, Worang RL, Syarief R, Miftahudin M (2009) The quality of physic nut (*Jatropha curcas*) seeds affected by water activity and duration of storage. Microbiology 3:139–145
Diener UL, Cole RJ, Sanders TH, Payne GA, Lee LS, Klich MA (1987) Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annu Rev Phytopathol 25:249–270
DOR (2013) Vision 2050. ICAR - Indian Institute of Oilseeds Research (IIOR), Andhra Pradesh, p 33
DRMR (2015) Vision 2050. ICAR - Directorate of Rapeseeds-Mustard Research, Bharatpur, p 26
Ellis MB (1971) Dematiaceous hyphomycetes, 1st edn. Commonwealth Mycological Institute, Kew. ISBN-13: 978-0851986180, 608 p
Enikuomehin OA (2010) Seed sorting of sesame (*Sesamum indicum* L.) by salt density and seed-borne fungi control with plant extracts. Arch Phytopath Plant Prot 43(6):573–580

Escamilla D, Rosso ML, Zhang B (2019) Identification of fungi associated with soybeans and effective seed disinfection treatments. Food Sci Nutr 7(10):3194–3205
Ghosh T, Biswas MK, Aikat K (2018) A review on seed borne mycoflora associated with different oilseed crops and their management. Int J Pure App Biosci 6(1):1526–1538
Ghotekar SN, Hedawoo GB (2010) Seed-borne mycoflora of cabbage and cauliflower. In: National Conf. on Curr. Scenario on Global Challenges in Germplasm & Biodiversity (Abstract), p 47
Ghugal SI, Thakre RP (2014) Incidence of microorganisms in mustard seeds during storage & its toxic effect on seed germination and seedling diseases. Int J Life Sci Spec Issue A2:69–72
Goldblatt LA (1969) Aflatoxin. Scientific background, control and implications. Academic Press, New York, NY. 472 p
Goodman JJ, Christensen CM (1952) Grain storage studies. XI. Lipolytic activity of fungi isolated from stored corn. Cereal Chem 29:299–308
Gupta SK (2002) Seed borne diseases of linseed 'flax' (*Linum usitatissimum* L.). Dissertation, University of Allahabad, Allahabad
Gwary DM, Mailafiya DM, Jibrin TJ (2006) Survival of *Colletotrichum sublineolum* and other seed-borne fungi in sorghum seeds after twenty months of storage. Int J Agric Biol 8:676–679
Habib A, Sahi ST, Ghazanfar MU, Ali S (2007) Location of seed-borne mycoflora of eggplant (*Solanum melongena* L.) in different seed components and impact on seed germinability. Int J Agric Biol 9:514–516
Harrington JF (1967) Seed and pollen storage for conservation of plant gene resources. FAO Techn Conf Explor Util Conserv. Plant Gene Resource 13(3):1–22
Henselov M, Hudecov D (2001) Differences in the microflora of scarified and unscarified seeds of *Karwinskia humboldtiana* (Rhamnaceae). Folia Microbiol 46:543–548
Ijaz A, Anwar SA, Riaz A, Khan MSA (2001) Seed-borne pathogens associated with wheat and their role in poor germination. Pak J Phytopathol 13:102–106
Ismail M, Irfan M, Riaz A (2004) Seed-borne mycoflora of safflower (*Carthamus tinctorius* L.) and their impact on germination. Mycopathologia 2(1):51–54
Ismaiel AA, Papenbrock J (2015) Mycotoxins: producing fungi and mechanisms of phytotoxicity. Agriculture 5:492–5377
Jones FN (2003) Alkyd resins. Ullmann's encyclopedia of industrial chemistry. Wiley, Weinheim
Jonsyn FE (1988) Seed-borne fungi of sesame (*Sesamum indicum* L) in Sierra Leone and their potential aflatoxin/mycotoxin production. Mycopathologia 104(2):123–127
Jovicevic B (1980) Contribution to the knowledge of harmful mycoflora on seed and seedling of wheat, maize and sunflower. Zaštita Bilja 31:101–119
Jyoti, Malik CP (2013) Seed deterioration: a review. Int J Life Sci Biotechnol Pharm Res 2(3):374
Kakad SA, Parate RL, Bramhankar SB, Pawar RD, Wasnik DG, Dinkwar GT, Bambal SV, Isokar SS (2019) Evaluation of fungicides and botanicals against major seed borne fungi of groundnut. Int J Chem Stud 7(1):1949–1952
Kakde RB, Chavan AM (2011) Extracellular lipase enzyme production by seed-borne fungi under the influence of physical factors. Int J Biol 3:95–100
Kakde RB, Badar KV, Pawar SM, Chavan AM (2012) Storage mycoflora of oilseeds: a review. Int Multidiscipl Res J 2(3):39–42
Kapoor N, Arya A, Siddiqui MA, Kumar H, Amir A (2011) Physiological and Biochemical changes during seed deterioration in aged seeds of Rice (*Oryza sativa* L.). Am J Plant Physiol 6:28–35
Kaur J, Chahal SS, Aulakh KS (1990) Differential efficiency of different methods in detection and location of seed-borne fungi in sunflower. Plant Dis Res 5(1):53–58
Khan SAJ, Khanzada AK, Sultana N, Aslam M (1988) Evaluation of seed health testing techniques for the assessment of seed borne mycoflora of rice. Pak J Agric Res 9:502–505
Khanzada KA, Rajput MA, Shah GS, Lodhi AM, Mehboob F (2002) Effect of seed dressing fungicides for the control of seed borne mycota of wheat. Asian J Plant Sci 1:441–444
Khattak I, Hussain S, Ali A, Shah SJA (1993) Efficacy of various methods employed for the study of seed borne microflora of soybean. Sarhad J Agric 9:45–48

Kubiak K, Korbas M (1999) Occurrence of fungal diseases on selected winter wheat cultivars. Postepy w Ochronie Roslin 39:801–804
Kumar K, Singh J, Saksena HK (1984) Fungi associated with sesame seeds- their nature and control. Indian Phytopath 37:330–332
Kumar P, Chatli MK, Mehta N, Singh P, Malav OP, Verma AK (2017) Meat analogues: health promising sustainable meat substitutes. Crit Rev Food Sci Nutr 57:923–932
Lantos F, Lantos J, Lantos E (2002) An unusual twist to the blotter test: the Lantos method. Pract Oil Anal:7
Limonard T (1966) A modified blotter test for seed health. Neth J Plant Pathol 72:319–321
Lodhi SA, Naeem A (1955) Some seed-borne fungi from Pakistan. Trans Br Mycol Soc 38:240–242
Lumpungu K, Baelenge B, Bitijula M (1989) The effect of groundnut seed coat on the development of pathogenic fungi. Tropicitura 7(4):128–131
Mangena P, Mokwala PW (2019) The influence of seed viability on the germination and *in vitro* multiple shoot regeneration of soybean (*Glycine max* L.). Agriculture 35(9):1–12
Manoharachary C, Kunwar IK (2006) Seed borne fungal pathogens of Soybean. J Basic Appl Mycol 5(I&II):1–8
Meena M (2005) An introductory study of seed mycoflora and powdery mildew disease of linseed. Dissertation, Indira Gandhi Krishi Vishwavidyalaya, Raipur
Mirza MY (2009) Oil-seed program. NARC, Islamabad. http://www.parc.gov.pk/isub Dirision/NARCCSI/oil.html
Mishra RR, Kanaujia RS (1973) Studies on certain aspects of seed-borne fungi. II. Seed-borne fungi on certain oil-seeds. Indian Phytopath 25:284–294
Mondal GC, Nandi D, Nandi B (1981) Studies on deterioration of some oil seeds in storage. I: Variation in seed moisture, infection and germinability. Mycologia 73(1):157–166
Morar MV, Dancea Z, Bele C, Salegean D, Beke A, Baonca I (2004) An app roach upon the qualities of the raw material and raw oil from sunflower seeds resulting in process of low capacities. Buletinul-Universitatii-de-Stiinte -Agricole-siMedicina-Veterinara-Cluj-Napoca-Seria-Agri 60:381–384
Mukherjee PS, Nandi SK, Nandi B (1992) Deteriorative changes in groundnut seeds in storage. J Mycopathol Res 30(2):113–119
Mumtaz B, Fatima S (2017) Evaluation of Mycoflora and Mycotoxins contamination of mustard seeds (*Brassica juncea* L.). Epitome Int J Multidiscipl Res 3(1):11–15
Muskett AE (1948) Technique for the examination of seed for the presence of seedborne fungi. Trans Br Mycol Soc 30:74–83
Nahar S, Mushtaq M, Hashmi MH (2005) Seed-borne mycoflora of sunflower (*Helianthus annuus* L.). Pak J Bot 37(2):451–457
Nandi D, Mondal GC, Nandi B (1982) Studies on biodeterioration of some oilseeds. III. Effect of different storage temperatures and relative humidities on seed moisture, germinability and infection. Seed Sci Technol 10:141–150
Neergaard P (1979) Seed pathology, vol 1. MacMillan Press Ltd, London, p 839
Negedu A, Ameh JB, Umoh VJ, Atawodi SE (2012) Lipolytic activity of some fungal species on castor oil. Afr J Food Agri Nutr Dev 12(6):6686–6699
Noble M, Richardson MJ (1968) An annotated list of seedborne diseases, 2nd edn. ISTA, Washington, DC, p 231
Oladipupo OA (2011) Effect of seed-Borne fungi on percentage germination and health of Groundnut seedlings. Dissertation, College of Plant Science and Crop Production
Onwueme IC, Sinha TD (1991) Field crop production in Tropical Africa. CTA, Ede, pp 337–343
Oyewole OL, Owoseni AA, Faboro EO (2010) Studies on Medicinal and toxicological properties of Cajanus cajan, Ricinus communis and Thymus vulgaris leaf extracts. J Med Plant Res 4(19):4–8
Papavizas GC, Christensen CM (1958) Grain storage studies. 26: Fungus invasion and deterioration of wheat stored at lower temperatures and moisture contents of 15–18 per cent. Cereal Chem 35:27–34

Qasem SA, Christensen CM (1958) Influence of moisture content, temperature, and time on the deterioration of stored corn by fungi. Phytopathology 48:544–549
Qumberani TK (2001) Effect of storage fungi on seed quality parameters of different mustard varieties. FAO AGRIS, Rome, p 430
Ramakrishna N, Lacey J, Smith JE (1991) Effect of surface sterilization, fumigation and γ irradiation on the microflora and germination of barley seeds. Int J Food Microbiol 13:47–54
Ramesh BV, Hiremath SV, Naik MK, Amaresh YS, Lokesh BK, Vasudevan SN (2013) Study of seed mycoflora of soybean from north eastern Karnataka. Karnataka J Agric Sci 26(1):58–62
Ranjan KS, Sahay SS, Sinha AK (1992) The influence of storage structure on aflatoxin contamination in wheat and mustard. J Stored Prod Res 28:221–224
Rao TV, Rajeswari B, Keshavulu K, Varma VS (2015) Studies on seedborne fungi of soybean. SSRG Int J Agric Environ Sci 2(1):16–24
Raper KB, Thom C (1949) A manual of *Penicillia*. Williams and Wilkins, Baltimore, MD, p 875
Rasheed S, Dawar S, Ghaffar A, Shaukat SS (2004) Seed borne mycoflora of groundnut. Pak J Bot 36(1):199–202
Reddy MJ (1993) Varietal differences in seed mycoflora of sunflower. Seeds Farms 15:17–20
Reddy V, Immanuelraj K (2017) Area, production, yield trends and pattern of oilseeds growth in India. Econ Aff 62:327–334
Reddy MN, Rao AS (1980) Damping off of groundnut caused by 2 *Fusarium* spp., *F. solani* and *F. oxysporum*. Indian J Mycol Plant Pathol 10(1):65–66
Rizzo G, Baroni L (2018) Soy, soy foods and their role in vegetarian diets. Nutrients 10(1):43
Saleh AKM, Latif MA, Khan MAI, Rahman H, Uddin MK (2003) Prevalence of fungi in mustard seeds grown and stored at different locations of Dhaka Region, Bangladesh and their control. Pak J Biol Sci 6:995–997
Sarabhoy AK, Agarwal DK (1983) Fungal diseases of soybean and their management. Int J Trop Plant Dis 1:13–19
Schery RW (1972) Plants for man. Prentice-Hall, Inc, Englewood Cliffs, NJ
Seung-Hun Y, Mathur SB, Neergaard P (1982) Taxonomy and pathogenicity of four seed-borne species of *Alternaria* from sesame. Trans Br Mycol Soc 78(3):447–458
Sharma KD (1977) Biochemical changes in stored oilseeds. Indian J Agric Res 11:134–144
Siddiqui S (2013) Seed borne mycoflora of Indian mustard (*Brassica juncea* L.). LAP Lambert Academic Publishing, Saarbrücken. ISBN-13: 9783659323409
Sinclair JB (1975) Bacterial and fungal diseases of soybean in the tropics and subtropics. In: Proc. Workshop for Trop. and Subtrop. Conditions (Puerto Rico), pp 112–114
Singh BP, Shukla BN, Sharma YK (1974) Post infection changes in chemical composition of groundnut (*Arachis hypogaea* L.) seed. J Res Punjab Agric Univ Ludhiana 33:74–75
Singh AK, Manibhushan, Bhatt BP, Singh KM, Upadhyaya A (2013) An analysis of oilseeds and pulses scenario in Eastern India during 2050-51. J Agric Sci 5(1):241–249
Singh R, Singh AK, Kumar P (2014) Performance of Indian mustard (*Brassica juncea* L.) in response to integrated nutrient management. J Agric Search 1(1):9–12
Singh AK, Singh AK, Choudhary AK, Kumari A, Kumar R (2017) Towards oilseeds sufficiency in India: present status and way forward. J Agric Search 4:80–84
Solanke RB, Kore SS, Pensalwar SN (1997) Effect of fungicidal seed treatment on seed variability and mycoflora during storage of sorghum. J Mycol Plant Pathol 27(3):297–298
Srivastava S, Sinha A (2013) Estimation of moisture content and percent germination of stored and infested *Jatropha curcas* L. seeds. Allahabad Farmer LXVIII:63–72
Srivastava AK, Singh VN, Pathak KK (1981) Seed mycoflora of stored mustard and its control by fungicides. Proc Indian Natl Sci Acad B47(1):96–98
Srivastava S, Sinha A, Srivastava CP (2011) Screening of seed-borne mycoflora of *Jatropha curcas* L. Res J Seed Sci 4:94–105
Suleiman MN, Omafe OM (2013) Activity of three medicinal plants on fungi isolated from stored maize seeds (*Zea mays* L.). Global J Med Plant Res 1(1):77–81
Sundaresh HN, Hiremath (1978) Studies on seed mycoflora of soybean. Curr Res 10:178–179

Thakur RP, Rao VP, Navi SS, Garud TB, Agarkar GD, Bharati B (2003) Sorghum grain mold: variability in fungal complex. Int Sorg Mill Newslett 4:104–108
Thapa S, Baral R, Thapa S (2019) Status, challenges and solutions of oil-seed production in India. Res Rev J Agric Allied Sci 8(1):27–34
Thom C, Raper KB (1945) Manual of *Aspergilli*. William and Wilkins Co., Baltimore, MD
Toole EH (1939) Seed longevity and seed storage. US Dept Agric Bur Pl Ind Bull 5:15
Upadhyay RS, Singh DB (1978) Seed mycoflora of mustard and their responses to storage and seed treatments with fungicides. J Sci Res BHU 29:171–181
Vaidehi BK (2002) Seed mycoflora of sunflower - a perspective. Front Micro Biotech Plant Pathol:25–40
Vereshchagin AG, Novitskaya GV (1965) The triglyceride composition of linseed oil. J Am Oil Chem Soc 42:970–974. https://doi.org/10.1007/BF02632457
Verma PK, Saxena MK (2012) Allelopathic potential of Lantana camara L. to check seed borne fungal infection in mustard (*Brassica campestris*) and chickpea (*Cicer arietinum*). Int J Rec Sci Res 3(10):805–808
Vidhyasekaran P, Govindaswamy CV (1968) Role of seed-borne fungi in paddy seed spoil.i-e. III. I'roducuon of c.irbon dioxide, fatty acids and reducing sugars. Indian Phytopathol Soc Bull 4:71–78
Vijayalakshmi M, Rao AS (1985) Fungal infection of sunflower seeds under different conditions of storage. Indian Phytopath 38(2):315–318
Ward HS, Diener UL (1961) Biochemical changes in shelled peanuts caused by storage fungi, I. effects of *Aspergillus tamarii*, four species of *A. glaucus* group, and *Penicillium citrinum*. Phytopathology 51:244–250
Williams RJ, McDonald D (1983) Grain molds in the tropics: problems and importance. Annu Rev Phytopathol 21:153–178
Wilson C (1947) Concealed damage of peanuts in Alabama. Phytopathology 37:657–668
Zohary D, Hopf M, Weiss E (2012) Domestication of plants in the old world: the origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin, 4th edn. Oxford University Press, Oxford, p 168. ISBN 978-0199549061

Seed-Borne *Alternaria helianthi* Leaf Blight in Sunflower

15

P. Ahila Devi and L. Gnanasing Jesumaharaja

Abstract

Sunflower leaf blight and seed-borne nature caused by *Alternaria helianthi* is a major disease of Tamil Nadu. When the infection was severe, yield loss will go up to 80–85%. Of late, the disease has become endemic and severe in sunflower belts of Tamil Nadu with variation in the intensity. The management of this disease by cultural methods and by using toxic chemicals has reduced the biodiversity of soil microbes which results in resistance development in pathogen. Hence the current investigation aims for eco-friendly management strategies.

Keywords

Alternaria helianthi · Sunflower · Seed-borne

15.1 Introduction

Sunflower (*Helianthus annuus* L.) termed as 'Golden girl of American Agriculture' is the principle oilseed crop in the globe which ranks third in area behind groundnut and soybean. Sunflower belongs to the family Asteraceae (Compositae), one of the earliest domesticated and cultivated members in the United States.

Sunflower is approximately cultivated in 25.24 mha around the world with the total production of 35.64 million metric tones and a productivity of 1424 kg/ha. Major cultivating countries are Russia, Ukraine, India, Argentina, the United States and China occupying about 68% of the total sunflower cultivable area around the

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world. The status of sunflower cultivation in India with an area more than 2.03 mha with the production over 1.11 million metric tones and the productivity of 542 kg/ha. State-wise sunflower growers were Tamil Nadu, Karnataka, Andhra Pradesh and Maharashtra. In recent years, the crop cultivation gains momentum in the states, viz. Punjab, Haryana and Uttar Pradesh which were earlier unaware. In Tamil Nadu, sunflower is cultivated in 25,800 ha approximately with total production of about 34,300 tones and productivity of 1329 kg/ha.

Among a number of biotic stresses, vulnerability to diseases is the important constraint for prosperous production. *Alternaria* leaf and stem blight, rust, powdery mildew, downy mildew, collar rot and necrosis are the important diseases infecting sunflower in India. Among the abovementioned diseases, leaf blight incited by *Alternaria helianthi* is considered to be a potential destructor of sunflower production in India (Gopalakrishnan et al. 2010).

A. helianthi is a seed-borne, necrotrophic pathogen causing circular spots on the leaves, stem, flower head and petioles of sunflower. Infection of the plants at early growth stage causes high yield loss, and infection during flowering results in higher level of seed infection (Santha Lakshmi Prasad et al. 2010).

This disease drastically reduces the flower size, seeds per head, yield per plant, test weight and oil content (Balasubramanyam and Kolte 1980a). Loss of yield lies between 11.30% and 73.33% depending on the severity of infection (Reddy and Gupta 1977).

In Tamil Nadu, under severe epiphytotic condition, the disease causes 80% or more yield loss. For better production, the use of quality seeds ensures uniform and rapid growth from seedlings and also better yield. Sunflower is a very sensitive crop to adverse environment. Oxidation of oil content deteriorates the seeds in storage (Wilson and McDonald 1986).

Biological control is a most reliable strategy to manage crop diseases. Effectiveness depends not only on the organisms used but also the strategies and methods utilized for introduction and population levels' maintenance and their activities.

Seed treatment is one of the most suited and popular methods for introducing biocontrol agents. Application of beneficial microorganisms to seed in proposition which has better effect and minimal impact of environment to the combination (McQuilken et al. 1998).

Various seed treatment techniques were followed for improved crop production. Seed invigoration is one of the strategies which include osmohardening, hardening, osmoconditioning, hydropriming, biopriming and matri-priming (Windauer et al. 2007).

Seed priming with biocontrol organisms serves as an important tool to manage soil- and seed-borne diseases. Successful application of beneficial microorganism through the seed in economically viable method is the first step to improve the health of the crop. Seed coated with microorganisms have the ability to colonize and flourish well in the rhizosphere.

Therefore this study is taken up with the following objectives that focus on the use of efficient antagonistic organism in the management of leaf and stem blight caused by *Alternaria* in sunflower:

1. Assessment of seed-borne *A. helianthi* prevalence in sunflower in Tamil Nadu
2. Location of seed-borne *A. helianthi* in sunflower seeds
3. Screening of biocontrol agents isolated from the rhizosphere of sunflower and new fungicides against *A. helianthi* in vitro
4. To test the efficacy of biocontrol agents and the fungicides on incidence of *A. helianthi*, seed potential, and seedling vigour

15.2 Results

Sunflower (*Helianthus annuus* L.) is the most important oilseed crop in India. Leaf blight of sunflower that occurs all over the growing parts in the world is an important foliar disease.

15.2.1 Distribution and Economic Importance

To assess the incidence of sunflower leaf blight and seed-borne disease, an intensive survey was conducted in different sunflower cropped regions in Tamil Nadu. The data reveals that isolate I_3 collected from Naranapuram of Tirunelveli district was the most virulent by recording 72% disease index (PDI) with maximum disease grade of 6.48 on the susceptible sunflower variety CO_3 followed by I_1 collected from Chittampatti of Madurai district recorded 65.55 PDI with disease grade of 5.91. Sunflower leaf blight incidence is reported from all the sunflower-growing countries (Kolte 1984; Sackston 1988). *Alternaria* has the capacity to cause disease over a wide temperature range, thus becoming a threat to sunflower production over the globe (Islam and Maric 1978; Sackston 1988). According to Reddy and Gupta (1977), yield loss varies from 11.3% to 73.2%. Mathur et al. (1978) and Balasubramanyam and Kolte (1980a, b) reported a severe reduction in yield attributes, viz. plant height, girth of the stem, size of the head, seeds count/head, seed test weight, seed yield and oil recovery percentage. Disease incidence of 95% to 100% is reported in Karnataka by Hiremath et al. (1990).

Leaf spot, blight caused by *A. helianthi*, is a menace to profitable sunflower cultivation throughout the growing regions (Theerthaprasad 2003). Favourable weather may result in yield losses up to 62% (Chattopadhyay 1999). Mahalinga et al. (2002) reported a drastic reduction of seed and oil yield to an extent up to 80% and 17%. In addition to reducing seed and oil yield, *Alternaria* affects the germination capacity and vigour of seedlings drastically (Chander Rao 2003).

15.2.2 Symptomatology

The *Alternaria* infects the aerial parts of plant, viz. leaf, petiole, stem and floral parts including the seeds. The disease initially appears as small brown spot scattered on the lower leaf lamina, later, spot size increase intermingle with each other sleeve major area in the leaf (1–2.5 cm in dia), dark brown margin surrounding with yellow halo. Necrotic abrasions are also noticed on the petioles, sepals and stem. Under severe infections, the entire head and the seeds are also affected (Kolte and Mukhopadhyay 1973; Anilkumar et al. 1974).

Chavhan et al. (2008) revealed that the disease symptoms include brown to black, concentric circles or round on leaves and flower heads. Spots may have yellow halo and sometimes coalesce with each other and become large necrotic zones that result in premature fall of the leaf. During favourable condition, infection spreads throughout the plants resulting in blighting of sunflower.

15.2.3 Morphology of the Pathogen

15.2.3.1 Conidial Morphology of *A. helianthi*

Variability study within the isolates, i.e. morphological variations of different isolates, was deliberated.

15.2.3.2 Length of Conidia

The length of conidia varies from 38.62 to 62.16 μm among the different isolates. The maximum length of conidia is observed in the isolate I_3 (62.16 μm) followed by I_1 (61.15 μm). The minimum length of conidia is observed in I_7 (38.62 μm). The remaining isolates' conidial lengths are recorded between the ranges of 40.32 and 58.59 μm (Fig. 15.1).

15.2.3.3 Conidial Width

The conidial width varied from 10.00 to 15.60 μm. While it was maximum in I_3 isolate (15.60 μm) followed by I_1 (13.87 μm), the rest of the isolates' conidial widths range from 10.44 to 13.34 μm (Fig. 15.2).

15.2.3.4 Conidial Beak Length

The beak length of the conidia varied from 14.55 to 24.50 μm. The maximum beak length was observed in I_3 (24.50 μm) followed by I_1 (20.20 μm). While the remaining isolates' beak length ranges between 14.55 and 19.22 μm.

15.2.3.5 Conidial Cells

The highest number of conidial cells observed in I_3 isolate (three to eight cells) succeeds by I_1 isolate (three to seven cells). The rest of the isolates' conidial cells range from two to eight to three to four, while the minimum numbers are observed in I_7 isolate.

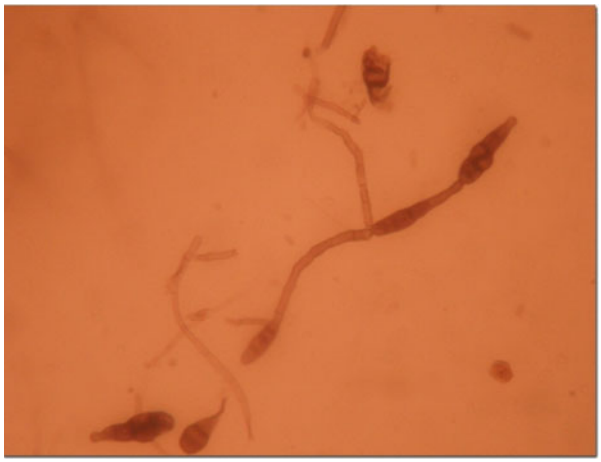

Fig. 15.1 Conidial cells and beak length of *Alternaria helianthi*

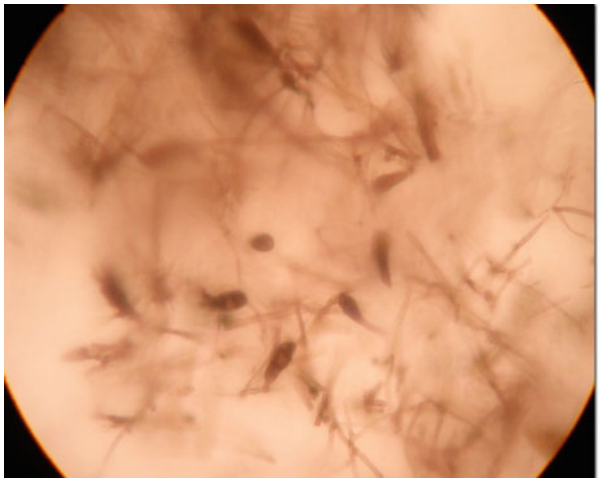

Fig. 15.2 Conidia of virulent isolate of *Alternaria helianthi*

- Tubaki and Nishihara (1969) illustrated the pathogen morphology. Cylindrical-shaped conidiophores, yellow to pale grey in colour, upright or curvaceous, five septation, geniculate, single or branched with a size of 25–80 × 8–11 µm. The conidia were singly produced on conidiophore which was cylindrical to ellipsoidal in shape, upright or curved and yellow to brown in colour with one to seven transverse septation, and up to three longitudinal septa were present. Predominantly constricted at transverse septa, measured up to 40–110 × 13–28 µm (Figs. 15.1 and 15.2).

15.2.4 Seed Microflora of Sunflower

Agarwal and Singh (1974) experimented the microflora content of 14 sunflower varieties in the seed and recorded *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Verticillium* sp. Jhamaria et al. (1974) found seven different fungi, viz. *Cunninghamella*, *Alternaria*, *Rhizoctonia*, *Curvularia*, *Aspergillus*, *Rhizopus* and *Chaetomium*, from sunflower seeds. Among the different fungi found, *Aspergillus*, *Rhizopus* and *Rhizoctonia* were present in large numbers. Chohan and Kaur (1975) registered 19 fungi which were mostly present in the seeds of four sunflower varieties (EC-68413, Sunrise, local and EC-68414). Among the 19 fungi, the predominant one is *Alternaria tenuis*, succeeded by *Aspergillus flavus* followed by *A. niger*, and then the fourth most is *Rhizopus arrhizus*. Anilkumar and Urs (1976) documented *Aspergillus* sp., *Rhizoctonia* sp., *Penicillium* sp., *Rhizopus* sp. and *Fusarium* sp. on sunflower cv. EC-68414. Kanwar et al. (1979) listed ten fungi, viz. *Rhizopus arrhizus*, *R. stolonifer*, *Chaetomium* spp., *Aspergillus candidus*, *Botrytis cinerea*, *Verticillium albo-atrum*, *Alternaria* sp., *Curvularia lunata*, *Fusarium moniliforme* and *Sclerotium rolfsii* associated with sunflower seeds.

Sackston (1981) and Morris et al. (1983) reported internally seed-borne feature of *Alternaria helianthi* in seeds and seed transmissible up to 22.9%. Pathogen detected all portions of seed, endosperm showing higher content of more than 65% and embryo has the pathogen up to 25–30%. Seed to plant infection observed more than 50% (Raut 1985). Hiremath et al. (1993) also described *A. helianthi* as an internally seed-borne fungi in sunflower seeds. Shtienberg (1994) isolated *A. alternata* from the surface of the sunflower seeds and showed that, out of 16 samples of healthy seeds, five samples showed infection. The presence of *A. helianthi* was detected in the pericarp, endosperm and embryo by employing component plating technique. Sowing the infected seeds causes seedling blight indicating the role of seed-borne inoculum.

Germination reduced by 32.8% in naturally infected seed and 19.0% in artificially inoculated seed, respectively. Shoot and root lengths of seedlings were also reduced in both cases. The vigour index was very low in naturally infected seeds (400.0). Artificially inoculated seed recorded a vigour index of 533.5, whereas in control it was 1799.8.

15.2.5 Implications of Seed-Borne Sunflower Diseases and Their Economic Impacts

Many researchers recorded that the importance of fungi carried in the seed generates numerous infections of sunflower (Hiremath et al. 1993). Chohan and Kaur (1975) desolated different fungi associated with seeds of several sunflower varieties.

A study on microflora of sunflower by Anilkumar and Urs (1976) found predominant association of various species of *Aspergillus*, *Penicillium*, *Rhizopus* and *Alternaria*. Ramegowda (1980) described varietal divergence plays a vital role in

the types of pathogens associated and also the constituent at the end of the storage period.

Leaf blight is a significant sunflower disease in the most cropped regions constitutes for more than 80% of yield loss. Devaluation of yield observed were from 28% to 80%, and the oil content losses hold an extent to 31–34% in India (Balasubramanyam and Kolte 1980a, b).

The seed-borne infestation of *Macrophomina phaseolina* was recorded in sunflower by several workers (Sadashivaiah et al. 1986). The most significant pathogen is *M. phaseolina*; it is seed-borne in sunflower which produces the most prominent symptoms like charcoal rot, root rot and most devastating seed rot (Raut 1987).

Plasmopara halstedii an important fungi causing downy mildew in sunflower persist as mycelium or oospore in the seeds from naturally infected plants (Novotel'nova 1966). Seeds obtained from downy mildew infested plants were found to be unhealthy, poorly developed, and lack of colour, and these seeds have very less germination capacity, if germinated unable to produce good healthy seedlings for a better crop production (Doken 1989).

15.2.6 Methods to Test Seed Health

Considerable methods were employed to test the seed health and were developed by researchers to detect and diagnose the pathogens carried by seeds. Special importance was given to those methods economically feasible, simple and easy to perform, highly sensitive, efficient and reproducible (Neergaard 1977).

Limonard (1968) suggested a replacement method for 2-4 D blotter, i.e. deep freeze blotter method, to withhold germination of seeds to interpret and describe the type of fungus present. Jeffrey et al. (1985) proposed modified agar method for disclosure of *A. helianthi*, and Krishnappa and Shetty (1990) recommend whole mount's technique. Sadashivaiah et al. (1986) and Raut (1987) documented blotter method with higher counts than PDA method in sunflower seeds infested *M. phaseolina*.

Shetty et al. (1980) recognized the presence of *Sclerospora graminicola* spores which elicit downy mildew of bajra by seed washing test.

15.2.7 Location and Impart in Seed

Raut 1985, reported *A. helianthi* from seeds of naturally grown crop in water agar. Sahu et al. 1991, described the association of pathogen on crop debris. In 10% of samples, pathogens mostly present in the seed coat, followed by 2% in endosperm and cotyledon rarely found 0.8% on the plumule and radical (Krishnappa and Shetty 1990).

Raut (1985) identified the presence of leaf blight fungi 65% on the endosperms, followed by 25–30% on seed embryo. The pathogen produces spots on cotyledons, leaves within 10–15 DAI in the water agar test for the symptom production. The

typical symptoms on cotyledons were reddish brown near the margin while on the leaves brown colour circular spots with ash centre.

Flowering stage was highly harmed by pathogen. Vulnerability reduces as the stage of flowering advances. In context to the charcoal rot disease caused by *M. phaseolina* transmission, test indicate severely infested seeds loose the capacity germinate due to rotting of seed. Furthermore if germinate produce discoloured seedlings which gets collapsed by rot.

Sadashivaiah et al. 1986, found pre- and post-emergence seedlings' death is most severe as well as devastating result of rot. Pre- and post-emergence death of young seedlings in Bhendi was the cause of pathogens present inside embryo, but no mortality found by the pathogen present outside embryo. *M. phaseolina* was found on the coat of soybean seed not inside the seed which has black spots collected from plants stem-inoculated in vitro, and those seeds fail to germinate (Gangopadhyay et al. 1970). The fungal hyphae was found in almost parts of the *P. vulgaris* seeds. Sclerotia were found in the cotyledons in advanced stages of infection (Shama 1991).

An experiment was conducted to find the dissemination of sunflower downy mildew pathogen from infected seed seedlings. The result revealed that seedlings were symptom less (latent infection). Fourteen to 89% infection noticed when the infected seeds used and kept at 20 °C and greatly latent noticed frequently when the plants grow along with the infected plant debris. Infected seeds give rise to seedlings which evince symptoms of *P. halstedii*. The pathogen mostly infests the ovary and the pericarp, not in the embryo (Doken 1989).

15.2.8 Yield Loss

The sunflower leaf blight can cause both quantitative and qualitative losses. *A. helianthi* reduced the seed yield and size of flower head by 17–26%. *A. helianthi* infection reduced 1000 seed weight, seeds per ear head by 50–76%, respectively, and also the seed weight (Balasubramanyam and Kolte 1980a, b). The effect of Alternaria blight and Rhizoctonia root rot caused increasing losses in seed yield (Bhowmick and Singh 1977).

Kolte (1984) reported that oil yield loss was ranging from 17% to 33% with 5.2% reduction in oil content. *A. tenius* decreased the oil content and iodine value but increased saponification number and free fatty acid content (Alaga and Akueshi 1986; Prasad and Singh 1983).

15.2.9 Susceptibility of Host

The aim of the experiment is to find the difference on susceptibility among the different age of sunflower plants. In general, aged plants are highly susceptible to the disease; however 70-day-old plants recorded the highest PDI of 72.22 followed by 60 days old plants with 63.42 PDI, while the lowest PDI of 13.00 was found in

20-day-old plants. Pathogen which harms the flowers leads to disintegration of head and seed (Kolte 1984). Plants inoculated with *A. helianthi* at 60 days showed significant infection in seeds (Godoy and Fernandes 1985), and seed infection was favoured during flowering stages of crop during winter season crop (Jasbir Kaur et al. 1991). *A. helianthi* infection was severe during flowering to early seed setting. *A. alternata* reduced the size of flowers, seed number per head and seed weight up to 84%. *A. alternate* caused 50% reduction in flower head size.

15.2.10 Epidemiology of the Disease

Acimovic (1979) reported that the most favourable temperature for *A. helianthi* infection was 25 and 30 °C. *Alternaria* leaf spot in sunflower was more frequent and severe in areas with long wet summer together with daily mean temperature of 25 and 30 °C (Allen et al. 1983). High humidity is essential for the infection, and cultural debris provides initial inoculum for severe infection.

15.2.11 Method of Inoculation

Two pairs of leaves in seedlings at V8 stage of growth were inoculated with *A. helianthi* which was cultured and maintained on agar medium with leaf extract up to 10 days with a spore density of 1–2 spores/cm^2 with 48-h dew period for screening against *A. helianthi* resistance (Kong et al. 1995). The spore suspension with pin prick method was the best method of inoculation for *A. alternata* in onion leaves recording 57.0 PDI followed by spore injection. Further, opined spraying spore suspension about 5×10^5 spores per millliter of *A. alternation on* detached young shoots of Minneola exhibited manifestations on leaves and twigs, peculiar characteristic of disease symptom after 48 h, and proved pathogenicity by spraying leaves of 6-month-old potted *Camellia japonica* cv. Burnside plants with a spore and mycelia suspension prepared by using a mixture of three isolates at a concentration of 1×10^5 mL CFU/mL.

15.2.12 Variability of the Pathogen

15.2.12.1 Morphological Characters

Muthulakshmi (1990) found that the isolates of *A. tenuis* causing fruit rot of chilli varied in their morphological characters. The sporulation was moderate in the majority of the isolates while it was profuse in isolate 1 and isolate 6. The colour of the conidia was brown to dark brown. The number of cell per conidium and conidial length and width also varied among isolates. Kannan (1992) reported that the individual conidial length of the isolates of *A. alternata* inciting onion blight disease significantly varied from 10 to 43 µm, and the width varied from 5 to 15 µm.

Babu (1994) observed that 20 *Alternaria solani* major incitant for tomato early leaf blight, the length and width of the conidia significantly varied from each other. He reported that the spore length of *A. solani* significantly varied from 158.72 to 197.58 μm and width from 12.19 to 16.6 μm. The beak length ranged from 47.38 to 65.82 μm among the isolates.

Ranganatha et al. (2003) found variations in morphological of *A. helianthi* grown on media with different wild *Helianthus* species extracts. Reduced growth of fungal mycelium observed in semisynthetic media enriched with wild sunflower (*H. tuberosus* L., *H. hirsutus* and *H. occidentalis*) leaf extracts in comparison with cultivated sunflower extract in terms of deformed conidia.

A huge discrepancy observed in growth, spore production and infection capacity between the *Alternaria helianthi* from divergent geographical regions Tamil Nadu (Coimbatore), Andhra Pradesh (Kurnool), Telangana (Hyderabad), Maharashtra (Akola and Latur) and Karnataka (Raichur) (Anonymous 2002).

Sharma et al. (2005) described the pathogen causing leaf spot (*A. alternate*) of apple was differed in their morphology. Isolate 1 showed sparse aerial mycelium and produced abundant olivaceous conidia on a dark mycelium mat on the surface of the medium. Isolate 2 had abundant grey mycelium and produced fewer numbers of conidia (Figs. 15.3 and 15.4).

15.2.12.2 Culture Characters

In Vitro Experiment on Carbon Sources for Growth Media to Culture *A. helianthi* (I_3)

With the view to find out the utilization of carbon source for the growth of *A. helianthi*, an experiment was conducted with six carbon sources; the result showed that the growth rate differed significantly depending upon the carbon sources in solid and liquid media. The maximum colony diameter of 6.56 cm and mycelial dry weight of 2.12 g were found in glucose substituted medium.

Fig. 15.3 Mycelium of virulent isolate of *Alternaria helianthi*

Fig. 15.4 Different isolates of *Alternaria helianthi*. (1) Chittampatti, (2) Aruppukottai, (3) Naranapuram, (4) Madurai, (5) Sankarankovil, (6) Rajapalayam, (7) Kovilpatti, (8) Omalur, (9) Lakshmipuram, (10) Perur

In Vitro Experiment on Nitrogen Sources for Growth Media to Culture *A. helianthi* (I_3)

With the view to find out the utilization of nitrogen source for its growth, an experiment was conducted with seven nitrogen sources; the result showed that the growth rate differed significantly depending upon the nitrogen source in solid and liquid media. The maximum colony diameter and mycelial dry weight were found in potassium nitrate 7.51 cm on 9 cm petri dish and dry weight of 3.47 g succeeded by sodium nitrate recorded 7.22 cm and 2.82 g, and the least colony diameter and mycelial dry weight were found in ammonium molybdate with 1.92 cm and 0.18 g. In the other form of nitrogen, the colony diameter and mycelial dry weight ranged from 2.19 to 3.74 cm and 0.47–0.70 g.

15.3 Management of the Disease

15.3.1 Chemical Control

15.3.1.1 Evaluation of Fungicides Under In Vitro Condition

Crisan and Stelota (1970) observed ferbam, maneb and captan at 0.05–0.25% highly enforce reduced mycelia growth on *A. alternata*. Utikar et al. (1979) revealed that zineb and mancozeb were superior to copper oxychloride, carbendazim and aureofungin when tested against *A. alternata*.

Twelve fungicides were assessed under in vitro on *Alternaria helianthicola* leaf spot causing fungi of sunflower. Thiram 0.15% was highly effective in reducing

growth and germination of spore. Patil et al. (1992) experimented eight fungicides using paper disc method on *Alternaria helianthi*; the most promising result was given by ziram concentrations, viz. 0.1%, 0.2% and 0.3% succeeded by copper oxychloride and mancozeb. Wadiphasme et al. (1994) evaluated six nonsystemic and three systemic fungicides against *A. helianthi* in vitro by poison food technique, and the results revealed better control by mancozeb and then copper oxy chloride. Kamalalakshmi (1996) reported in both poisoned food technique and spore germination assay; mancozeb (0.2%) was the most effective fungicide against *A. alternata* (leaf blight of jasmine) followed by aureofungin (200 ppm) and copper oxychloride (0.25%).

In Vivo Evaluation of Fungicides

Kamalalakshmi (1996) evaluated mancozeb (0.2%) was significantly the greater in reducing leaf blight in Jathimalli provoked by *A. alternate* supervene by aureofungin (200 ppm) and copper oxychloride (0.25%). Sumathi (1997) found that mancozeb (0.2%) spray was the efficient suppressing sesame leaf blight caused by *A. solani*.

Amaresh (1997) observed promising results of fungicides, viz. 0.2% chlorothalonil, 0.2% mancozeb and 0.1% cyproconazole under field condition against leaf blight. As in case of yield, highest recorded in chlorothalonil and cyproconazole treatments succeeded by mancozeb. Mancozeb 0.2% was found better in the protection schedule for rust and leaf blight of sunflower.

Rao (2000) expressed 63% yield improvement on treatment with bayleton for leaf blight on sunflower. Mancozeb has the greater cost-benefit ratio and propiconazole 0.1% highly efficient fungicide on leaf blight of sunflower (Mesta et al. 2003).

15.3.2 Biological Management

15.3.2.1 Efficacy of Bacterial Antagonists Against *Alternaria* spp.

To find out the fungistatic efficiency of *Trichoderma* spp., seven isolates for antagonistic capacity on leaf blight pathogen were tested. All the *T. viride* isolates showed varying degree of antagonism towards *A. helianthi*. Isolate *T. viride*1 recorded the highest inhibition (58.29%) on mycelial growth which observed 3.60 cm over control 8.62 cm followed by *T. viride*3.

Current investigation was to find efficacy of *Pseudomonas* isolates against pathogenic fungi. Five *Pseudomonas fluorescens* isolates, a *Bacillus subtilis* tested for their antagonistic effect. Among them *Pf1* documented highest inhibition of 63.30% against pathogen mycelial growth of 3.13 cm over control 8.53 cm.

Basim and Katircoioglu (1990) examined antagonistic activity of *Bacillus subtilis* on *Alternaria* sp., viz. *A. alternata* and *A. solani* in dual culture. The results revealed that two isolates, viz. *B. subtilis* AB-2 and AB-27, performed well. Leifort et al. (1992) found fluorescent *Pseudomonas* and *Bacillus* spp. have a greater level of antagonistic capacity. Zaspel and Sussir (1992) studied *B. subtilis* and *Pseudomonas putida* against *Pythium ultimum* and *Alternaria* sp. Application of antagonistic

bacteria like *Pseudomonas* against *Alternaria* leaf spot in tomato and lucerne diminished severity (Casida and Lokezic 1992). Amaresh (2000) observed decreased mycelial growth in *A. helianthi* on *Pseudomonas fluorescens* and *Trichoderma harzianum* treatments. Mohan et al. (2001) observed that *P. fluorescens* was antagonistic to *A. palandui*.

15.3.2.2 Seed Priming

Seed priming the beast pre-sowing techniques, seeds were partially soaked and dried to invigourative cause on field emergence, extended to yield (Corleto and Mallik 1974). Hegarty (1970) also reported that priming would improve the velocity of seed quality in concern with emergence of seed and establishment under suboptimal conditions. Nascimento and Pereira (2007) reported that in summer, crops sowing with primed seed give better establishment and performance in field condition.

15.3.2.3 Biopriming

The term 'bioprime' was introduced by Callan et al. (1991) who used it to describe a method of infecting maize seeds with a strain of *P. fluorescens* and reported that combination seeds with biocontrol and priming will enhance efficacy of biocontrol agents. They also observed that sweet corn bioprimed and *P. fluorescens* coated increased 10–10,000-fold bacterial count and depend on the level of inoculum. *Pseudomonas fluorescens* provided protection against damping off as good equal to metalaxyl fungicide MZ 72 WP. In *P. fluorescens* bioprimed seeds enhanced the ability of *Pseudomonas fluorescens* on *Pythium* sp. to reduce infection on sweet corn. The effects of biopriming on both physiology of seed to relieve chilling injury and bacterial growth to provide pathogen control may be involved in this response.

Singh (2003) proposed an upgraded technique to prime the seed with *Trichoderma harzianum* and *P. fluorescens*, pre-sowing treatment to improve establishment of seedlings. The technique reveals 1 kg seeds coated with *Trichoderma* powder suspension (10 g *T. harzianum* and 10 g sieved powder FYM in 50 mL water blended with 5 g arabica gum) and incubated at 27–32 °C under moist environment for 24–48 h best suited for rice, wheat and vegetable crops.

Biopriming of *P. fluorescens* isolates improved bajra crop by 20–75% increase in resistance against downy mildew and 22% increase in yield (Niranjan Raj et al. 2004).

Mohamedy (2004) revealed *T. harzianum* bioprimed okra seeds at 1.25 and 2.5 mg/L lowered 72% pathogen incidence and also in field conditions seeds bioprimed with *T. harzianum* and *B. subtilis* lowered pre-emergence at 68.4% and post-emergence at 64.4% on 45 and 60 DAS.

Jensen et al. (2004) demonstrated carrot seeds produced organically with *Alternaria* infestations bioprimed with *Clonostachys rosea* promised good establishment.

Mohamedy et al. (2006) observed *T. harzianum* bioprimed cowpea seeds with diminished root rot in field and fresh pod yield elevated. Singh and Singh (2008) found sorghum seeds primed with *T. harzianum* have fewer incidence of disease.

Kaymak et al. (2008) suggested PGPR biopriming of radish (*Raphanus sativus* L.) seeds more consistent boosting germination under high saline conditions.

Mougy and Abdel Kader (2008) found greater reduction in pre- and post-emergence disease incidence on faba bean seed biopriming with anyone of *Trichoderma* sp., *Bacillus* sp. and *Pseudomonas* sp.

Rao et al. (2009) indicated that biopriming with *P. fluorescens* before sowing sunflower seeds could be economical, eco-friendly substitute for chemical method of seed treatment and provides enhanced resistance of seedlings against *A. helianthi*, etc.

Chandra Nayaka et al. (2010) revealed *T. harzianum* biopriming on reduced infection of *Fusarium verticillioides* and also fumonisin production which increased germination, field emergence and vigour of seedling and yield.

Moeinzadeh et al. (2010) revealed biopriming of sunflower seeds with *P. fluorescens* UTPf 76 and UTPf 86 has greater possibility to grow, colonize and produce plant growth regulators which enhance seedling growth and development.

15.4 Conclusion

Sunflower leaf blight and seed-borne disease incited by *Alternaria helianthi* serious pathogen in Tamil Nadu. An economically important disease of sunflower causes severe seed yield loss. The ill effects of toxic chemicals over biodiversity of soil microbes and resistant development by the pathogen. Currently biocontrol method is gaining more important recognition as a feasible method in management of diseases. Hence this study included the collection of isolates, studying their morphological and cultural variability and virulence use of botanicals including plant oils and sunflower phylloplane microorganisms against the disease.

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References

Acimovic M (1979) Appearance of Alternaria sp. Up to now unknown causes of sunflower spottiness in Yugoslavia. Bilten Za Biljna Ulja I masti 6:3–9

Agarwal VK, Singh OV (1974) Fungi associated with sunflower seeds. Indian Phytopathol 27:240–246

Alaga AE, Akueshi CO (1986) Changes in oil and free fatty acid contents of sunflower seeds inoculated with *Alternaria tenuis* Auct., *Curvularia lunata* (Wakker) Boedjin, *Fusarium moniliforme* Shed and *Macrophomina phaseolina* (Tassi.) Goid. *Phytopathol Mediterr* 25:44–46

Allen SJ, Brown JF, Kochman JK (1983) The infection processes, sporulation and survival of *Alternaria helianthi* on sunflower. Ann Appl Biol 102:413–419

Amaresh YS (1997) Studies on foliar diseases of sunflower (*Helianthus annuus* L.) with special reference to Alternaria leaf blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara. Dissertation, University of Agricultural Sciences, Dharwad, p. 117

Amaresh YS (2000) Epidemiology and management of Alternaria leaf blight and rust of sunflower (*Helianthus annuus* L.). Dissertation, University of Agricultural Sciences Dharwad, p. 1–320

Anilkumar TB, Urs SD (1976) Seed microflora of sunflower and its control. Mysore J Agric Sci 10:424–427

Anilkumar TB, Urs SD, Seshadri VS, Hegde RK (1974) Alternaria leaf spot of sunflower. Curr Sci 43:93–94

Anonymous (2002) DOR research highlights 1997-2000. Directorate of Oilseeds Research, Hyderabad, pp 70–75

Babu S (1994) Studies on leaf blight of tomato (*Lycopersicon esculentum* Mill.) caused by *Alternaria solani* (EII. and Mart.) Jones and Grout. Dissertation, Tamil Nadu Agricultural University, Coimbatore, India, p. 193

Balasubramanyam N, Kolte SJ (1980a) Effect of Alternaria blight on yield components, oil content and seed quality of sunflower. Indian J Agric Sci 50:701–706

Balasubramanyam N, Kolte SJ (1980b) Effect of different intensities of Alternaria blight on yield and oil content of sunflower. J Agric Sci 94:749–751

Basim H, Katircoioglu YJ (1990) Studies on *in vitro* antagonistic effect of some *B. subtilis* isolates against important plant antagonistic fungi. Turkia 11. In: Proceedings of the second Turkish national congress of biological control, pp 109–118

Bhowmick TP, Singh A (1977) Combined effect of Rhizoctonia root rot and Alternaria leaf blight on sunflower. Indian Phytopathol 30:195–197

Callan NW, Mathre DE, Miller JB (1991) Field performance of sweet corn seed bioprimed and coated with *Pseudomonas fluorescens* ab254. Hortic Sci 26:1163–1165

Casida LE, Lokezic FL (1992) Control of leaf spot disease of alfa-alfa and tomato with application of the bacterial predators, *Pseudomonas* strains 679-2. Plant Dis 76:1217–1220

Chander Rao S (2003) Hybrid seed production technology in sunflower In: sunflower in India, Directorate of oil seeds Research, Hyderabad. Indian council of Agriculture Research, New Delhi, p 112

Chandra Nayaka S, Niranjana SR, Uday Shankar AC, Niranjan Raj S, Reddy MS, Prakash HS, Mortensen CN (2010) Seed biopriming with novel strain of *Trichoderma harzianum* for the control of toxigenic *Fusarium verticillioides* and fumonisins in maize. Arch Phytopathol Plant Protect 43:264–282

Chattopadhyay C (1999) Yield loss attribute to Alternaria blight of sunflower (*Helianthus annuus* L.) in India and some potentially effective control measures. Int J Pest Manag 45:15–21

Chavhan RL, Chakrabarty PK, Patil FS, Patil HB, Singh SK, Khadi BM (2008) Association of new fungal species with leaf spot and blight of sunflower and cloning of their ribosomal RNA genes. Indian Phytopathol 61(1):70–74

Chohan JS, Kaur J (1975) Seed borne mycoflora of sunflower and control of seed borne pathogens. Indian J Mycol Plant Pathol 6:210–211

Corleto A, Mallik A (1974) Influenza della tecnica del "presoaking" dei semi e di differenti periodi di mancanza di acqua disponibile nel tereno sulla produzione del sorgo da grarella. X Simp Inter Agron 20-24:256–268

Crisan A, Stelota M (1970) Investigation on *A. alternata* on red pepper. In: Contrtii, Bet. Univ. Babes - Bolyai. Cluj., pp 13–21

Doken MT (1989) *Plasmopara halstedii* (Farl.) Berlese et de Toni in sunflower seeds and the role of infected seeds in producing plants with systemic symptoms. J Phytopathol 124:23–26

Gangopadhyay S, Wyllie TD, Luedders VD (1970) Charcoal rot disease of soybeans transmitted by seeds. Plant Dis Rep 54:1088–1091

Godoy JRDE, Fernandes NA (1985) *Alternaria helianthi* (Hansf.) Tubaki and Nishihara on sunflower susceptibility and seed infection. Summa Phytopathol 11:186–187

Gopalakrishnan C, Manivannan N, Vindhiyavarman P, Thiyagarajan K (2010) Evaluation and Identification of Alternaria leaf spot resistant sunflower genotypes. Electron J Plant Breed 1 (2):177–181

Hegarty TW (1970) The possibility of increasing field establishment by seed hardening. Horti Res 10:59–64
Hiremath PC, Kulkarni MS, Lokesh MS (1990) An epiphytotic of Alternaria blight of sunflower in Karnataka. Karnataka J Agric Sci 3:277–278
Hiremath PC, Lokesh MS, Kulkarni MS (1993) Seed borne nature of *Alternaria helianthi* and its effect on seed germination of sunflower. Karnataka J Agric Sci 6:68–69
Islam U, Maric A (1978) *Alternaria helianthi* (Hansf.) Tubaki and Nishihara on sunflower in Yugoslavia and some culture characteristics of the fungus. Zaštita Bilja 29:219–236
Jeffrey KK, Lipps PE, Herr LJ (1985) Seed-treatment fungicides for control of seed borne *Alternaria helianthi* on sunflower. Plant Dis 69:124–126
Jensen MB, Knudsen I, Madsen M, Jensen DF (2004) Biopriming of infected carrot seed with an antagonist, *Clonostachys rosea*, selected for control of seedborne *Alternaria* spp. Plant Dis 94 (6):551–560
Jhamaria SL, Sharma KB, Gupta RBL (1974) Fungi intercepted from sunflower seeds and their control. Indian J Mycol Plant Pathol 5:212–213
Kamalalakshmi C (1996) Studies on leaf blight disease of Jathimalli *Jasminum grandiflorum* L. incited by *A. alternata* (Fr.) Keissler. agg. Dissertatino, Tamil Nadu Agricultural University, Coimbatore, India, p 199
Kannan R (1992) Studies on leaf spot on onion (*Allium cepa* L.) caused by *Alternaria alternata* (Fr.) Keissler. Dissertation, Tamil Nadu Agricultural University, Coimbatore, India, p 144
Kanwar ZS, Khanna PK, Thareja ML (1979) Seed mycoflora of sunflower and their control. Pesticides 13:41–44
Kaur J, Chahal SS, Aulakh KS (1991) Effect of different crop seasons on the incidence of *Alternaria helianthi* in sunflower seeds. Plant Dis Res 6:83–84
Kaymak HC, Goveng I, Yarali F, Donmez MF (2008) The effects of biopriming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. Turk J Agric For 33:173–179
Kolte SJ (1984) Diseases of annual edible oilseed crops, vol III. CRC Press, Boca Raton, FL. 96 pp
Kolte SJ, Mukhopadhyay AN (1973) Occurrence of some new sunflower disease in India. Pestic Abs News Summ 19:392–396
Kong GA, Kochman JK, Brown JF (1995) A greenhouse assay to screen sunflower for resistance to *Alternaria helianthi*. Appl Biol 127:463–478
Krishnappa M, Shetty HS (1990) Location of *Alternaria* species in sunflower seeds. Plant Dis Res 5:203–204
Leifort C, Sigee DC, Epton HAS, Stanley R, Knight C (1992) Isolation of bacteria antagonistic to post harvest fungal disease of cold stored *Brassica* spp. Phytoparasitica 20:158–163
Limonard J (1968) Ecological aspects of seed health testing. Int Proc Seed Test Assoc 33:1–8
Mahalinga DM, Jayalakshmi SK, Ganadhar C (2002) Chemical control of Alternaria blight of sunflower. J Mycol Plant Pathol 33:132–133
Mathur SJ, Prasad N, Agrawat JM, Chippa HP (1978) Estimation of losses due to Alternaria blight of sunflower. Indian J Mycol Plant Pathol 8:15
McQuilken MP, Halmer P, Rhodes DJ (1998) Application of microorganisms to seeds. Microbiol Rev Can Microbiol 44(2):162–167
Mesta RK, Sunkad G, Katti P (2003) Chemical control of Alternaria blight of sunflower. In: Extended Summaries of National Seminar on Stress Management in Oilseeds for attaining self-reliance in vegetable oils, January 28–30, held at DOR, Hyderabad, pp 149–151
Moeinzadeh A, Sharif-Zadeh F, Ahmadzadeh M, Heidari Tajabadi F (2010) Biopriming of sunflower (*Helianthus annuus* L.) seed with *Pseudomonas fluorescens* for improvement of seed invigoration and seedling growth. Aust J Crop Sci 4(7):564–570
Mohamedy RSR (2004) Biopriming of okra seed to control damping-off and root rot diseases. Annals Agric Sci Ain Shams Univ Cairo 49(1):339–356

Mohamedy RSR, Abd Alla MA, Badiaa RI (2006) Soil amendment and seed biopriming treatments as alternative fungicides for controlling root rot diseases on cowpea plants in Nobaria province. Res J Agric Biol Sci 2(6):391–398
Mohan K, Seetharaman T, Ebebnezer EG (2001) Management of leaf blight disease of onion caused by *Alternaria porri* by plant extracts, plant oils and biocontrol agents. NHRDF Newslett XXI (3-4):11
Morris JB, Yang SM, Wilson L (1983) Reaction of *Helianthus* species to *Alternaria helianthi*. Plant Dis 67:539–540
Mougy NS, Abdel Kader M (2008) Long term activity of biopriming seed treatment for biological control of faba bean root rot pathogens. Aust Plant Pathol 37:464–471
Muthulakshmi P (1990) Studies on fruit rot of chillies (*Capsicum annum* L.) caused by *Alternaria tenuis* Nees. Dissertation, Tamil Nadu Agricultural University, Coimbatore, India, p 139
Nascimento WM, Pereira RS (2007) Preventing thermo-inhibition in carrot by seed priming. Seed Sci Technol 35:504–507
Neergaard P (1977) Seed pathology. MacMillan Press, London, p 1187. vols I and II
Niranjan Raj S, Shetty NP, Shetty HS (2004) Seed biopriming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. Int J Pest Manag 50(1):41–48
Novotel'Nova NS (1966) Downy mildew of sunflower. USSR, Nauka, p 112
Patil MK, Kulkarni S, Hegde Y (1992) *In vitro* bioassay of fungicides against leaf spot of safflower. Curr Res 21:60
Prasad T, Singh BK (1983) Effect of relative humidity of oil properties of fungal infects sunflower seeds. Biol Bull India 5:85–88
Ramegowda S (1980) Studies on seed viability in relation to storage in sunflower. Dissertation, University of Agricultural Sciences, Bangalore, 120 pp
Ranganatha ARG, Reddy AVV, Sujatha M, Sudhakarbabu SN, Prayaga L, Basappa H, Rao SC, Nagaraj G (2003) Diversification of parental base and development of elite lines, populations, varieties and hybrids. DOR Annual Report 2002-03. Directorate of Oilseeds Research, Hyderabad, pp 8–11
Rao SC (2000) Management of Alternaria leaf spot in sunflower. Annual Report 1999-2000. Directorate of Oilseeds Research, Hyderabad, p 54
Rao MSL, Kulkarni S, Lingaraju S, Nadaf HL (2009) Biopriming of seeds: a potential tool in the integrated management of *Alternaria* blight of sunflower. Helia 32:107–114
Raut JG (1985) Location of *Alternaria helianthi* in sunflower seed and its transmission from seed to plant. Indian Phytopathol 38:522
Raut JG (1987) Detection of *Macrophomina phaseolina* in sunflower seeds. Seed Res 15:102–103
Reddy PC, Gupta BM (1977) Disease loss appraisal due to leaf blight of sunflower incited by *Alternaria helianthi*. Indian Phytopathol 30:569–570
Sackston WE (1981) The sunflower crop and disease: progress, problems and prospects. Plant Dis 65:643–648
Sackston WE (1988) First report of *Alternaria helianthi* on sunflower in Canada. Plant Dis 72:801
Sadashivaiah AS, Ranganathaiah KG, Nanjegowda D (1986) Seed health testing of *Helianthus annuus* with special reference to *Macrophomina phaseolina*. Indian Phytopathol 39:445–447
Sahu BK, Ghemawat MS, Agrawat JM (1991) Survival of *Alternaria helianthi*, the leaf-spot and blight pathogen of sunflower in Kota, India. Acta Phytopathol Entomol Hungar 26:321–326
Santha Lakshmi Prasad M, Sujatha K, Rao C (2010) Seed transmission of *Alternaria helianthi*, Incitant of leaf blight of sunflower. J Mycol Plant Pathol 40(1):63–66
Shama SM (1991) Histopathology of bean seeds infected with *Macrophomina phaseolina*. Alex J Agric Res 36:249–261
Sharma JN, Gupta D, Bhardwaj LN, Kumar R (2005) Occurrence of Alternaria leaf spot (*Alternaria alternata*) on apple and its management. Int Plant Dis Manag:25–31
Shetty HS, Mathur SB, Neergaard P (1980) *Sclerospora graminicola* in pearl millet seeds and its transmission. Trans Br Mycol Soc 74:127–134

Shtienberg D (1994) Achene Blemish syndrome. A new disease of sunflower in Israel. Plant Dis 78:1112–1116

Singh US (2003) Integration of fungal and bacterial antagonists for the management of important plant disease. G. B. Pant University of Agriculture & Technology, Pantnagar, pp 44–47

Singh Y, Singh US (2008) Biocontrol agents of anthracnose in sorghum. J Mycol Plant Pathol 38 (3):488–491

Sumathi P (1997) Biological management of Sesame (*Sesame indicum* L.) leaf blight caused by *Alternaria sesame* (Kawam) Mohanty and Behra. Dissertation, Tamil Nadu Agricultural University, Coimbatore, India, 159 p

Theerthaprasad D (2003) Simple and rapid *in vitro* screening method for Alternaria blight tolerance in sunflower using toxin of *Alternaria helianthi*. J Mycol Plant Pathol 33:45–50

Tubaki K, Nishihara N (1969) *Alternaria helianthi* (Hansf.) Comb. Nov. Trans Br Mycol Soc 53:147–149

Utikar PG, Padulae DN, More BB (1979) Effect of temperature on the biological activity of *A. alternata* (Fr.) Keissler. Indian Phytopathol 32:272–273

Wadiphasme SS, Ingole DV, Fulzele GR (1994) Evaluation of fungicides in vitro against *Alternaria helianthi* inciting leaf blight of sunflower. J Maharashtra Agric Univ 19:361–363

Wilson DO, McDonald MB (1986) The lipid peroxidation model of seed ageing. Seed Sci Technol 14:269–300

Windauer L, Altuna A, Arnold RB (2007) Hydrotime analysis of *Lesquerella fendleri* seed germination responses to priming treatments. Ind Crop Prod 25:70–74

Zaspel I, Sussir (1992) Biological control of damping off in Pinus sylvestris L. with bacterial antagonists. Bull OILB/SROP 15(1):118–120

Interspecific Hybridization Among *Vigna mungo* (L.) Hepper (Black Gram) and Wild *Vigna* Species

16

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Abstract

Black gram (*Vigna mungo* (L.) Hepper) is a legume commonly used as proteinaceous food and forage. With the aim of improvement of black gram production and disease-resistant varieties, we initiated present investigation to select appropriate genome donor from crop wild relatives (CWRs) of Asian *Vigna*. In the present study, about 1163 interspecific crosses among black gram and wild *Vigna* species were attempted. The maximum crossability was recorded between black gram and its wild progenitor, *V. silvestris* (11.76%) and *V. sahyadriana* (9.18%). The F1 hybrids were also produced from crosses involving black gram and *V. radiata*, *V. hainiana*, *V. sublobata*, and *V. stipulacea*. The crosses among *V. mungo* × *V. trilobata* and *V. mungo* × *V. trinervia* var. *trinervia* showed the least crossability with an unfilled pod. We propose that this study will help in the selection of highly compatible *Vigna* species for black gram improvement program.

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Breeding · Crossability · Crop wild relatives · Pollination · Resistance

16.1 Introduction

Black gram (*Vigna mungo* (L.) Hepper) is an important grain legume of South and Southeast Asian countries (Kaewwongwal et al. 2015). It is commonly known as urd, udid, urad, or mash. The plant is thought to be originated in Central Asia and distributed throughout the tropical areas of Asia and Africa. It is also cultivated as fodder crop in the USA and Australia (Jansen 2006). Black gram is the third important grain legume after gram and tur in India. The black gram was cultivated over an area of 5.03 million hectare, and the recorded productivity is at an average of 652 kg/ha in 2017–2018 (DAC and FW 2018). The major black gram production-contributing states are Andhra Pradesh, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, and Utter Pradesh.

The improvement of pulse production there is a need to produce new disease-resistant, high-yielding varieties. Interspecific hybridization in different species is an important method for plant breeding program, to incorporate the desirable trait/s from one species to another, to increase genetic variation and disease resistance, and to elucidate the taxonomic relationship among species. Due to narrow genetic base, the progress of *Vigna* breeding was comparatively slow in the past (Smartt 1979; Tomooka et al. 2011). The major goal of black gram breeding is to produce high-yielding varieties which can be accomplished by integration of different breeding objectives such as resistance to various biotic and abiotic stresses (Smartt 1979; Bisht et al. 2005; Singh et al. 2006; Kumar et al. 2011; Pratap et al. 2013). Several authors reported the integration of yield components coupled with resistance to insect pests and mung bean yellow mosaic virus (MYMV) (Fujii et al. 1989; Gill et al. 1983; Subramanian and Muthiah 2001; Srinives et al. 2007). The transfer of these characters to cultivated genotypes could be useful for the development of high-yielding varieties. The crop wild relatives (CWRs) constitute diverse genetic resources which are helpful to broaden the genetic base of crops. The utilization of CWR in crop improvement programs is found to be difficult due to the presence of crossability barriers between CWR and crop plants. However, CWRs are proved to be one of the major resources to introduce desired traits in crop varieties (Stalker 1980).

For establishment of successful hybrids, selection of appropriate genotypes and direction of crosses are prerequisites (Smartt 1979; Bharathi et al. 2006; Krishnasamy et al. 2008). There will be successful fertilization between diverse species within the same genus if both parents are compatible. The successful completion of a series of events following pollination requires a perfect mingle of coordination between genes and gene complexes of male and the female parents (Rieseberg and Willis 2007). Several authors have reported the crossability barriers in the Asiatic *Vigna* species during interspecific hybridization (Chavan et al. 1966; Biswas and Dana 1975; Ahn and Hartmann 1978a, b; Renganayaki 1985; Pandae

et al. 1990; Audilakshmi and Chandel 1990; Fatokun 1991; Dar et al. 1991; Ganeshram 1993; Subramanian and Muthiah 2001; Bharathi et al. 2006; Krishnasamy et al. 2008; Thiyagu et al. 2008; Pandiyan et al. 2010). The study aims to check the crossability between cultivated urdbean and wild *Vigna* species to create distant hybrids with improved yield and tolerance to various biotic and abiotic stresses.

16.2 Material and Methods

16.2.1 Plant Materials

Four cultivated and 11 wild *Vigna* species were obtained from the National Bureau of Plant Genetic Resource (NBPGR), New Delhi (Fig. 16.1, Table 16.1). All the *Vigna* species were maintained in the Botanical Garden at Department of Botany, Shivaji University, Kolhapur, and at insect-proof net house of NBPGR, New Delhi.

16.2.2 Pollination Technique

The hybridization experiments were conducted during the months of August to October. During the flowering period, the young flowers of the maternal parent were emasculated in the evening 4–6 pm, the day before blooming. The flowers 1 day prior to opening were chosen for emasculation. The remaining flowers and pods were removed from the plant. The selected flowers were emasculated, and the precautions were taken to avoid self-pollination. At the time of flower opening, the flowers were pollinated with the pollen of male parent. The pollen grains were dusted on stigma surface. The pollinated flowers were bagged and labeled with tags. The data on the number of flowers crossed, pod set, percentage of pod set, number of seeds obtained, number of seeds germinated, germination percentage, and number of plants raised were recorded for respective crosses (Table 16.2).

16.3 Result and Discussion

The crossability in *Vigna* species is one of the important experiments to establish the gene flow. The crossability studies estimate outcrossing frequencies, determining the possibility of gene transfer in closely related species and incorporation of traits from CWR to cultivated *Vigna*. In the present study, the attempts were made to obtain interspecific hybrids of the genus *Vigna*. The crossability study is a prerequisite for the successful establishment of distant hybrids (Umamaheswari 2002; Krishnasamy et al. 2008). An understanding of crossability relationship among the species had been helpful not only in choosing methods for producing F1 hybrids but also in tracing gene pools and the relationship among cultivated and wild species. The distant hybrids of *V. mungo* and its wild relatives were reported by many workers

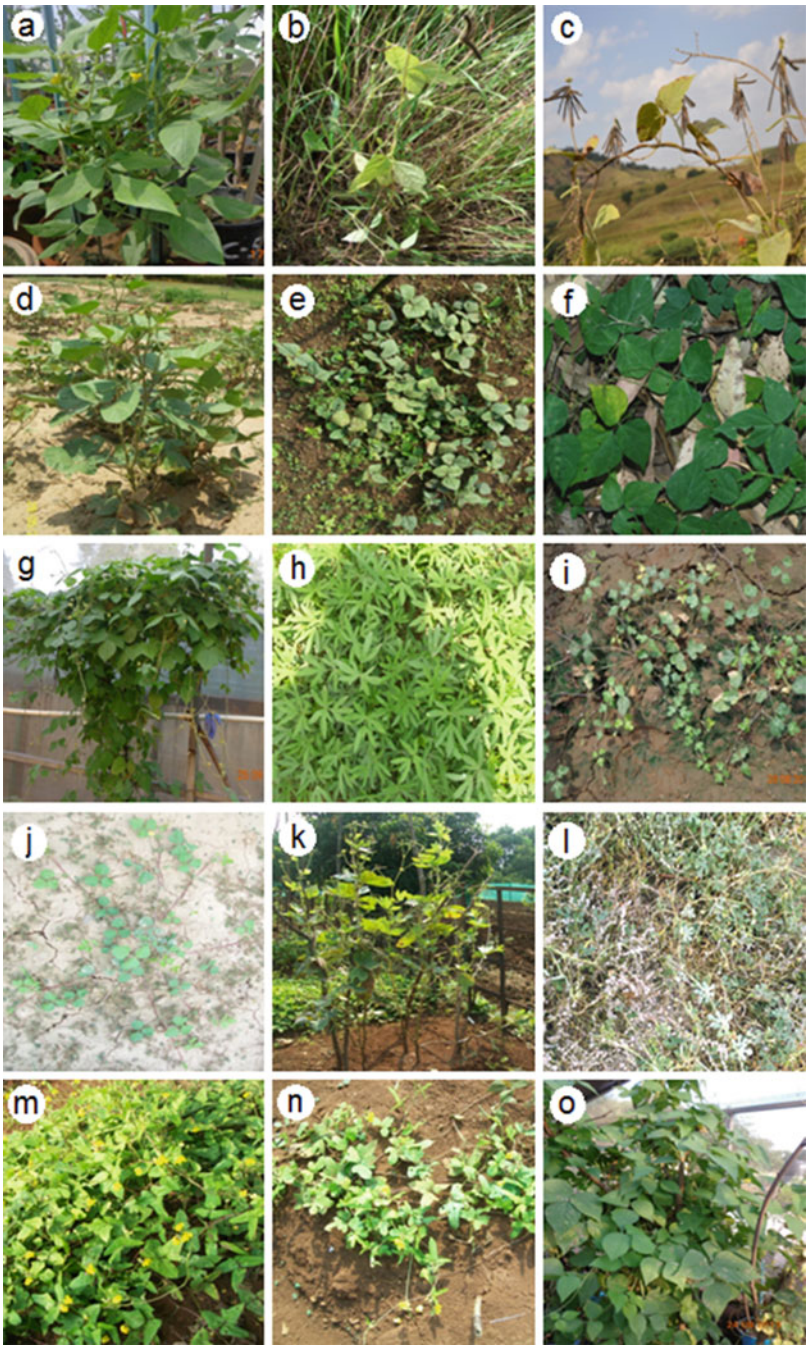

Fig. 16.1 Cultivated and wild *Vigna* species used in the present study: (**a**) *V. mungo*; (**b**) *V. silvestris*; (**c**) *V. sahyadriana*; (**d**) *V. radiata*; (**e**) *V. sublobata*; (**f**) *V. subramaniana*; (**g**) *V. hainiana*; (**h**) *V. aconitifolia*; (**i**) *V. trilobata*; (**j**) *V. stipulacea*; (**k**) *V. khandalensis*; (**l**) *V. indica*; (**m**) *V. trinervia* var. *trinervia*; (**n**) *V. trinervia* var. *bourneae*; (**o**) *V. umbellata*

Table 16.1 Details of *Vigna* species used in present study with their biological status and potential source

Section	Taxa	Wild/ cultivated	Source	Reference
Ceratotropis	*V. mungo*	Cultivated	Source of protein	Mahajan et al. (1988)
	V. silvestris	Wild	Drought tolerant	Iseki et al. (2018)
	V. sahyadriana	Wild	–	–
	V. radiata	Cultivated	Source of protein and essential amino acids like lysine and tryptophan	Zhu et al. (2018)
	V. sublobata	Wild	Wide genetic base	Tomooka et al. (2000)
			Resistance to bruchids	Miyagi et al. (2004)
			High methionine content	Babu et al. (1985)
			High photosynthetic efficiency and drought tolerance	Ignacimuthu and Babu (1985)
			MYMV resistance	Pal et al. (2000)
	V. subramaniana	Wild	MYMV resistance	Pal et al. (2000)
	V. hainiana	Wild	Resistance to bruchids	Babu et al. (1985)
			MYMV resistance	Babu et al. (1985)
Aconitifoliae	*V. aconitifolia*	Cultivated	Source of protein	Adsule (1996)
			Drought tolerance	Jain and Mehra (1980)
			Heat tolerance	Tomooka et al. (2001)
	V. trilobata	Wild	Tolerance to drought stress	Iseki et al. (2018)
	V. stipulacea	Wild	Resistance to powdery mildew	Tomooka et al. (2006)
			Tolerance to drought stress	Iseki et al. (2018)
	V. khandalensis	Wild	Tolerance to drought stress	Iseki et al. (2018)
	V. indica	Wild	Tolerance to drought stress	Iseki et al. (2018)
Angulares	*V. trinervia* var. *trinervia*	Wild	Resistance to bruchids	Srinives et al. (2007)
	V. trinervia var. *bourneae*	Wild	Resistance to bruchids	Srinives et al. (2007)
	V. umbellata	Cultivated	Resistance to bruchids	Tomooka et al. (2000)
			MYMV resistance	Pandiyan et al. (2010), Sudhaa et al. (2013)

(Renganayaki 1985; Pandae et al. 1990; Ganeshram 1993; Subramanian and Muthiah 2001).

In the present study, the crossing program involving 1163 crosses was attempted between urdbean and different wild *Vigna* species. The genus *Vigna* is a group of self-pollinated plants. The successful pod set was observed in the six interspecific crosses with *V. mungo* as ovule parent. The rate of flower shedding after emasculation and after pollination is very high. The shedding of flowers (30–40%) after emasculation in *V. mungo* was reported by Verma et al. (1991).

1. *V. mungo* × *V. silvestris*
   The cultivated urdbean variety UG-414 was crossed with wild *V. silvestris* (BB2656). The cross was fully compatible. Out of 119 flowering buds crossed, the 14 pods were set, and remaining buds were dropped after pollinations. The total of 57 viable seeds was obtained after the successful crossing. Among the 57 seeds of F1 hybrid of *V. mungo* × *V. silvestris*, 32 hybrid seeds (56.14%) were germinated and showed 43.75% hybrid lethality. The highest pod set and crossability were recorded in the cross *V. mungo* × *V. silvestris*. The highest pod set percentage, hybrid seed germination, and lowest hybrid lethality were observed in cross *V. mungo* × *V. silvestris*. The cross between *V. mungo* and its wild relative, *V. silvestris*, is successful. These observations support the view of Audilakshmi and Chandel (1990) and Pandae et al. (1990) that *V. silvestris* is the putative progenitor of *V. mungo*. Apart from our studies, successful crosses between *V. mungo* and *V. silvestris* were reported by Dwivedi and Singh (1985) and Audilakshmi and Chandel (1990). The cross was found fully compatible, and F1 generations were produced. The hybrids were successfully established showing that the post-zygotic isolating mechanism was not operating between these two species. Therefore, the hybrids obtained could serve as the superior base for urdbean improvement.
2. *V. mungo* × *V. sahyadriana*
   The *V. sahyadriana* is a recently known species of section *Ceratotropis* (Aitawade et al. 2012). Therefore, the cross was not attempted earlier. In this cross, 98 pollinations were attempted, out of which 9.18% cases, pods were formed, while 38 viable seeds were obtained. Among the 38 seeds of F1 hybrid of *V. mungo* × *V. sahyadriana*, 15 hybrid seeds (39.47%) were germinated and showed 60% hybrid lethality. The hybrids of *V. mungo* × *V. sahyadriana* were established successfully for the first time. Therefore, *V. sahyadriana* will be used for the improvement of urdbean varieties.
3. *V. mungo* × *V. radiata*
   The cross between two cultivated species, *V. mungo* and *V. radiata* (PDM11), showed 2.86% crossability. The 105 pollinations produced only three pods with 22 seeds, among which some seeds were shriveled at maturity. Among the 22 seeds of F1 hybrid of *V. mungo* × *V. radiata*, eight hybrid seeds (36.36%) were germinated and showed 37.5% hybrid breakdown. The F1 hybrids of this cross were unable to germinate due to sterile seeds (Subramanian 1980). The cross between *V. mungo* × *V. radiata* was reported to be unsuccessful due to

Table 16.2 Crossability studies in *V. mungo* (UG414; female parent—♀) with wild and cultivated Asiatic *Vigna* species

Sr. No.	Male parent (♂)		Number of flowers crossed	Pod set	Crossability (%)	Number of seeds obtained	Remark	Number of plants germinated	Germination (%)	Number of plants raised	Hybrid lethality (%)
	Species	Accession No.									
1	*V. silvestris*	BB2656	119	14	11.76	57	Viable seeds	32	56.14	18	43.75
2	*V. sahyadriana*	SUK156	98	9	9.18	38	Viable seeds	15	39.47	6	60
3	*V. radiata*	PDM11	105	3	2.86	22	Viable seeds/ shriveled seeds	8	36.36	3	62.5
4	*V. sublobata*	BB2680	74	3	4.05	12	Viable seeds/ shriveled seeds	3	25.00	1	66.66
5	*V. subramaniana*	SUK170	66	2	3.03	3	Shriveled seeds	1	33.33	0	100
6	*V. hainiana*	BYM13-01B	78	4	5.13	11	Viable seeds/ shriveled seeds	3	27.27	1	66.66
7	*V. aconitifolia*	SUK79	76	2	2.63	3	Viable seeds/ shriveled seeds	1	33.33	0	100
8	*V. trilobata*	SUK78	84	2	2.38	4	Shriveled seeds	1	25.00	0	100
9	*V. stipulacea*	SUK105	94	2	2.13	5	Shriveled seeds	1	20.00	1	0

(continued)

Table 16.2 (continued)

Sr. No.	Male parent (♂)		Number of flowers crossed	Pod set	Crossability (%)	Number of seeds obtained	Remark	Number of plants germinated	Germination (%)	Number of plants raised	Hybrid lethality (%)
	Species	Accession No.									
10	*V. khandalensis*	SUK22	75	3	4.00	4	Nonviable seeds	0	0.00	–	–
11	*V. indica*	SUK32	69	1	1.44	0	–	–	–	–	–
12	*V. umbellata*	RBL6	68	2	2.94	8	Viable seeds/ shriveled seeds	2	25.00	0	100
13	*V. trinervia* var. *trinervia*	SUK101	81	1	1.23	0	–	–	–	–	–
14	*V. trinervia* var. *bourneae*	SUK128	76	1	1.31	4	Nonviable seeds	0	0	–	–

embryo abortion (Miyazaki 1982; Bhanu et al. 2018). In the present study, out of 22 hybrid seeds, only eight seeds were able to germinate showing high percentage of hybrid lethality in cross between *V. mungo* × *V. radiata*. The success was limited as only few hybrid seedlings survived and produced normal fertile plants in the F1 generation (Verma and Singh 1987; Gupta et al. 2002).

4. *V. mungo* × *V. sublobata*
   The above cross involved the cultivated urdbean and wild putative progenitor form of mung bean, *V. sublobata* (BB2680). The cross *V. mungo* × *V. sublobata* showed 4.05% crossability. Out of 74 flowering buds of urdbean pollinated with *V. sublobata*, only three pods were produced. The three pods gave 12 seeds among which some seeds were shriveled. Among the 12 seeds of F1 hybrid of *V. mungo* × *V. sublobata*, three hybrid seeds (25%) were germinated and showed 66.66% hybrid lethality. The hybrids with partial fertility were noticed by several authors (Chavan et al. 1966; Biswas and Dana 1975). Audilakshmi and Chandel (1990) observed high sterility in the pollen of F1 hybrid of this cross. The crossability of *V. mungo* × *V. sublobata* showed existence of crossability barriers between *V. mungo* and a wild progenitor of mung bean, *V. sublobata*.
5. *V. mungo* × *V. subramaniana*
   The cross was not attempted due to the taxonomic uncertainty of *V. subramaniana*. The 66 flowering buds were pollinated with *V. subramaniana*, of which only two pods reached maturity. The seeds of were shriveled. The F1 hybrids were unable to reach maturity. The crossability of *V. mungo* × *V. subramaniana* showed existence of crossability barriers between *V. mungo* and *V. subramaniana*.
6. *V. mungo* × *V. hainiana*
   The *V. hainiana* is the wild putative progenitor of both urdbean and mung bean (Babu et al. 1985). The pod set among pollinated buds took place in 5.13% cases, while four pods (5.13%) reached maturity. The four pods were filled with 11 viable as well as shriveled seeds. Among the 11 seeds of F1 hybrid of *V. mungo* × *V. hainiana*, three hybrid seeds (27.27%) were germinated and showed 33.33% hybrid breakdown. The cultivated *V. mungo* crossed with *V. hainiana* resulted into hybrid F1 seeds with low hybrid lethality. From these results, it can be concluded that the *V. hainiana* is closely related to the urdbean which was also reported by Babu et al. (1985) and Vir et al. (2009).
7. *V. mungo* × *V. aconitifolia*
   The cross between two cultivated species, *V. mungo* and *V. aconitifolia* (SUK79), showed 2.63% crossability. The 76 pollinations produced two pods with only three seeds, among which some seeds were shriveled at maturity. Among the three seeds of F1 hybrid of *V. mungo* × *V. aconitifolia*, only one hybrid seed (36.36%) was germinated and showed 100% hybrid lethality.
8. *V. mungo* × *V. trilobata*
   The cross between *V. mungo* × *V. trilobata* showed 2.38% crossability. The 84 pollinations produced only two pods with only four seeds, among which three seeds were shriveled after maturity. Among the four seeds of F1 hybrid of

V. mungo × *V. trilobata*, one hybrid seed (25%) was germinated and 100% hybrid lethality. Also, least pollen fertility (4.5%) of F1 hybrids was observed by Dana (1966).

9. *V. mungo* × *V. stipulacea*
   The cross *V. mungo* × *V. stipulacea* involved the cultivated urdbean and wild underutilized species of section *Aconitifoliae*, *V. stipulacea* (SUK105). The cross *V. mungo* × *V. stipulacea* showed 2.13% crossability. Out of 94 flowering buds of urdbean pollinated with *V. stipulacea*, only two pods were successfully set. The five seeds that were produced in this cross produced shriveled seeds. Among the five seeds of F1 hybrid of *V. mungo* × *V. stipulacea*, only one hybrid seed (20%) was germinated and attained maturity.
10. *V. mungo* × *V. khandalensis*
    In this cross, an endemic *V. khandalensis* (Umdale et al. 2018) was used as a pollen parent. The 75 pollinated buds produced only three pods with only four seeds. The F1 seeds of *V. mungo* × *V. khandalensis* were shriveled after maturity. All four seeds of F1 hybrid were unable to germinate and showed 100% hybrid lethality.
11. *V. mungo* × *V. indica*
    The *V. indica* is a new species of section *Aconitifoliae* (Dixit et al. 2011). The crossability of *V. indica* and other *Vigna* species was not attempted earlier. In the present study, 69 pollinations were attempted, out of which only one pod was set. The pod of *V. mungo* × *V. indica* cross showed shriveled immature seeds. The result showed that the cross between *V. mungo* and *V. indica* was incompatible and crossability barriers exist between these two species.
12. *V. mungo* × *V. umbellata*
    The cross between *V. mungo* × *V. umbellata* showed 2.94% crossability. The 68 pollinations produced only two pods with eight seeds, among which some seeds were shriveled after maturity. Among the eight seeds of F1 hybrid of *V. mungo* × *V. umbellata*, two hybrid seeds (25%) were germinated but showed 100% lethality after germination. Also, least pollen fertility of F1 hybrids was observed by Biswas and Dana (1975). Verma et al. (1991), out of eight crosses involving *V. mungo* and *V. umbellata*, only two hybrids were successfully established in single cross, suggesting that the pod set depends on species chosen for hybridization. Similarly, the genotype also plays an important role in successful hybridization (Rashid et al. 1987; Verma and Singh 1987; Pal et al. 2005).
13. *V. mungo* × *V. trinervia* var. *trinervia*
    The cross *V. mungo* × *V. trinervia* var. *trinervia* showed 1.23% crossability. Out of 81 flowering buds of urdbean pollinated with *V. trinervia* var. *trinervia*, only one pod was successfully set. The pod of *V. mungo* × *V. trinervia* var. *trinervia* cross showed shriveled immature seeds. The result showed that there exists crossability barrier between these two species.
14. *V. mungo* × *V. trinervia* var. *bourneae*
    The cross *V. mungo* × *V. trinervia* var. *bourneae* showed 1.31% crossability. Out of 76 flowering buds of urdbean pollinated with *V. trinervia* var. *bourneae*,

Table 16.3 Crossability studies in *V. mungo* (UG414; female parent—♀) with wild and cultivated Asiatic *Vigna* species

Name of cross	Reference
V. mungo × *V. silvestris*	Reddy and Singh (1989), Audilakshmi and Chandel (1990), Pandae et al. (1990)
V. mungo × *V. sahyadriana*	Present study
V. mungo × *V. radiata*	Subramanian (1980), Gosal and Bajaj (1983a, b), Verma and Singh (1986), Gupta et al. (2002)
V. mungo × *V. sublobata*	Audilakshmi and Chandel (1990)
V. mungo × *V. subramaniana*	Present study
V. mungo × *V. hainiana*	Present study
V. mungo × *V. aconitifolia*	Present study
V. mungo × *V. trilobata*	Dana (1966)
V. mungo × *V. stipulacea*	Present study
V. mungo × *V. khandalensis*	Present study
V. mungo × *V. indica*	Present study
V. mungo × *V. umbellata*	Biswas and Dana (1975), Ahn and Hartmann (1978b), Bhanu et al. (2018)
V. mungo × *V. trinervia* var. *trinervia*	Present study
V. mungo × *V. trinervia* var. *bourneae*	Present study

only one pod was successfully set. The pod of *V. mungo* × *V. trinervia* var. *bourneae* cross showed four shriveled immature seeds. The seeds of F1 hybrid were unable to germinate. The lowest pod set and crossability were recorded by the cross *V. mungo* × *V. trinervia* var. *bourneae*. The result showed that there exists crossability barrier between these two species.

The cross between *V. mungo* and wild *Vigna* species *V. silvestris*, *V. sahyadriana*, *V. sublobata*, and *V. hainiana* gave hybrids. These results are in agreement with the reports of Gopinathan et al. (1986), Monika et al. (2001), Umamaheswari (2002), and Sidhu and Satija (2003) (Table 16.3). The cross between *V. mungo* and *V. umbellata* gave shriveled seeds showing low germination percentage and high hybrid lethality. A variety of isolating mechanisms were found to operate between *Vigna* species (Singh 1990). After pod set, in most cases embryo aborted before reaching maturity. In the present study, the crosses between the cultivated species and wild species of the same section gave successful hybrids which further confirmed the close relationships between species involved in the cross. However, the crosses between the cultivated species and wild species of other sections do not give fruitful results which confirm the sectional delineation of species of subgenus *Ceratotropis* (Baudoin and Marechal 1988).

The lower percentage of pod setting obtained from this study, even though a large number of flowers were pollinated, indicated that the level of cross compatibility depended on the varieties used and/or on environmental conditions. Present

investigation and earlier reports suggest that the failure of crosses could occur due to failure of germination of the pollen, failure of pollen tube growth on stigma, failure of fertilization, and embryo abortion (Ahn and Hartmann 1978a, b). Low pod set observed in the present study could be due to the operation of pre-fertilization barriers (Umamaheswari 2002). The common pre-fertilization barriers are found to occur in many distant crosses. These barriers are influenced by delayed pollen germination and growth of the pollen tube of one species on the stigma of another species (Monika et al. 2001; Krishnasamy et al. 2008). It was suggested to use techniques such as bud pollination, application of growth hormones, in vitro fertilization, protoplast fusion to promote pollen germination, and embryo rescue for successful establishment of hybrids (Waster and Marth 1949; Yasiri and Coyne 1964; Gosal and Bajaj 1983a, b; Chen et al. 1983; Verma et al. 1991).

16.4 Conclusion

The present investigation on interspecific hybridization involving urdbean and other *Vigna* species has revealed that the crossability, hybrid viability, and hybrid lethality in those hybrids. The higher crossability in case of *V. silvestris* and *V. sahyadriana* suggests that these two species are closely related to urdbean. Further, we suggest the use of bud pollination, application of growth hormones, and in vitro fertilization; protoplast fusion to promote pollen germination and also embryo rescue could be utilized for the production of interspecific hybrids.

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References

Adsule RN (1996) Moth bean (*Vigna aconitifolia* (Jacq.) Marechal). In: Food and feed from legumes and oilseeds. Springer, New York, NY, pp 203–205

Ahn CS, Hartmann RW (1978a) Interspecific hybridization between mungbean *Vigna radiata* L. Wilczek. and adzuki bean *Vigna angularis* Wild. Ohwi and Ohashi. J Am Soc Hortic Sci 103:435–438

Ahn CS, Hartmann RW (1978b) Interspecific hybridization among four species of the genus *Vigna* Savi. In: Report of first international mungbean symposium. Univ. Philippines, Los Banios, pp 240–246

Aitawade MM, Sutar SP, Rao SR, Malik SK, Yadav SR, Bhat KV (2012) Section *Ceratotropis* of subgenus *Ceratotropis* of *Vigna* (Leguminosae-Papilionoideae) in India with a new species from Northern Western Ghats. Rheedea 22:20–27

Audilakshmi B, Chandel KPS (1990) Interspecific hybridization and crossability studies among cultivated and closely related wild *Vigna* species in Indian Gene Centre. Indian J Plant Genet Resour 3:46–60

Babu CR, Sharma SK, Johri BM (1985) Leguminosae-Papilionoideae: Tribe-Phaseoleae. Bull Botan Surv India 27:1–28

Baudoin JP, Marechal R (1988) Taxonomy and evolution of the genus *Vigna*: mungbean. In: Proceedings of second international symposium. AVRDC, Bangkok, Shanhua, Taiwan

Bhanu AN, Singh MN, Srivastava K (2018) Crossability studies of interspecific hybridization among *Vigna* species. Biomed J Sci Tech Res 2:1–7

Bharathi A, Vijay Selvraj KS, Veerabadhiran P, Subba Lakshami B (2006) Crossability barriers in mungbean (*Vigna radiata* L. Wilczek): with its wild relatives. Ind J Crop Sci 1:120–124

Bisht IS, Bhat KV, Lakhanpaul S (2005) Diversity and genetic resources of wild *Vigna* species in India. Genet Resour Crop Evol 52:53–68

Biswas MR, Dana S (1975) Black gram × rice bean cross. Cytologia 40:787–795

Chavan VM, Patil GD, Bhapkar DG (1966) Improvement of cultivated *Phaseolus* species-need for interspecific hybridization. Indian J Genet Plant Breed 26:152–154

Chen NC, Baker RL, Honma S (1983) Interspecific crossability among four species of *Vigna* food legumes. Euphytica 32:925–937

DAC & FW (2018) Pulses revolution - from food to nutritional security. Crops Division, Government of India Ministry of Agriculture & Farmers Welfare Department of Agriculture, Cooperation & Farmers Welfare, New Delhi

Dana S (1966) Interspecific hybrid between *Phaseolus mungo* L. × *P. trilobus* Ait. J Cytol Cytogenet 1:61–66

Dar GM, Verma MM, Gosal SS, Brar JS (1991) Characterization of some interspecific hybrids and amphiploids in *Vigna*. In: Sharma B, Mehra RB (eds) Golden jubilee celebration symposium on grain legumes. Indian Society of Genetics and Plant Breeding, New Delhi, pp 73–78

Dixit TM, Sutar SP, Yadav SR, Bhat KV, Rao SR (2011) Vigna indica, a new name for Vigna trilobata var. pusilla and a note on section Aconitifoliae in India. Rheedea 21:1–7

Dwivedi S, Singh DP (1985) Inheritance of resistance to yellow mosaic virus in a wide cross of black gram (*V. mungo* (L.) Hepper). Z Pflanzenzuchtg 95:281–284

Fatokun CA (1991) Wide hybridization in cowpea: problems and prospects. Euphytica 54:137–140

Fujii K, Ishimoto M, Kitamura K (1989) Pattern of resistance to bean weevil Bruchidae in *Vigna radiata-mungo-sublobata* complex inform the breeding of new resistant varieties. Appl Entomol Zool 24:126–132

Ganeshram S (1993) Evaluation of some genotypes interspecific hybrids and derivatives of greengram (*V. radiata* (L.) Wilczek) × Black gram (*Vigna mungo* (L.) Hepper) crosses. Dissertation, Tamil Nadu Agricultural University, Coimbatore

Gill AS, Verma MM, Dhaliwal HS, Sandhu TS (1983) Interspecific transfer of resistance to mungbean yellow mosaic virus from *Vigna mungo* to *V. radiata*. Curr Sci 52:31–33

Gopinathan MC, Babu CR, Shivanna KR (1986) Interspecific hybridization between rice bean (*Vigna umbellata*) and its wild relative (*V. minima*): fertility - sterility relationships. Euphytica 35:1017–1022

Gosal SS, Bajaj YPS (1983a) In vitro hybridization in an incompatible cross-black gram × green gram. Curr Sci 52:556–557

Gosal SS, Bajaj YPS (1983b) Interspecific hybridization between *Vigna mungo* and *Vigna radiata* through embryo culture. Euphytica 32:129–137

Gupta VP, Plaha P, Rathore PK (2002) Partially fertile interspecific hybrids between a black gram × green gram derivative and adzuki bean. Plant Breed 121:182–183

Ignacimuthu S, Babu CR (1985) Significance of seed coat pattern in *Vigna radiata* var. *sublobata*. Proc Indian Acad Sci 94:561–566

Iseki K, Takahashi Y, Muto C, Naito K, Tomooka N (2018) Diversity of drought tolerance in the genus *Vigna*. Front Plant Sci 9:729

Jain HK, Mehra KL (1980) Evolution, adaptation, relationships and use of the species of *Vigna* cultivated in India. In: Summerfield RJ, Bunting AH (eds) Advances in legume science. Volume 1. Proceedings of the International Legume Conference, Kew, UK, pp 459–468

Jansen PCM (2006) *Vigna mungo* (L.) Hepper. In: Brink M, Belay G (eds) Record from protabase. PROTA (Plant Resources of Tropical Africa), Wageningen

Kaewwongwal A, Kongjaimum A, Somta P, Chankaew S, Tarikar Y, Srinivas P (2015) Genetic diversity of the black gram [*Vigna mungo* (L.) Hepper] gene pool as revealed by SSR markers. Breed Sci 65:127–137

Krishnasamy T, Palaniappan J, Nagasamy N (2008) Pollen pistil interaction in interspecific crosses of *Vigna* spp. Cytologia 73:251–257

Kumar S, Imtiaz M, Gupta S, Pratap A (2011) Distant hybridization and alien gene introgression. In: Pratap A, Kumar J (eds) Biology and breeding of food legumes. CABI, Oxfordshire, pp 81–110

Mahajan R, Malhotra SP, Singh R (1988) Characterization of seed storage proteins of urdbean (*Vigna mungo*). Plant Food Hum Nutr 38:163

Miyagi M, Humphry M, Ma ZY, Lambrides CJ, Bateson M, Liu CJ (2004) Construction of bacterial artificial chromosome libraries and their application in developing PCR-based markers closely linked to a major locus conditioning bruchid resistance in mungbean (*Vigna radiata* L. Wilczek). Theor Appl Genet 110:151–156

Miyazaki S (1982) Classification and phylogenetic relationships of the *Vigna radiata-mungo-sublobata* complex. Bull Natl Inst Agric Sci Ser D 33:1–61

Monika K, Singh P, Sareen PK (2001) Cytogenetic studies in mungbean-ricebean hybrids. J Cytol Genet 2:13–16

Pal SS, Singh JJ, Singh I (2000) Transfer of YMV resistance in cultivar SML32 of *Vigna radiata* from other related *Vigna* species. Plant Dis Res 15:67–69

Pal SS, Sandhu JS, Singh I (2005) Exploitation of genetic variability in interspecific cross between *Vigna mungo* × *V. umbellata*. Indian J Pulses Res 18:9–11

Pandae KS, Raghavanshi S, Prakesh P (1990) Induced high yielding amphiploid of *Vigna radiata* × *Vigna mungo*. Cytologia 55:249–253

Pandiyan M, Senthil N, Ramamoorthi N, Muthiah AR, Tomooka N, Duncan V, Jayaraj T (2010) Interspecific hybridization of *Vigna radiata* × 13 wild *Vigna* species for developing MYMV donar. Electron J Plant Breed 1:600–610

Pratap A, Malviya N, Tomar R, Gupta DS, Kumar J (2013) *Vigna*. In: Pratap A, Kumar J (eds) Alien gene transfer in crop plants. Volume 2. Achievements and impacts. Springer, New York, NY, pp 163–189

Rashid KA, Smart JP, Haq N (1987) Hybridization in genus *Vigna*. In: Proc 2nd Int Symp Bangkok, Thailand, pp 205–214

Reddy KR, Singh DP (1989) Transgressive segregation in the wide and varietal crosses of black gram (*Vigna mungo* L. Hepper). Indian J Genet Plant Breed 49:131–134

Renganayaki K (1985) Studies on genetic differentiation between three species of *Vigna* Savi. Dissertation, Tamil Nadu Agricultural University, Coimbatore

Rieseberg LH, Willis JH (2007) Plant speciation. Science 317:910–914

Sidhu N, Satija CK (2003) Cytomorphological characterization of amphidiploids of *Vigna radiata* × *V. umbellata*. Crop Improv 30:25–32

Singh DP (1990) Distant hybridization in genus *Vigna*: a review. Indian J Genet Plant Breed 50:268–276

Singh M, Bisht IS, Sardana S, Gautam NK, Hussain Z, Singh BB, Dwivedi NK (2006) Asiatic *Vigna*. In: Dhillon BS, Saxena S, Agrawal A, Tyagi RK (eds) Plant genetic resources: foodgrain crops. Narosa Publishing House, New Delhi

Smartt J (1979) Interspecific hybridization in grain legumes- a review. Econ Bot 33:329–337

Srinives P, Somta P, Somta C (2007) Genetics and breeding of resistance to bruchids (*Callosobruchus* spp.) in *Vigna* crops: a review. NU Sci J 4:01–17

Stalker HT (1980) Utilization of wild species for crop improvement. Adv Agron 33:111–147

Subramanian D (1980) Interspecific hybridization in *Vigna*. Indian J Genet Plant Breed 40:437–438

Subramanian A, Muthiah AR (2001) Interspecific hybridization between *Vigna radiata* (L.) Wilczek and *V. mungo* (L.) Hepper. Legum Res 24:154–158

Sudhaa M, Anusuya P, Nawkar GM, Karthikeyan A, Nagarajana P, Raveendrana M, Senthil N, Pandiyan M, Angappan K, Balasubramanian P (2013) Molecular studies on mungbean (*Vigna radiata* (L.) Wilczek) and ricebean (*Vigna umbellata* (Thunb.)) interspecific hybridisation for Mungbean yellow mosaic virus resistance and development of species-specific SCAR marker for ricebean. Arch Phytopathol Plant Protect 46:503–517

Thiyagu K, Jayamani P, Nadarajan N (2008) Pollen pistil interaction in inter-specific crosses of *Vigna* species. Cytologia 73:251–257

Tomooka N, Kashiwaba K, Vaughan D, Ishimoto M, Egawa Y (2000) The effectiveness of evaluating wild species, searching for sources of resistance to bruchid beetle in the genus *Vigna* subspecies *Caratotropis*. Euphytica 115:27–41

Tomooka N, Vaughan DA, Xu RQ, Kashiwaba K, Kaga A (2001) Japanese native *Vigna* genetic resources. Jpn Agric Res Q 35:1–9

Tomooka N, Kaga A, Vaughan DA (2006) The Asian *Vigna* (*Vigna* subgenus *Ceratotropis*) biodiversity and evolution. In: Sharma AK, Sharma A (eds) Plant genome: biodiversity and evolution. Part C Phanerogams (Angiosperms-Dicotyledons), vol 1. Science Publishers, Enfield, NJ, pp 87–126

Tomooka N, Kaga A, Isemura T, Vaughan D (2011) Chapter 15. *Vigna*. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, legume crops and forages. Springer, Berlin, pp 291–311

Umamaheswari D (2002) Wide hybridization in the genus *Vigna*. Dissertation, Tamil Nadu Agricultural University (TNAU), Coimbatore

Umdale SD, Chavan JJ, Ahire ML, Kshirsagar PR, Gaikwad NB, Bhat KV (2018) *Vigna khandalensis* (Santapau) Raghavan et Wadhwa: a promising underutilized, wild, endemic legume of the Northern Western Ghats, India. Genet Resour Crop Evol 65:1795–1807

Verma RPS, Singh DP (1986) Problems and prospects of interspecific hybridization involving green gram and black gram. Indian J Agric Sci 56:535–537

Verma RPS, Singh DP (1987) Problems and prospects of interspecific hybridization involving greengram and black gram. Indian J Genet 37:130–132

Verma MM, Dar GM, Brar JS, Gosal SS (1991) Wide hybridization in *Vigna*. In: Golden Jubilee Calibrations: Symposium on Grain Legumes, Indian society of Genetics and Plant Breeding, Indian Agricultural Research Institute, New Delhi, pp 9–11

Vir R, Bhat KV, Lakhanpaul S (2009) Analysis of population substructure, genetic differentiation and phylogenetic relationship among selected Asiatic *Vigna* Savi species. Genet Resour Crop Evol 56:783–795

Waster RE, Marth PC (1949) Some effect of a growth regulator mixture in controlled cross pollination of lima beans. Proc Am Soc Hort Sci 53:315–318

Yasiri SAA, Coyne DP (1964) Effect of growth regulators on pod abscission and embryo abortion. Crop Sci 4:431–433

Zhu YS, Shuai S, FitzGerald R (2018) Mung bean proteins and peptides: nutritional, functional and bioactive properties. Food Nutr Res 62:12

Technological Advances in Agronomic Practices of Seed Processing, Storage, and Pest Management: An Update

17

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Abstract

The seed is a rudimentary embryonic form of a plant present in a capsulated form that has an ability to grow up into a mature plant. Also the seed is the basic unit of production in agriculture, and therefore, seed health is an important factor in agriculture. Moreover, seeds with superior potency make contributions almost to 30% of the excessive harvest. However, factors such as seed characteristics, pests, microbial contamination, geographical locations, moisture, temperature, and storage have a direct influence on seed viability, germinations, and quality. Therefore, it is important to monitor the series of the process starting from seed collection to seed testing and storage. In the absence of high-quality seeds, farmers continue to use their own seeds. Ideally, this retained produce cannot be substituted for high-quality seeds because it lacks genetic vigour and has poor germination. Thus, the availability of certified seeds of right varieties has become crucial. In this chapter, we elaborate about current agronomic practices, technological advancements in seed processing, treatment, qualitative parameters necessary for a healthy seed, seed storage practices, and testing parameters for certified seeds.

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Keywords

Seed quality · Genetic purity · Development · Seed composition · Physical purity · Seed moisture · Seed germination

17.1 Introduction

Seeds are the origin of the plant kingdom which influences the world's food needs. The world's hunger is fulfilled via Angiospermae (the flowering plants), a class of vegetation which is an essential source of food for animals and other dwelling organisms on earth. For this reason, the production of fine seed and ensuring its quality are essential. The true quality seeds have the significant capability of increased on-farm productivity and by the way to improve food security. Seeds with superior potency make contributions almost to 30% excessive harvest.

While referring to storage life, it is the mean of length of time required for the seed to survive (Roberts and Feast 1972). Not all seeds or varieties in a genetic group are intended to survive for the specific time period under specific conditions. The records of the Ministry of Agriculture reveal that food grains' wastage in India is worth around INR 44,000 Crore every year. For this reason, the advanced practices in seed management are demanding to boom shelf-life of the seeds. The basic difference between raising a crop for seed and for its produce (seed or any other economic part) is with reference to how the final product will be used. The crop raised for its seed should, therefore, be done with the utmost care, as the product will be used for raising the next generation. Moreover, the yield of the crop very much depends on the health of the seed.

17.2 Seed Development

The seed development process begins within the flower. The male part of the flower is called *stamen*, and the female part is called *pistil*. In certain plants, the flower may contain both the pistil and stamen (e.g. beans); however, in others, they may occur in different flowers (as in corn). Fertilization takes place when male gametes fuse with the female egg in the ovule and the ovules emerge into the seeds. In *self-pollinated* plants such as cotton and wheat, pollen fertilizes the stigma of the same flower. In *cross-pollinated* plants, such as corn, alfalfa, carrots, and onions, the pollen grains pollinate the flowers of other plants but not the flowers of the plant from which they originate.

Early developmental stages of strong plants depend on the condition of growth. The quality of the seed is primarily dependent on environmental conditions and harvesting method. For a specific period of time, seeds remain viable, ranging from a few weeks to many years. Lotus seeds have the maximum viability of 1000 years. In many plants, the freshly shed seeds become dormant due to the presence of growth inhibitors, the deficiency of food, minerals, and enzymes, etc.

17.3 Chemical Composition of Seed

Seeds possess different types of chemicals and are used as food reserves for the next generation of plants. Therefore it serves as a vital part of our food supply. Seeds possess three major classes of nutrients: proteins, carbohydrates, and lipids. Certain seed oils including peanut oil are particularly well suited to cooking. Others such as jojoba have good lubrication property. Seed oils are also used for making soap, printing inks, paint, etc. The levels of seed storage reserves can be altered through careful breeding and selection. It may be possible to breed a 'designer' seed plant with the required chemical composition to meet human nutritional and industrial needs using biotechnological approach.

17.4 Basic Types of Seed

Based on the *cotyledon* number *of* the seed, a seed is classified into two basic types: *monocots* contain one cotyledon (e.g. grasses), and *dicots* contain two cotyledons (e.g. leguminous plants). Seeds are made up of three basic structures, i.e. seed covering (*seed coat*), embryonic root (*radicle* and *shoot* or *plumulei*), and supporting tissues (the *cotyledons* and *endosperm*).

Lately, synthetic seeds are the scenarios evolving from biotechnology innovations and plant tissue culture. These synthetic seeds are literally the 'seeds' that are not formed by sexual fertilization which is essential for the normal seed. Such seeds contain essentially embryos and are genetically uniform which provides the growers greater economic yields and greater harvests (Copeland and McDonald 1999).

17.5 Seed Quality

Seed quality includes genetic purity and physical purity (Tripp et al. 1997).

17.5.1 Genetic Purity

Genetic purity refers to the trueness to type. A seed must be pure and stable with the same characteristics that are used for varietal distinction; otherwise it would be simple to 'create' new varieties without any effort in breeding to generate a more productive variety. Genetic purity should be accomplished and preserved for higher agronomic performance and to ensure quality in productivity. Morphological comparison has been traditionally used for evaluating genetic purity. Therefore, genetic purity has a direct influence on crop yield and revenue. Because the genetic basis of most morphological traits remains unknown, morphological characters provide, at best, an indirect means of assessing genetic purity. There now exist a variety of laboratory-based technologies (e.g. biochemical characterization using seed storage

protein or isozyme polymorphisms and/or DNA polymorphisms) for distinguishing and identifying varieties de novo. These molecular and biochemical methods can measure genetic distances and are well suited for determination of genetic conformity between varieties.

17.5.2 Physical Purity

The physical quality refers to the absence of inert matter, seed debris, insect-damaged seed, and diseased seeds. The seeds should have uniform size, weight, and colour and should be free from stones, leaves, twigs, flowers, other crop seeds, debris, and dust. It also should be devoid of discoloured, damaged, shrivelled, mottled, moulded, and empty seeds. The seed should be easily identifiable as a type of the specific category. Physical purity could be obtained by proper cleaning and grading of seeds. The seedling plant from the seed should resemble its mother in all aspects. Physiological quality of the seed includes components like germination capacity, viability, vigour, and characteristics related to dormancy.

The quality of the seed depends very much on its health. The seed should not be infested with insect pests or infected with fungi as these will reduce the physiological quality and physical quality of the seed for long-term storage. Seed health also includes the deterioration status of the seed which is expressed through low vigour status of the seed. The seed quality characters are directly influenced by the seed health and warrant their reliability for the production of elite seedlings (Danesh et al. 2014). Seed health is a very essential sign for measuring seed quality. Seeds may act as a spreading agent of pathogens to crop. Moreover, pathogen-infected seeds result in low or complete loss of germination or abnormal seedling development and low vigour that leads to quality and yield loss (Bishaw et al. 2013).

17.6 Qualitative Parameters Determining the Seed Quality

17.6.1 Seed Moisture

The seed moisture is the most critical factor in maintaining seed viability during storage. Therefore, the seed must be dried to safe moisture content (Tatipata 2009).

17.6.2 Seed Germination

Germination is a complex process by which the seed is able to give rise to a typical seedling when planted under normal sowing conditions. The liveliness of a seed is referred to as viability. The quantity of liveliness for production of good seedling or the potential of seed for the production of seedling with regular root and shoot under favourable condition is called germinability. The primary component in crop cultivation is that seed needs to germinate. A seed without viability is no more seed and

in no manner can be used for crop production. Germination of seed must be as such which can make a certain establishment of optimum plant stand for desirable production (Baskin and Baskin 2004). So germination of the seed and the primary growth of seedling are the most important stages for any plant species to establish (Vibhuti et al. 2015).

17.6.3 Viability

A viable seed is defined to possess the capability to germinate under favourable conditions, whereas a non-viable seed does not have the ability to germinate when the favourable conditions persist, even after treating the seed in order to remove the dormancy (Cheah and Osborne 1978). As the seeds produce crops, the viability refers to the measurement of the fitness of the seed to yield the desired crop (Pratap and Sharma 2010).

17.6.4 Vigour

Vigour of a seed can be described as the state of active health and natural strength after planting, which allows the continuation of germination swiftly under an extensive range of growing conditions. It has also been described as the capability for speedy uniform germination and fast growing of seedling under preferred field conditions (Rajjou et al. 2004). Seed vigour is the energy or stamina of the seed in generating elite seedling. It is the sum total of all seed parameters that allows its regeneration under any given conditions. Therefore, seed vigour determines the extent of the overall performance of seed at some point of germination and seedling emergence.

The seed which carries good qualitative traits properly at sowing is termed as excellent seed, and based on the degree of overall performance in the production of elite seedling, it is categorized as high-, medium-, and low-vigour seed. The difference in seed vigour may be expressed as the rate of emergence, uniformity of emergence, and loss of seed germination. For this reason, it is understood that all viable seeds are no longer be germinable; however, all germinable seed could be viable. Similarly, all vigorous seeds could be germinable, but all germinable seeds need not be vigorous. Physiologically high-quality seed may be achieved through the right choice of the seed (matured seed) used for sowing and by means of being concerned for high-quality characters during drying and storage. Hence selection of the seed primarily based on seed vigour is vital for raising ideal plantation (Ambika et al. 2014).

The subsequent conceptual parameters have emerged which make clear the meaning of vigour in terms of seed, seedling, and plant's overall performance:

- Speed of germination
- Uniformity of germination and plant development under non-uniform condition

- Capability to emerge via crusted soil
- Germination and seedling emergence from cold, moist, and pathogen
- Normal morphological development of seedlings
- Storability under optimum or damaging conditions

17.6.5 Dormancy

The viable seed that lacks the ability to germinate at appropriate conditions is referred to as dormancy. The capability of the seeds to postpone their own germination until the availability of suitable time and condition is one of the important survival mechanisms. Dormancy of the seed is the approach through which plants survive and adapt to their surroundings (Baskin and Baskin 2004).

17.7 Current Agronomic Practices

17.7.1 Site Preparation

Agricultural site preparation is a very important aspect of agronomic practices. The crop for seed purposes should be raised on the land which has the right type of soil suitable for the crop to be raised. For example, it will not be advisable to raise rice crop on sandy soil, as the soil should have sufficient moisture retention ability to grow. Similarly, we can't grow millet on black cotton soil, as waterlogging is injurious to millet. Choosing a suitable site is very important before planting. A fairly level land helps in reducing the cost of land preparation and preventing soil erosion (Karlen et al. 2003). The site preparation process is categorized into three types, namely, pre-planting, planting, and post-planting operations. As the soil is the medium for plants' growth, good soil results in good yield. Therefore, the choice of the soil types is essentially considered while choosing the farm site or location.

17.7.2 Selection of Crop Variety

Identification and maintenance of crop diversity are recognized as a global priority. Only varieties recommended for the area of interest should only be cultivated to achieve highly potent good quality seed. It is also ideal to cultivate only varieties whose demand is ensured; otherwise, the products will not be lifted (Abay et al. 2008).

17.7.3 Germination Improvisation Techniques

To improve the germination rate of the seeds, various pretreatment methods are currently in practice and listed below.

17.7.3.1 Hydration of Seeds

Seed hydration refers to the combined process that includes hydration and redrying of seeds which permits routine handling. Seed hydration results in the increased rate of germination, elevated uniform emergence, germination on various environments such as adverse weather and soil conditions, and improved seedling vigour and growth (Bradford 1986). Before seedling, the seeds are soaked in water to enhance the rate of germination. In 1981, Gray introduced gel for this purpose that results in pre-germination at 20 °C, and this practice is known as fluid drilling. Gel protects the germinated seed as well as maintains the moisture content in seeds.

17.7.3.2 Hydropriming

Hydropriming is one of the hydration processes which differs from normal seed hydration process involving soaking of the seed in water along with re-drying until the initial moisture they had. In this technique, no chemicals are used. For the seeds which are to be planted in the condition of stress like salinity and drought-affected environments, hydropriming treatment can be used (Janmohammadi et al. 2008).

17.7.3.3 Osmopriming

This type of seed hydration is to control or regulate the uptake level of the water as well as prevent radical protrusion which is achieved under controlled aerated conditions. It uses (1) low water potential osmotic solution, e.g. polyethylene glycol, and (2) solution of salts such as potassium chloride (KCl), potassium nitrate (KNO_3), magnesium sulphate ($MgSO_4$), calcium chloride ($CaCl_2$), sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), and potassium phosphate (K_3PO_4) which are most commonly used for osmopriming.

17.7.3.4 Hormonal Priming

Two compounds (1) indole-3-butyric acid (IBA), which belongs to auxin, and (2) kinetin, which belongs to cytokinin, are commonly used plant growth hormones. Among them, cytokinins play a vital role that begins from seed germination to biological ageing or senescence (Riefler et al. 2006). Priming with these plant growth hormones is called hormonal priming, e.g. the optimum concentration of cytokinins increases crop's germination, yield, and growth.

17.7.3.5 Vernalization

Vernalization is a method of artificial exposure of the seeds to low temperatures in order to stimulate germination. By mimicking the cold temperate zone, germination can be induced to occur earlier than normal or in warm climates that lack the requisite seasonal chilling. This is a golden method for enhancing the germination of seeds.

17.7.4 Thinning

It is a general technique in the agronomical field which is the elimination of any parts of the plant without cutting the entire plant, to create a space for the cultivation of other crops. The main target of the method is to rearrange the crop or trees for their growth potential with preferred shape and organization. Five different approaches were in use: (1) low, or thinning from below; (2) crown, or thinning from above; (3) selection, or diameter-limit thinning; (4) free thinning; and (5) mechanical thinning (Nyland 1996; Smith et al. 1997). These thinning processes are preferably carried out within 2 weeks after the soil is moistened.

17.7.5 Fertilization and Irrigations

The poor crop will result in developing seed with poor quality and affects the performance of the crop raised from it. The usage of chemical fertilizers influences plant growth at a considerable rate (Kahrl et al. 2010).

High seed rates increase plant population and yield. The report of Malik et al. (2009) estimated that delay in sowing automatically decreases the germination rate (m^{-2}), grain numbers ($spike^{-1}$), and weight; however the high seed rate also didn't influence the yield of the grain. Therefore, the optimum recommended seed rate should be followed. Higher or lower seed rates both reduce the quality of the produce. Moreover, the seedling rate strongly influences the intra- and interplant competition for environmental resources, i.e. light, water, and nutrients during crop development.

17.7.6 Disease and Pest Control Measures

Pesticides and insecticides are widely used to control the pest that causes damage to the crops (Horne and Page 2008). The beneficial microbes are used as a biocontrol agent to minimize the diseases in plants (Huffaker 1984). According to Barzman et al. (2015), there are eight principles in disease and pest control measures that have been implied by farmers worldwide. They are the following:

17.7.6.1 Prevention and Suppression

The prevention and/or suppression of harmful organisms should be achieved or supported among other options especially by:

- Crop rotation
- Use of adequate cultivation techniques (e.g. stale seedbed technique, sowing dates and densities, undersowing, conservation tillage, pruning, and direct sowing)
- Use, where appropriate, of resistant/tolerant cultivars and standard/certified seed and planting material

- Use of balanced fertilization, liming, and irrigation/drainage practices
- Preventing the spreading of harmful organisms by hygiene measures (e.g. by regular cleansing of machinery and equipment)
- Protection and enhancement of important beneficial organisms, e.g. by adequate plant protection measures or the utilization of ecological infrastructures inside and outside production sites

Monitoring

Harmful organisms must be monitored by adequate methods and tools available. Such adequate tools should include observations in the field as well as testing at laboratory, forecasting and early diagnosis systems, as well as the use of the advice from professionally qualified advisors.

Decision-Making

Based on the results of the monitoring, the professional user has to decide whether and when to apply plant protection measures. Robust and scientifically sound threshold values are essential components for decision-making. For harmful organisms, threshold levels defined for the region, specific areas, crops, and particular climatic conditions must be taken into account before treatments.

Non-chemical Methods

Sustainable biological, physical, and other non-chemical methods must be preferred to chemical methods if they provide satisfactory pest control.

Pesticide Selection

The pesticides applied shall be as specific as possible for the target and shall have the least side effects on human health, non-target organisms, and the environment.

Reduced Pesticide Use

The professional user should keep the use of pesticides and other forms of intervention to levels that are necessary, e.g. by reduced doses, reduced application frequency, or partial applications, considering that the level of risk in vegetation is acceptable and they do not increase the risk for development of resistance in populations of harmful organisms.

Anti-Resistance Strategies

Where the risk of resistance against a plant protection measure is known and where the level of harmful organisms requires repeated application of pesticides to the crops, available anti-resistance strategies should be applied to maintain the effectiveness of the products. This may include the use of multiple pesticides with different modes of action.

Evaluation
Based on the records on the use of pesticides and on the monitoring of harmful organisms, the professional user should check the success of the applied plant protection measures.

17.7.7 Roguing

This is an important step in seed production. Roguing means removal of unwanted weeds and other crop plants in a seed production plot to control the chances of cross-pollination occurrence which may deteriorate the genetic purity of the succeeding crop. Therefore, roguing should be done at regular intervals starting at a very early stage of the crop which is very essential before the flowering starts.

17.7.8 Emasculation

Certain crops require special operations such as emasculation for raising the hybrid seed crop. Emasculation is the removal of male parts from a hermaphrodite crop. This is essential for the hybrid seed production programme, as it makes convenient the pollination process using the desired male parent. The mechanism of genetic male sterility is commonly used in maize, pearl millet, and sorghum to name a few (Matzk and Mahn 1994).

17.7.9 Harvesting and Threshing

It is a crucial step in the seed production process. Harvesting should be done only when the crop is fully ripe. Early harvesting is likely to give the product with lower reliability. In many cases, early harvesting results in unripe seeds which may not germinate. Moreover, threshing should be done using appropriate methods. Improper threshing may result in broken seeds. Similarly, the threshing should be undertaken in a clean environment, because threshing is the stage where maximum contamination and mixture with other seeds are possible. Therefore the threshing equipment should be thoroughly cleaned (Warghane and Rajkumar 2017).

17.7.10 Seed Processing

In agriculture, the term seed processing includes cleaning, drying, seed treatment, packaging, and storage. It is the process of eliminating the unwanted parts of the crops like weeds, seeds of other crops, and inactive materials of the plant. After this process, the seeds become uniform in size and are allowed to have enough moisture. Finally, the seeds are tested and sealed. Thus by seed processing, the physical quality

of the seed is improved. Seed processing methods include debearding, winnowing, grading, treatment with appropriate plant protection chemicals, etc.

17.7.11 Cultural Practices

All the activities that are carried out in the farm area before, at the time, and after the process of the seedling are known as cultural practices. The cultural practices that are recommended for the crop should be strictly followed. The basic fundamental is to raise a crop with practices as follows:

- The optimum time of sowing should be followed for raising the seed crop. Therefore, the time of sowing is influenced by the change in the season and climatic conditions. Moreover, an early sowing or late sowing deteriorates not only the yield of the product but also the quality of the seed.
- Maximizing the length of the growing season to increase yields is most preferred.
- In some of the seed manufacturing schedule, a peculiar method of sowing is monitored which is similar to the hybrid seed manufacturing schedule, in which male to female ratio is conserved.

17.8 Seed Treatment Practices

Seed treatments are performed on seeds after they are removed from the parent crop. Improvising the methods of pest management (e.g. brown rust diseases), increase in the productivity, improved sprouting, protrusion of buds from seed coating, treatment of seed using lime and seawater, and seed priming techniques are the practices targeted by these current practices.

Seed treatment for the prevention of plant diseases, whether accidental or experimental, dates many years back. Previous studies have revealed that the seed viability was affected by factors like temperature, light, seed moisture, O_2 and CO_2 concentrations that prevail around seeds, and, in addition to storage conditions, seed collection time, development of seed, viability on the initial time, the seed's physical condition at the time of harvest, etc. (Harrington 1972; Willan 1985; Schmidt 2000; McCormack 2004). According to Hartmann and Kester (1975), light, water, temperature, and exchange of gases are the main factors that affect germination of seedlings. For each seed of different species, these factors react differently, and hence for seeds of all species, the storage condition and the pretreatment procedures cannot be in common.

Seed treatment processes such as seed pelleting, film coating, inoculation, and priming were practised worldwide in order to enhance the plant ability, distribution of nutrients, germination, and seed storage by means of using adhesive films, herbicides, fungicides, growth promoters, and biological agents on the surface of the seed (Thomas et al. 2003; Balbinot and Lopes 2006). A carrier of chemical materials was coated on the seed in order to support seedling growth and to enhance

seed performance (Scott 1989; Rehman et al. 2011). If needed, the seed may be treated with appropriate chemicals and microbial cultures to ensure uniform germination and growth (Murungu et al. 2004).

Seed Disinfection
Seed disinfection is the removal of fungal spores that are present inside the deeper tissues and on the outer seed coat. In order to eradicate the spores effectively, the fungicide should breach the seed. Some of the previous studies demonstrated that the usage of hypochlorite solution at various concentrations has shown the remarkable disinfection activity against different species. Do Rego et al. (2009) proved that the effective disinfection for Cereus jamacaru is 0.5% sodium hypochlorite solution. Many studies reveal the various effects of effective disinfectants, such as 70% ethanol for half an hour followed by immersion in 2% chlorine for 10 min (de Moraes et al. 2010), 3.5% sodium hypochlorite solution (Oyebanji et al. 2009), 70% ethanol along with 3% sodium hypochlorite, etc.

Seed Disinfestation
Seed disinfestation is the killing of surface organisms (fungi and bacteria) and insects that contaminate the surface of the seed without causing infection. In this practice, some of the methods like soaking, chemical dips, and fungicidal application in the form of dust, liquid, or slurry are well suitable (Schmidt 2000).

Seed treatment is broadly categorized into three types, namely, (1) mechanical method, (2) biological method, and (3) chemical method.

17.8.1 Mechanical Method of Seed Treatment

Mechanical method of seed treatment is the removal of infectious materials mixed with seeds by mechanical means. In order to remove the pathogens from the surface of the seed, the seeds are mechanically cleaned before seedling. Likewise, seed coating, pelleting, and scarification are some of the methods that are done mechanically to protect the seeds against pests and to enhance the germination. Herewith we have briefly described a few of those.

17.8.1.1 Magnetic Field
Magnetic fields are used for seed treatments where the exposure of non-standard seeds to magnetic field increases the germination as well as improves their quality. Research reports reveal that the magnetic field impacts the growth of the plants at their initial stage after the germination (Aladjadjiyan 2002). According to Morejon et al. (2007), priming with magnetic field-treated water proved to improve the plant growth and seed germination.

17.8.1.2 Plasma Treatment

Using plasma in the field of agriculture as well as in medicine is a modern and emerging advancement (Sosnin et al. 2004; Akitsu et al. 2005; Hayashi et al. 2011; Klämpfl et al. 2012). Both germinations of seed and the growth of plant are given priority in the agricultural aspects. Applying plasmas along with the gases such as cyclohexane, aniline, and helium influences and enhances the germination and growth mechanism (Volin et al. 2000; Jiayun et al. 2014). Atmospheric plasma, microwave plasma, and magnetized plasma methods are some of the adopted seed treatments to enhance seed development and plant growth (Sera et al. 2010; Zhou et al. 2011). Many studies revealed that the reactive oxygen species (ROS) generated by water vapour plasma have a great impact on the thiol groups that control the quality of plant development using redox reaction (Henselová et al. 2012). Non-thermal plasma radiations are one among the advanced techniques performed which replace a number of traditional techniques such as scarification, stratification, and priming (Dhayal et al. 2006). Moreover, seed destruction and use of chemical up to zero degrees that is eco-friendly are the main aspects of using plasma treatments to seeds.

Henselová et al. (2012) reported that plasma alters the enzymatic activity and also sterilizes the seed surface while exposing to plasma. It also alters (delaying or boosting) the seed germination chemically by depositing on seed surfaces (Volin et al. 2000). Low-density radio frequency (RF) discharges and microwave discharges are the recent and important plasma-related analysis that were reported by Sera et al. (2010) and Bormashenko and Grynyov (2012). Recent studies also reveal the discharge of coplanar barrier and the atmospheric pressure in plasma treatment of seeds (Filatova et al. 2013). The seeds of crops such as wheat, maize, radish, oat, safflower, and blue lupine were investigated for various seed germination patterns. The results revealed that while radio frequency plasma was exposed for 130 min in combination with argon, the safflower seeds show a 50% enhanced germination rate (Sera et al. 2010). In another study by Ling et al. (2014), cold plasma-treated soybean seeds with 0–120 W for 15 s and found positive effects on both seed germination and seedling growth.

17.8.1.3 Sound Treatment

A sound is a wave form of energy which falls under the frequencies between 20 Hz and 20 kHz. The audible sound is used for priming and was tested in different plants (Chowdhury et al. 2014). Sound waves differ with each other in several aspects such as frequencies, exposure periods, and sound pressure levels (SPLs) and distances between the source of the sound and the target. Sound waves impact the plant growth by increasing the plant plasma membrane H^+-ATPase activity and increasing the soluble sugar and protein content and amylase activity in the callus. Research studies conducted in different environmental setups such as open field as well as greenhouse conditions and different ranges of sound frequencies that were audible and different sound pressure ranges reveal that sound waves ranging between 1 kHz and 100 dB exposed up to 1 h at a distance of 0.20 m from the source considerably stimulate the cell division and cell wall fluidity in the callus. Moreover, it also expressively boosts

protective enzyme activity and endogenous hormonal activity (Hassanien et al. 2014).

17.8.1.4 Ultrasound Treatment

Ultrasounds (US) are acoustic waves with higher frequencies (>20 kHz). Moreover, research reports reveal that the ultrasound-treated plants exhibit an increased germination percentage, seed vigour index (SVI), and length of the root and shoot than the non-treated plants. The ultrasonic seed treatment effects on the seed germination and growth of seedlings were analysed using four ultrasonic factors, namely, sonication time (minutes), sonication temperature (°C), output power (watts), and seed soaking time (hours). The changes in malondialdehyde (MDA), superoxide dismutase (SOD), and peroxidase (POD) activities were recorded in recent studies. As a result, ultrasonic treatment is proved to have the potential to improve seedling growth. Also, the positive effects of the US treatment on the aged seeds were observed with an enhanced germination percentage (GP) and growth of seedlings (GS) (Liu et al. 2016).

Ultrasound at 40 kHz applied on germination of vegetable seeds for a duration of 5–60 min by Goussous et al. (2010) and found that the treated seeds showed the highest growth rate index at 45 min. Risca et al. (2007) used ultrasound to promote germination of Norway spruce seeds, and the study revealed 5–8% increase in shoot length, 32% increase in root length, and up to 40% increase in germination with the 50 and 60 s of ultrasound treatment. Likewise, seed germination and protocorm development increased with increased ultrasonic duration at the frequency of 42 kHz of 135 Watts for the duration of 3–10 min.

17.8.1.5 Gamma Radiation Treatment

With emerging trends in agriculture, gamma radiation acts as a tool for improving the plant characteristics like maturity, grain, yield, product quality, and salinity tolerance during unfavourable circumstances that may depend on the level of irradiation (Kiong et al. 2008). This radiation can also prevent the infestation of pathogens on agricultural products and helps in the conservation and storage of seeds (Melki and Sallami 2008). Yilmaz and Boydak (2006) reported the positive impacts on germination of Chinese cabbage by applying a low dose of gamma radiation (up to 20 Gy).

17.8.1.6 Ultraviolet Radiation Treatment

The impacts of the ultraviolet radiations are due to the fact that biomolecules interact with water that creates free radicals which manipulate biomolecules and trigger the cell that activates the antioxidant system (Rogozhin et al. 2000; Sudhakar and Murugesan 2011) to act against upcoming stresses (Wi et al. 2007; Ashraf 2009).

17.8.1.7 Ozone Treatment

Sudhakar et al. (2011) reported that the effect of ozone (O_3) gas on tomato seeds plays a vital role in inhibiting dormancy by reduced abscisic acid (ABA) level.

17.8.2 Chemical Method of Seed Treatment

Chemicals are used for controlling diseases of the seeds by means of seed treatment with additives in combinations with insecticide and fungicide. Some seed treatment substances are available in combinations of fungicide and insecticide. However, seed treatments of chemical method didn't show remarkable changes in the development of root, drought-proofing, or crop yield (Sharma et al. 2015). Restriction of using arsenic during 1808 has made a remarkable improvement in the seed treatment history. Moreover, advancements in the technologies of agriculture propel the seed companies to use seed treatment methods that improve the crop yield. Chemical seed treatment methods are classified into two types based on the following:

1. Formulation
2. Function or purpose

17.8.2.1 Classification Based on the Formulation

Formulations of seed treatment are of four basic types; they are as follows:

True Liquids

The true liquid formulation contains emulsions as minute globules in the form of suspensions probably the water-soluble suspension. It may be diluted to improve seed coverage, e.g. metalaxyl-M, methylmercury, and carbaxin.

Flowables

Pre-dispersed immiscible particles produce a colloidal suspension of flowable which is basically present in a flowable consistency which may contain dyes, mordants, and flourishing compounds.

Soluble or Wettable Powder

Wettable or soluble powders are made to readily dissolve in water for application on the seeds. They are able to form a true solution in water, and insoluble wettable powders are mixed with wetting agents to form instant formulation. This formulation generates dust at the site of usage due to its lower affinity to bind with the seeds, e.g. dioctyl sodium sulfosuccinate and anionic surfactant blend.

Dust

Dust or powders are the formulations designed to apply on the seed when they are in the dry state. These dust formulations can be readily applied to coat a few kinds of seeds like peanuts and grasses. The limitations of this practice include exposure of workers to dust powders. Some of the dust powders are uniconazole and sulphur powder.

17.8.2.2 Classification by Function or Purpose (Target)

The seed treatment chemicals are broadly classified into three types based on the purpose or function such as fungicides, insecticides, and non-pesticidal conditioning aids.

Fungicides

Fungicides are chemical compounds that particularly control the fungal pathogens which may be transmitted through the surface of the seed, internal tissue of the seed, and pre- and post-emergence soil-borne and airborne modes. Few fungicides have the ability to control soil-borne as well as the seed-borne microbes effectively. Moreover, some fungicides move into the parts of the growing plant at the time of seed germination, and seedlings begin to grow; hence, they are systemic in nature.

Basically, the fungicides are classified under six categories based on chemical structure which are (1) inorganic, (2) organometallic, (3) antibiotics, (4) carbamate, (5) chlorinated hydrocarbons, and (6) miscellaneous organics.

Insecticides

An insecticide is a chemical or biological compound which controls insect pests. One method of applying insecticide is fumigation that controls the insects in the stored seed in order to control soil insects that get attached to the seed and seedlings. Insecticide simply controls insects throughout the plant by moving all over the plants for a little period of time. Insecticides are categorized based on their chemical structure into four categories: They are (1) naturally occurring, either mined or botanical, (2) chlorinated hydrocarbons, (3) organic phosphate, and (4) carbamates. In some cases, fungicides can be applied in addition to insecticides in order to treat or process the seed.

List of Chemicals Widely Used for the Seed Treatments

2,2-dichlorovinyl dimethyl phosphate, arsenicals, dichlorodiphenyltrichloroethane (DDT), dimethoate, fenthion, lindane, malathion, nicotine, phorate, phosphamidon, pyrethrins, rotenone, ryania, sulphur, and trichlorfon are chemicals widely used for seed treatments.

17.8.3 Biological Methods of Seed Treatment

Biological seed treatments can be defined as the seed treatment which involves utilization of herbal formulation, bacteria or fungi to control soil and seed pests, and pathogens as an alternative to harmful chemicals. Biological seed treatments are attaining an increased interest because of less toxicity and impacts on humans as well as the environment and also phytotoxicity problems due to the excess use of pesticides. Moreover, instead of protecting the plants during the seed/seedling stage, biological seed treatments protect the plant throughout its entire life cycle.

17.8.3.1 Seed Coating

Seed coating is explained as applying inert substance above the surface of the seed uniformly. Seed coating protects the seed from the seed-borne and the soil pathogens. In industries, polymer seed coating is widely used. Plasticizers, polymers, and commercially available colourants are precisely applied as film formulations (Ni 1997). Generally, seed coating requires an intermediate layer coating material such as binders (e.g. polyvinyl alcohol, gypsum, clay, etc.) or fillers (e.g. clay, polyvinyl acetate, and vermiculite). Seed agglomeration is one of the advanced coating technologies which aids in sowing a variety of seeds on a seed lot or a number of seeds on diverse seed lots in account with varieties or species (Sikhao et al. 2015). For the biological seed treatments, handling and application require sterile environments, which prevent the infection of beneficial microbes by pathogenic microorganisms which is not an important factor in traditional seed treating. Therefore sterilizing the equipment thoroughly is essential to free the undesirable microbes.

Advanced seed coating technologies currently in practice are as follows:

Temperature-Activated Polymer

The latest seed coating technology uses a polymer that is activated by temperature and as a consequence controls the water permeability of the seed. This is an interesting technology in which the seed germinates when the soil gets warm above 55 °F. This remarkable coat of polymer is a combination of natural fatty acids which prevents the absorption of water during the cool temperatures. When the temperature rises, the polymer gets altered and allows the seed to absorb water. These new coatings aid in controlling the germination of the seed in cold and in protecting the seeds from germinating under unfavourable environmental conditions. This seed coating technology is more precise in time and uniform emergence even sooner than the normal planting from a few days up to 4 weeks faster resulting in minimal risk of storage loss.

Flowable Suspensions (FS) and Emulsions (ES)

The flowable suspensions (FS) and emulsions (ES) are recent advancements to minimize hazardous dust, with improved stability in the formulation and increased seed adhesion. The seed is directly applied with the flowable concentrate liquid suspension formulation which is available as ready for application (RFA) products. At present flowables are available in suspensions of solids. A pigment of red colour is added as a marker for safety on dressed seed in flowable suspensions (Castro 1998). Emulsions (ES) are another type of recent seed coating which consists of an oil-in-water emulsion formulation which provides us with a stable seed treatment in both direct and after dilution methods.

Microencapsulation

Microcapsule seed treatment is a seed treatment with a little modification of flowable emulsion formulations that converts the droplet of an emulsion into capsule through

microencapsulation method. This avoids the potential skin irritancy problems which impact the operator while handling.

Gel Formulations

Gel formulations are recently used for special seed applications. The polymer film coating can be utilized for higher-value cereal seed, e.g. sugar beet and sunflowers; some of the high-value seeds may be pelletized and film-coated. These methods provide a vehicle for seed treatment of chemicals with improvement in the handling of the seed. The film coatings should be thicker enough to cover the seeds which may increase the value of the seed.

Nanogel-Based Seed Coating Formulation Technology

Nanogels are nanosized hydrogel arrangements with high crosslinked polymer (either co-polymerized or monomeric). The introduction of nanogel systems achieved its goal in delivering the ingredients in continuous, well-organized, and targetable manner. The various nanogel formulations have been articulated based on their easily changeable physical and chemical properties and availability of a wide range of polymer systems.

Water-Dispersible Granule (WG)

The new technology insists the conversion of water slurriable powder of the seeds into a water-dispersible granule (WG) (Nalepa and Hahn 2013). The surface-active detergents such as aliphatic alcohol ethoxylates and lignosulphonates along with some of the polyphosphates are utilized as flocculating agents that prevent the rapid deposition of pesticide particles at the time of seed treatment procedure.

Seed Encrustation Technology

Encrustation is one of the hybrid techniques which is transitional between film coatings and pelleting; this technique is widely used to boost up the plantability, and the cost is very low when compared with pelleting. This technology needs to be exploited in order to facilitate the precise sowing, uniform and vigorous emergence, development, and increased seed yield in small-seeded crops in general (e.g. rapeseed and mustard) (Yadav 2018).

17.8.3.2 Seed Pelleting

In seed pelleting, a thick layer of inert substances was applied on the surface of the seed which alters their shape and size. The inert substances used are limestone powder or clay and stickers which are used as binders that comprise polyoxyethylene glycol-based waxes and cellulose polymers. Similarly to seed encrustation technology, it is also used to enhance the plantability. Most of the seeds, mainly vegetable seeds, are not in the proper shape which delays the crop yield, and in other cases, the seeds are very small in size which results in the lack of accurate placement in the soil (Smith and Miller 1987). In order to overcome these problems, most of the companies offer seeds with a coating material which alters the seeds, making them heavier and rounder.

In this method, the seeds are added in a coating pan or drum which looks like a concrete blender. The seeds are added with an amalgam (clay, calcium carbonate, limestone, vermiculite, and talc); cementing compounds like gelatin, gum arabic, polyvinyl alcohol, polyoxyethylene glycol-based waxes, and methylcellulose; and some of the composites (inoculants, fungicides, etc.) supplemented to improve the seed activity (Taylor and Harman 1990). When a seed pellet is added with calcium oxide and peroxides, it minimizes the anaerobic damage by the release of oxygen while the seed is under flooded field conditions (Ollerenshaw 1985; Langan et al. 1986). Seed pelleting is considered a significant method for most of the vegetables including carrot, lettuce, onion, and various flowers.

17.8.3.3 Seed Dressing

It is the most frequent technique in seed pretreatment. The seed may be covered with a dry form, slurry, or liquid formulation. It can be prepared and applied at the farm itself or with a specific facility (Babiker 2004).

Seed enhancement by bio-priming substances is a process of soaking/coating the seed using the pure culture of particular species of bacterium or fungi or a group of bacterium/fungi (microbial consortium) with proper incubation under optimum (temperature, pH, moisture, etc.) conditions. Among various organisms, rhizobacteria (particularly *Bacillus* spp. and *Pseudomonas* spp.) are considered to be potent in overwhelming the diseases among various agricultural crops via induction of resistance channel known as 'induced systemic resistance' (ISR) (Van Loon et al. 1998; Thomashow and Weller 1998). Bio-priming seeds with *Bacillus subtilis* and *Pseudomonas fluorescens* are widely used to control seed and root rot diseases (Begum et al. 2010; El-Mohamedy and Alla 2013), and also it is the best alternative against chemical fungicides.

Chitosan Treatment

The chitosan is widely used in agriculture for having a strong fungistatic effect, and also it has other properties, i.e. it helps to increase the post-harvest life of vegetables and fruits and stimulates the plant growth, among others. Most of the studies assessed and confirmed the capability of chitosan treatment in order to provoke resistance against seed-borne fungal infections in plants (Martínez et al. 2015). In many studies, the activities of phenol component and enzymes such as ascorbate peroxidase (APX), guaiacol peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) against diseases were proved to enhance the defence mechanism in plants (Bautista-Baños et al. 2006; Pandey et al. 2018).

Priming

The priming techniques (Heydecker and Coolbear 1977) and osmoconditioning (Khan et al. 1977) are the methods of impregnating the seeds under ventilated low water osmotic pressure. Some of the materials used are polyethylene glycol (PEG), potassium nitrate (KNO_3), tripotassium phosphate (K_3PO_4), potassium dihydrogen phosphate (KH_2PO_4), magnesium sulphate ($MgSO_4$), sodium chloride (NaCl), glycerol, and mannitol which ultimately provide the seed nitrogen and other nutrients

involved in the protein synthesis at the time of propagation. After this process, the dried seeds are accepted for handling, storage, or planting. The method of drying somewhat decreases the propagation progress than the soaking (Brocklehurst et al. 1984), and also it causes seed injury during excessive temperatures. Hence, priming has to be carried out under regimented conditions. By using this technique, the seeds could be effectively stored for a short period of time without losing their characteristics (Thanos et al. 1989).

Solid Matrix Priming

The use of low-grade potential solid carriers is one of the leading approaches to hydrate the seeds (Kubik et al. 1988; Taylor et al. 1988), and this method is also known as matrix conditioning. The carrier used should have the following characteristics:

- A low matrix potential.
- It has to be slowly soluble in water.
- Great water holding capacity.
- Increased surface area.
- It should not have toxicity to the seed.
- It should easily stick onto the surface of the seed.

Some of the natural carrier elements include vermiculite, peat moss, sand, commercially available diatomaceous silica element, Micro-Gel and Celite, etc. (Khan 1992). Other materials which have a considerable osmotic effect are Leonardite shale, calcined clay, and bituminous soft coal (Taylor et al. 1988; Kubik et al. 1988). This method is commonly employed with the application of pesticides (fungicides, insecticides, or both) to the seeds in order to sanitize and cleanse the seed, soil, and airborne pathogenic microbes and insect pests.

17.9 Processing of Seeds

17.9.1 Seed Extraction

The seed extraction procedure is based on the types of crop. For soft fruits like tomatoes, it is sorted into small pieces, smashed, and fermented. In the case of cucumbers, it is sliced, and then the scraped seeds are fermented; however, in case of watermelons, the seeds are extracted along with the entire flesh of the fruit and then fermented. The abovesaid fruits have seeds with gel which acts as an inhibitor in the process of propagation and also makes the seed difficult to handle and dry. When fruits undergo decomposing, it results in fermentation; when it occurs in an organized means, the microbes (particularly yeasts) disrupt the gel which results in the removal of bacterial and fungal cells responsible for most seed-borne diseases. In this process, the temperature and time taken for fermentation are the most important factors. If the time of fermentation is short, the microbes will not be removed,

whereas if the time for fermentation is too long, it may result in maturation of sprouts. The temperature and time for fermentation are correlated with each other.

17.9.2 Seed Washing

After completion of fermentation, the seeds are washed in order to get rid of fruit pulp, fruit pieces, dead cells, and also low-quality seeds. The good quality seeds are identified which are heavy and settle down in the bottom, while the low-quality ones are light and have a tendency to float with the other waste materials. This process has to be repeated until the wash water converts into a clear state.

17.9.3 Seed Drying

It is the process of reducing the percentage of humidity without causing damage to the seed viability in order to facilitate the storage. The amount of humidity in seeds differs based on the type of grain, its chemical constituents, method of harvesting, humidity during harvest, environment's relative humidity, and variations in seasons. The seeds that are harvested as fresh have higher humidity which results in high respiration and growth of seed embryos, fungi, and insects. In order to avoid these problems, the seeds should be dried, and moreover, damage, heating, and invasion at the time of storing should be avoided (Sacilik 2007).

It also influences the early harvesting and seed storage for a long time (McCormack 2004). If the moisture on seed surface evaporates quickly, it influences the rate of seed dryness; at the same time, the evaporation depends on the temperature of dry air and optimum relative humidity. Moreover, up to 45 °C is considered as a safe temperature. The rate of moisture when the air moves towards the seed from the seed surface also influences the seed drying. The correlation between the safest drying temperature and initial moisture content of the seed is also accounted for while seed drying. It is safe and recommended to dry the seed at a temperature not greater than 40 °C so that the moisture contents do not decrease more than 18%. Though drying is essential for the viability of seed during storage, harmful factors may affect the drying process. The seed coat splits or hardens if drying is too fast, and this results in the prevention of the interior of the seed from drying. Revenue of the farmers is lost by prolonged drying of seeds due to weight loss. Therefore it is essential that the seeds should be cooled by forced ventilation after drying prior to storage (Sacilik 2007).

17.9.3.1 Natural Sun Drying

Farmers use to dry little quantities of seeds by using natural wind and sunlight by drying a thin layer of the threshed seeds which were spread on the floor or straw. The main advantage of sun drying is that no additional requirements are needed. The major disadvantages of sun drying are a delay in time, damage due to weather condition, and the possibility of mechanical mixtures (Boxall et al. 2002). Seed

germinability is influenced by the sunlight directly by the impact of ultraviolet radiation and high temperatures. Moreover, direct exposure of the seed to the sun for a long period affects the seed viability. Sun drying of seeds is mostly followed in tropical countries. When the crop is at the stage of harvesting, this method is employed. Few crops like maize are sun-dried in order to get rid of insect and prevent rodent infestation and fungal damage (Kiaya 2014).

17.9.3.2 Solar Drying

Solar drying implies the collection of sunlight and rays through a specifically designed unit for the removal of moisture. The temperature of the solar drying unit is higher (approximately 20–30 °C) than the open drying unit and hence results in a reduction of processing time.

The solar dryer is constructed in two designs. They are natural convection dryer and forced convection dryer. Usual conventional dryers utilize thermal gradients, and in advanced dryers, the air is forced through solar collecting pan and seed layers. For farm use, these dryers are very efficient as they contain a drying bin, solar chimney, and a solar stockpile (Boothumjinda et al. 1983). The solar collector possesses a dark plastic foil or layer of burnt paddy husk and is covered with transparent plastic foil. The drying bin consists of a perforated platform. However, the high structural profile, wind stability problem, and replacing plastic sheets at monthly intervals are the disadvantages of solar drying (Golob and Farrell 2002).

17.9.3.3 Forced Air Drying

This practice uses natural (unheated) or hot air which is applied to the layer of the seeds till the drying is finished. It is also called as dehydration and is categorized into two types: batch driers and continuous flow driers.

17.9.3.4 Batch Driers

In this method, the layer of seeds is arranged in a controlled environment, and the dry air is passed till the whole seed is dried, and then seeds are taken out and the process is repeated for subsequent batches. The merits of this method are that it is simple to carry out and suitable only when the quantity of the seed is low.

17.9.3.5 Continuous Flow Driers

In this practice, the stream of hot air is passed horizontally and vertically over the seed, and then the seed enters into a cooling chamber; these two procedures are continuously processed to dry the seeds. This method is suited for a large quantity of seeds.

17.9.4 Commercial Seed Cleaning Process

As the seed comes from the field, it always gets contaminated with other plants, weed, plant debris, soil, and stones, which should be processed in order to get healthy seeds for sowing. The cleaning process is done to remove inert matter,

seeds of weed plants, undesirable varieties of crops, and seeds of the same variety that get deteriorated or diseased and damaged. Traditionally, winnowing is the process of removing the straw, husk, and other light materials by threshing the seed. According to Schmidt (2000), sieves are involved in the manual cleaning for removing different sizes of seeds and heavier materials such as soils and stones. Cleaning minimizes the high volume to be handled and stored and also removes the moist from green plant material which produces heat while storage. Different types of crop need different types of machines and sequences in combination with cleaning. Usually, cleaning involves a series of steps, which includes three stages: pre-cleaning, cleaning, and grading.

17.9.4.1 Scalping

In the commercial seed cleaning process, two types (partial and main) of cleaning practices are carried out after the seeds are dried. This is a kind of elimination process, which removes granular materials. The fragments of stem/shoot are very tough to isolate from the seed throughout the successive operation (Marcar et al. 1995), in which pre-cleaning is the first step, in which forced air and huge sieved screens or cylinders are used to take out the massive material and sieves. So the scalping is a method to enable the movement of seed by using the machine. Vibrating screens or rotating cylinders are used to retain the chaffs, stem, pods, leaves, and any other big-sized materials.

17.9.4.2 Basic or Secondary Cleaning

At this stage of cleaning, forced air and agitating screens are involved in this process and are suitable for all kinds of seeds. It is more or less similar to the scalping but the next stage of cleaning. The air blast is used to separate the seeds of weeds or broken seeds with a series of screens separating the seeds that were either larger or smaller than the crop seeds. Some seed lots require additional treatment of cleaning for removing the adulterants that closely attach to the crop seeds after the basic cleaning process.

17.9.4.3 Wet Cleaning

The wet cleaning method is used to clean the plants that contain seeds in their fleshy parts. The seeds along with the flesh of a ripened fruit are removed separately from the flesh by collecting in a drum and rubbing aggressively with coarse sand. Then the content is filtered by a sieve and washed repeatedly under running water until the pieces of flesh and mucilage are washed off and then the seeds are dried at least for 10 days earlier to storage, e.g. coffee, cucumber, tomato, fruit trees, etc.

17.9.4.4 Dry Cleaning

Dry cleaning is suitable for the well-ripened seeds in a dry pod. The dry pods are harvested separately; otherwise the whole plant along with the pod is dried in shade after separating it completely from the soil and is subjected to threshing to collect the seeds.

17.9.4.5 Seed Grading

Grading removes cracked, damaged, or defective seeds, based on the size which results in lower germinability and vigour (Harty 1980). It is done by removing the seeds that are dissimilar than the required seed size using sophisticated machines in order to obtain uniform sized and shaped seed which eases the automated planting. It also increases the sales appeal by improving the appearance of processed seed. Mostly, seed cleaning machines simultaneously grade the seed based on uniform size and shape into first grade, second grade, etc.

17.9.4.6 Threshing

Threshing is a process of application of machine-driven force under a well-organized system along with a shearing motion. Seed threshing has been carried out by different approaches. For instance, plants that have pods like beans and okra are taken in a bag and knotted firmly and then are thrashed by a twisting motion, so that the plant material is redistributed evenly. Subsequently, the threshed seeds are subjected to gentle crushing and winnowed before storing, e.g. most cereals, paddy, millets, oilseeds, etc.

17.9.4.7 Winnowing

This is one of the typical techniques, in which the seeds are placed in a widespread bag or basket and tossed and chaffed (carried away by the wind) into the air. This method is very hard to follow, and the outcomes are not in accepted range due to the change of velocity and direction in the wind speed. To overcome this issue, a design was created which combined the technology of a dual-combine sieve. This has been a successful concept and shows promise in being an effective solution for improving the efficiency and quality of winnowing.

17.10 Seed Storage

Storage should be done only when the seed is at appropriate moisture level. Both lower and higher moisture levels are detrimental for the quality of the seed. The shelf life of the seeds at the time of storage depends on seed moisture content, temperature, relative humidity, initial viability, stage of maturity at harvest, and storage gas.

In general, the seeds are divided into two categories based on their storage characteristics: recalcitrant and orthodox. Recalcitrant seeds are desiccation intolerant (cannot be dried below approximately 40% seed moisture content without damage) and are typically characterized as large seeds with small embryos from tropical trees and shrubs (Chin and Roberts 1980). Recalcitrant seeds do not possess dormancy. If dried, they will lose their viability or even die. Although it led to successful plant reproduction, it made agricultural planning difficult because of the factor that seed lifespan was short. Orthodox seeds, in contrast, are desiccation-tolerant (may be dried to 5% seed moisture content material without damage), often manifest some types of dormancy, and are characteristic of most agriculturally

important crops found worldwide. Orthodox seeds represent most of the seeds found in the world and are among the most agriculturally important species.

There are four main kinds of seed storage; they're conditioned storage, cryogenic storage, hermetic storage, and containerized storage according to Copeland and McDonald (1995).

17.10.1 Conditioned Storage

Seeds belonging to most plant species may be effectively saved for numerous years by using carefully controlled temperature and relative humidity. Although such situations cost a high price for agricultural seed, they are very efficient in maintaining the germplasm as well as some costly seed stocks. The conditioned storage of seeds implicates the storing of seeds in a cool but dry environment for prolonged durations. During the long-time storage, seed viability may be damaged by high oxygen surroundings but benefited by excessive carbon dioxide environment. The seeds of maximum grain plants (such as barley, maize, sorghum, wheat, etc.) will hold suitable germination rate and vigour for approximately 365 days at moisture ranging from 12% to 13% under regular storeroom temperatures.

While a seed requires long-term storage, the moisture content of the seed material should not exceed 11%, whereas the temperature should no longer exceed 20 °C. However, the seeds of a few species (such as soybean and groundnut) lose their viability approximately after 365 days even if they were stored at 11–12% moisture content at temperatures of 20 °C or much less. Hence, such seeds should be stored properly in conditioned storage facilities for 18–20 months of 20 °C along with 50% relative humidity.

Conditioned storage places normally were constructed along with refrigeration and dehumidification systems. The refrigeration is essential to decrease the temperature of the room for seed storage, and dehumidification is vital due to the fact that it decreases the temperatures of the storage air and consumes much less water that increases the relative humidity of the air (Harty 1980).

17.10.2 Cryogenic Storage

It involves storing the materials at temperatures ranging from −80 to −196 °C, using ultra-deep-freeze facilities or liquid nitrogen. It is obvious that the intact seed would be the best form of the germplasm to cryostorage, as theoretically it should be merely a matter of thawing the propagules after storage and planting them out. However, it is impossible to freeze seeds having high water content characteristic like freshly shed or harvested condition, because cooling would be slow and accompanied by lethal ice formation. However, cryostorage of intact seeds can be achieved when the propagules are small enough and dry sufficiently rapidly to employ this technological approach.

This is the case for *Azadirachta indica* (Neem); the exo- and mesocarp are removed prior to desiccation, as retention of these coverings was associated with lethally slow dehydration. After cooling at an intermediate rate in cryotubes plunged into liquid nitrogen, 70–75% of the endocarp-enclosed seeds had retained viability when sampled over the 4 months that they were stored in this cryogen (Berjak and Dumet 1996). Whole seeds of *Wasabia japonica* have also survived cryopreservation utilizing the liquid nitrogen after quick dehydration to low water content (Potts and Lumpkin 1997), and similarly, those authors found that slow dehydration was deleterious. This presupposes that the tissues of nonorthodox seeds of individual species will withstand such drastic dehydration and retain viability at ambient temperature. Moreover it seems that generally temperate provenance is correlated with the ability of the seeds/seed organs to withstand such extreme dehydration.

During cryogenic storage, physiological activity may lose to a small extent; however, in contrast, the storage life of seeds extends. Although, when compared to the conditioned storage, cryogenic storage is secure and inexpensive, the quantity and capacity of the cryogenic tanks were limited. Therefore, cryogenic storage cannot be used practically to store commercial seeds, but to construct germplasm, it may be useful (Pritchard and Nadarajan 2008).

17.10.3 Hermetic Storage

Hermetic storage refers to storing and marketing the seeds in moisture-resistant or hermetically sealed containers in order to secure storage. Hermetic storage stores the seeds for more than 10 years. The hermetic storage eliminates the ambient air from the seeds and periodically introduces particular gases responsible for the extension of seed storage life such as carbon dioxide. The efficiency of the storage is directly proportional to the materials that were used to pack. Normal paper and cloth packing containers can be little effective, whereas laminate and polyethylene materials are more effective, and metal cans retain the seed moisture most effectively.

17.10.4 Containerized Storage

Control of humidity in seed storage areas needs huge capital for constructing specialized rooms with dehumidifiers. In case of lack of these sophisticated technologies, humidity is regulated by enclosing the container along with chemical dehumidifiers. In containerized seed storage, chemical desiccants like sulphuric acid, saturated salt solutions, or silica gel treated with cobalt chloride were used as indicator dyes to regulate the relative humidity of a closed container. The dye turns to purple from blue when the relative humidity exceeds 45%. It is cheaper and avoids the insect, rodent, as well as the moisture and therefore the seeds remain undamaged.

Typically the standards followed at present should be noticed in the management of seed storage, and the seed stocks need to be monitored, particularly for

heat-generating areas. Incoming and outgoing stocks must undergo a fumigation process. Floors should be well sanitized and free from dusts. Ventilation is recommended in between stocks. The storage building has to be maintained in a good condition.

17.10.5 Steps Essential for Good Storage Practice in Respect of Seeds/Grains

Pests can be controlled either behaviourally (probe traps, light traps, pitfall traps, etc.) or with numerous protective and healing techniques such as chemical and non-chemical methods. Series of steps should be taken for the better storage of grains. These steps include the following:

17.10.5.1 Before Storage

- Inspection for outflow of rainwater and abundance of drainage amenities
- Hygiene of the storage capacity and atmosphere
- Valuation of the capacity of the facility
- Treatment with pesticides
- Safety and fire extinguishers
- Maintenance of systems available

17.10.5.2 During Storage

- Maintenance of hygiene
- Guaranteeing ventilation wherever essential
- Inspection for leakage during rain
- Monitoring for pests and rodents regularly
- Look out for signs of deterioration
- Guaranteeing cut-off of defective seeds
- Prearrangement avoiding damage due to leakage of water.

17.10.5.3 After Receipt of Seed

- Assessment for variation and sturdiness of the quality.
- Assessment for infected seeds: If present, note the infestation type and range.
- Assessment of excess moisture: Preventive measures should be taken before the storage to avoid rancid odour.
- If any grain is found to be moist or broken, it has to be isolated and recovered with available facilities, and check the weight of the received seeds.

17.10.6 Factors Affecting Seeds' Storage Life

Matured seeds of regular size and look are suitable for storage and freed from physical damages and microbial contamination. The seeds should not be subjected to hot temperature and extreme moisture until the harvest. Hence, any preharvest

environmental factors that affect these seed traits also affect the seed's storability. Around 95% of the dry weight of the seed is composed of storage material, which is utilized for the germination of the seed and seedling until it produces its own nutrient by photosynthesis (Pollock and Toole 1961). An immature seed with an unproportioned composition of chemicals or that is mechanically damaged allows early invasion of microbes that may affect the storage. The seeds with higher viability resist unfavourable conditions of storage than comparable seeds of lower viability (Barton 1941).

17.10.6.1 Seed Deterioration

Seed deterioration is irresistible damage of the seed that cannot be stopped but can be controlled. Various factors including seed moisture content, relative humidity, mechanical damage, genetic characteristics and temperature of the storage environment, presence of microflora, seed maturity, etc. are involved in seed deterioration. Among them, the two most important factors were temperature and relative humidity. The relative humidity (RH) has a direct influence on the seeds' moisture in storage, and therefore, the moisture achieves a balance through gaseous water which surrounds the seed. Temperature is also an important factor which directly influences the moisture content in the air because comparably more water is held by the higher temperature than the lower temperature. In addition, while temperature increases, it improves the deterioration rate of the seeds.

Harrington (1972) identified the importance of the relationships and proposed two rules which explain about the seed deterioration. They are as follows:

Rule 1: Reduction of seed moisture up to 1% increases the lifespan of the seed by double the time.

Rule 2: Reduction of seed storage temperature up to 5 °C multiplies the lifetime of the seed by double the time.

Protein/Amino Acid Content

During the seed ageing, the total protein content reduces, and the amino acid content increases because of the protein disintegration into amino acids.

Nucleic Acids

During seed ageing, decreased DNA synthesis and increased DNA degradation take place. The degradation of DNA results in the incorrect translation and transcription of enzymes which were necessary for germination of seeds.

Membrane Permeability

The whole system of the membrane of a cell is called the cell membrane, and the plasmalemma comprises the inner membrane of the cell. Plasma membrane permeability is an important character of dry seeds and indicates the degree of membrane integrity and therefore the seed quality. Electrolyte leakage is a marker for seed membrane permeability. Higher electrolyte leakage indicates that seeds are deteriorated: the electrical conductivity (EC) of seed imbibition solutions increases

after ageing compared with that of fresh seeds (Ventura et al. 2012; Kumar and Mishra 2014). Moreover, seed vigour is closely related to membrane integrity, and therefore weakening of the cell membrane directly impacts the decline in seed vigour (Heydecker 1972).

17.10.6.2 Preharvest Effects

Preharvest effects may also affect seed viability. Roberts and Feast (1972) reviewed the impacts of the photoperiod, temperature, rainfall, mineral nutrition, and soil moisture on the viability of the seeds and found that these factors of preharvest affect the seed viability and development.

17.10.6.3 Effect of Provenance

Effect of provenance or location of production very lightly influences the seed vitality and seed storability. MacKay and Tonkin (1967) studied the period required for the seeds of four types of forage crops that were grown in different regions. In case of red clover seeds, the seeds grown in Canada required 4 years; however England- and New Zealand-grown seeds required only 3 years. Likewise in case of meadow fescue (persistent grass), seeds grown in America required nearly 7 years; however Danish-grown seeds required only 6 years. In the case of crested dogtail, it required 6 years for Irish-grown seeds; however New Zealand-grown seeds required only 3 years. These results proved a very significant variance among locations of production.

17.10.6.4 Impact of Weather

Weather is one of the vital factors for preharvest which affects the viability and storability of the seed. Excessive moisture, adverse weather, and freezing temperatures are threats and risks to the farmers and seed growers at the later stages of maturation of the seeds and post-maturation stage in the field. So far, many researchers revealed the impact of weather on seed germination. Among them, Dillman and Toole (1937) observed the impact of weather on seed germination by storing the seeds of four flax cultivars from 1929 to 1930 which were obtained in California. When comparing to the seeds of 1929, the seeds of 1930 displayed weather injury as well as a decrease in the weight from 36.4 to 45.0 kg bushel^{-1}.

MacKay and Tonkin (1967) discovered that the 50% of seed deterioration is reduced, while the seeds of barley, oats, and wheat are harvested at the right time. They performed this study for more than 26 years and attained the results that involved a huge number of samples using optimum hours of sunlight and the quantity of rainfall at Cambridge. Another study by Haferkamp et al. (1953) on the seeds of numerous species concluded that the vigorous mature seeds that were stored in a heat-protective building for 33 years at desirable conditions for storage near Lind, Washington, exhibited a long storage life. Harrington and Thompson (1952) revealed that the germination of lettuce seed is ominously affected in the region where the lettuce was grown particularly at 24 and 30 °C.

17.10.6.5 External Factors

Water

The seed which is in inactivated form is mostly dehydrated and has only 6–15% of water content within the cells, but the fact is that 75–95% of water is needed for all the metabolic activities by the active cells and also for the protoplasm activities. So the form of inactivated cells has to engross the water from the outside to convert into an active form and also to carry out the germination process. Moreover, water makes the seed coats softer, acts as a source for its bursting, and raises the absorbance character of the seeds, and for translocation of the embryo, the insoluble food turned into a soluble form. It also helps in the uptake of dissolved oxygen by the emerging embryo.

Oxygen

It is the most important content for all the living cells for respiration, through which energy is released for its growth. The seeds in the germination stage breathe energetically and acquire sufficient oxygen from the soil. For this reason, only, most of the seeds are seeded deeply in the soil or in marshy lands. The classical methods such as ploughing and hoeing mainly result in aeration of the soil which facilitates the germination at a high rate.

Suitable Temperature

For the dynamic metabolisms of protoplasm and seed germination, an adequate amount of awareness is needed. Even though a wide range of temperature (5–40 °C) influences the germination, the ideal temperature is about 25–30 °C, and in most of the seeds, germination ceases at 0 and 45 °C.

Storage Structure

The study of Justice and Bass (1978) proved that the storage of the seeds is disturbed by many reasons which include (e.g. high moisture, humidity, and temperature) conditions of the preharvest field, ripeness at the time of harvest, mechanical injury, fungi, pests, rodents, and seed treatment (fumigation). The seeds damaged mechanically have reduced storability and deteriorated faster and were more prone to fungi on storage area and get damaged during the treatment. When the moisture content and storage temperature elevate, the life of stored seeds declines. Harrington (1972) detailed that while rising the storage temperature from 0 to 50 °C, it reduces the seed's lifespan to half for each 5 °C. Moreover, in case of moisture content from 5% to 14%, reducing 1% of moisture content almost increases the seed storage life up to 200%.

The fungi especially the species of *Aspergillus* and *Penicillium* affect the stored seeds when the moisture and/or relative humidity is about 65–100% (which favours the rapid fungal growth), whereas the insects attack the stored seeds by damaging the endosperm and/or embryo having moisture content at the range of 9% (Justice and Bass 1978). Considering the pests (mice, rat) and birds, they eat, disseminate, mix, and pollute the seeds.

Some of the chemicals which are used for the treatment of the seeds for the long-term storage and for avoiding the mechanical damages may affect the seeds. During the treatment, the treatments that protect some crop seeds can affect other crop seeds. Moreover, extreme treatment rate also impacts the storability. In some cases, the fumigants affect the seed viability under certain conditions that include the moisture content, the range of temperature, the amount of fumigant used, and the time of exposure (Justice and Bass 1978).

17.10.6.6 Internal Factors

If the plants have immature embryo at the time of shedding of the seeds, it may not germinate until the embryo gets matured. Growth hormones in the freshly shed seed of some plants may be deficient which are essential for the growth of the embryo, and such seeds will synthesize the hormones if some time interval is provided.

17.10.7 Storage Loss

The storage loss of the seeds both qualitatively and quantitatively influences the economy. The quality loss of the seeds (deviations in structure, consistency, flavour, low quality in nutrition, and the occurrence of impurities) can be judged with the standards, and the quantitative loss can be judged by the loss in size and weight. Boxall et al. (2002) recorded the biological, chemical, biochemical, and mechanical losses of the seeds at the time of storage. There is a great correlation between physical factors and chemical and biochemical losses (Mohammed 2014). The main reasons involved in crop losses are due to less knowledge in equipment handling and skills in management (processing, packaging, transport, drying, and storage) (Boxall et al. 2002; Kiaya 2014).

17.10.7.1 Biological Losses

The crops may be affected by rodents, birds, insects, and microbes (bacteria and fungi). The microbial growth on the crop results in loss of weight, spoilage, and defects which decrease the market demand.

17.10.7.2 Chemical Losses

Loss in consistency, aroma, colour, and its nutritive significance is accounted as chemical loss of the seeds (Atanda et al. 2011).

17.10.7.3 Biochemical Losses

This type of loss may result from enzymatic activities, and it includes softening, discolouring, and unpleasant odour on the seeds.

17.10.7.4 Mechanical Loss

Rupture and damage at the time of handling and harvesting cause mechanical losses.

17.10.7.5 Physical Loss

Climatic conditions like increased temperature and humidity and inappropriate surrounding in the place of storage cause physical losses which involve weight loss.

17.10.7.6 Weight Loss

It is considered as a valuable loss because it results in reduced moisture which causes loss in the economy. To calculate the commercial loss of the seeds, shrinkage factor can be applied.

17.10.7.7 Nutritional Loss

The loss of nutritional value qualitatively and quantitatively affects the nutritional status of the human population. Primitive cause of this condition is triggered by pests feeding on seeds. The insects like *Plodia* and *Ephestia* feed selectively on the embryo of the seed and suck the nutrient contents. Most of the pests feed on bran of cereal seeds and reduce the vitamin content, e.g. *Liposcelis* spp. (Yousaf et al. 2016).

17.10.7.8 Loss of Seed Viability

The seed viability loss is influenced by infestation, moisture content, excessive respiration, light, and embryo-attacking pest and results in huge losses in germination as compared to others. Standard germination tests can be done for detecting seed loss (ISTA 1985).

17.11 Seed Testing and Certification

The seed material should be tested for purity, germinability, vigour, and health before sowing, and seed lot with good quality standard should be used for sowing. Numerous range of processes, techniques, and testings have been evolved vigorously due to the science of seed technology which is usual in the seed industry, together with handling and harvesting procedures which can be useful in reducing the seed deterioration (Copeland and McDonald 2001). Both qualitative and semi-quantitative seed health testing methods involve direct examination or inspection of dry seed which can identify the fruiting structures of fungi under stereomicroscope or physical appearance of fungal pathogens on the seed (Mathur and Jorgensen 1998).

Incubation test is one of the methods for seed health testing, in which the agar plate is used to incubate the seeds for a certain period under specific environmental conditions which allows the pathogens on the seed to grow if present (Warham et al. 1996). It detects the viable fungus even when the fungi are in the preliminary development phase. Seed-borne pathogens that were not detected by direct inspection or incubation methods can be identified by staining methods. Seed's germinability is detected by tetrazolium (TZ) assay which is the rapid alternative method implied for the evaluation of seed viability (Porter et al. 1947; Wharton 1955). Each and every breathing tissues convert TZ (2,3,5 triphenyl tetrazolium chloride), which

is a colourless compound, into formazan, a coloured (carmine red) water-insoluble compound by the transfer of hydrogen due to the catalytic activity of cellular dehydrogenase. Tetrazolium has a capability of entering both living and dead cells, but the formation of formazan was catalysed only by living cells and stains the sustainable seeds red, whereas the dead seeds (aged tissue) prevent formazan production and therefore remain unstained due to the absence of respiration.

Embryo count method is another method for seed testing which examines the dried seeds with naked eyes, magnification up to 30 times, reveals plant pathogens as sclerotic which were mixed seeds to form fungal bodies or ergots, which converts the seed into fungus structures (Andersen and Leach 2010). Enzyme-linked immunosorbent assays (ELISA) and immunofluorescence are some of the immunoassays which provide us with an easy approach to test the seed viability. Antigen (an antibody to a specific protein) of the pathogen such as soybean mosaic virus, bean pod mottle, soybean mosaic virus, and other viruses is added to a sample in ELISA test; a colour change shows the presence of infection (ISTA 1985). Above all, a seed production plot should be properly and timely inspected by an authorized agency so that good quality seed is produced. Hence, not everyone is expected to raise a crop (Dahiya et al. 2005).

17.11.1 Stages in Certification of Seeds

The certification has to be accomplished by six stages:

1. Receiving and scrutinizing the application
2. Verifying the source, variety, and other requirements of the seed in nurturing the crop
3. Inspecting the field to compare with the approved field standards
4. Supervising the processing and packing
5. Sampling and analysing the seeds to test its physical and genetic purity
6. Applying for certificate grant, labelling, and sealing

17.11.2 Seed Types Based on the Certification

There are generally five authorized classes of seeds, namely, nuclear seed, breeder seed, foundation seed, registered seed, and certified seed.

17.11.2.1 Nuclear Seed

The important aspect of this seed is that it has been retaining its vigour of original type or parent seed. Pure seed with genetic and physical purity is produced from the basic nucleus of seed stock by the authenticated breeder/institute/state agriculture university. A pedigree certificate is issued by the manufacturing breeder.

17.11.2.2 Breeder Seed

The clean seed which is produced by the particular handler or by the organization is known as breeder's seed. The newly produced seeds are allowed to grow on a huge surface and are 100% perfect in physical and genetic characteristics. A certificate of golden yellow colour is issued for this category of the seed by the manufacturing breeder.

17.11.2.3 Foundation Seed

Foundation seeds are the basis of registered or certified seed and defined as a pure seed inherently from breeder's seed under the well-organized management.

17.11.2.4 Registered Seed

The registered seed is the descendant of foundation seed which maintains acceptable genetic identity and purity, which a certifying agency approve and certify. Registered seed should produce certified seed. The registered seed is issued with a purple colour certificate.

17.11.2.5 Certified Seed

Certified seeds are the descendant of registered or foundation seed. C-1 group is the seed obtained from the first generation of certified seed; similarly, C-2 seeds are produced from C-1. Likewise, there are various classes of certified seeds. C-3 seeds are the certified seed of self-pollinated crops such as rice, wheat, and millet; however in order to produce cross-pollinated and open-pollinated varieties, crops such as cauliflower, maize, and radish, proper isolation distance, and intercrossing among varieties of the same species should be checked. In case of hybrids, the yield reduces beyond F1 progeny on successive generations even by using certified seed; therefore new planting of the seed on every season is essential.

17.12 Conclusion

Seeds are the primary resources of vegetation, food chain, and agriculture. The grower expects that the seeds sown should fulfil the basic requirement of being capable to grow like a healthy plant. Seed treatment is an emerging technology of modern agriculture for protection of crops which provide benefits to the farmer. Formulation applied for the seed treatment is determined by the suitability of active ingredients, storage stability, the feasibility of formulation type, seed distribution, availability of the machinery, seed retention time, compatibility with other products, operator safety aspects, commercial requirements, market culture for seed treatments, and competitive products in the market.

Hence the seed producers should concentrate in order to adopt all the biological processes which were elaborated in seed production and to minimize any harmful impacts on their succeeding germination. Moreover, good seed storage practices should be ensured for maximum seed viability resulting in maximum germination percentage. Modern seed science and agro-industry have made a great progress in

the satisfactory developments in our war against the pathogens and insects, and we hope that we may advance to a still stronger position in this continuing struggle.

References

Abay F, Waters-Bayer A, Bjørnstad Å (2008) Farmer's seed management and innovation in varietal selection: implications for barley breeding in Tigray, Northern Ethiopia. J Hum Environ 37 (4):312–321

Akitsu T, Ohkawa H, Tsuji M, Kimura H, Kogoma M (2005) Plasma sterilization using glow discharge at atmospheric pressure. Surf Coat Technol 193(1–3):29–34

Aladjadjiyan A (2002) Study of influence of magnetic field on some biological characteristics of *Zea mays*. J Cent Eur Agric 3:89–94

Ambika S, Manonmani V, Somasundaram G (2014) Review on effect of seed size on seedling vigour and seed yield. Res J Seed Sci 7(2):31–38

Andersen AM, Leach CM (2010) Testing seeds for seedborne organisms. U.S. Dept. of Agriculture, Washington, DC

Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 27(1):84–93

Atanda AS, Pessu OP, Agoda S, Isong IU, Ikotun I (2011) The concepts and problems of postharvest food losses in perishable crops. Rev Afr J Food Sci 5:603–613

Babiker AZ (2004) Evaluation of alternative drying methods and storage techniques on the storability of sorghum (*Sorghum bicolor*) seeds in the genebank. Dissertation, University of Khartoum, Sudan

Balbinot E, Lopes HM (2006) Efeitos do condicionamento fisiológico e da secagem na germinação e no vigor de sementes de cenoura. Rev bras sementes 28:1–8

Barton LV (1941) Relation of certain air temperatures and humidities to the viability of seeds. Boyce Thompson Inst Contrib 12(2):85–102

Barzman M, Bàrberi P, Birch AN, Boonekamp P, Dachbrodt-Saaydeh S, Graf B, Hommel B, Jensen JE, Kiss J, Kudsk P, Lamichhane JR (2015) Eight principles of integrated pest management. Agron Sustain Dev 35(4):1199–1215

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16

Bautista-Baños S, Hernandez-Lauzardo AN, Velazquez-Del Valle MG, Hernández-López M, Barka EA, Bosquez-Molina E, Wilson CL (2006) Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. J Crop Prot 25(2):108–118

Begum MF, Rahman MA, Alam MF (2010) Biological control of *Alternaria* fruit rot of chili by *Trichoderma* species under field conditions. Mycobiology 38(2):113–117

Berjak P, Dumet D (1996) Cryopreservation of seeds and isolated embryonic axes of neem (*Azadirachta indica*). Cryo-Letters

Bishaw Z, Struik PC, Van Gastel AJ (2013) Farmer's seed sources and seed quality: seed health. Int J Plant Prod 7:637–657

Boothumjinda S, Exell RH, Rongtawng S, Kaewnikom W (1983) Field tests of solar rice dryers in Thailand. In: Proc ISES Solar World Forum. Perth, Australia (Parkville ISES). Pergamon Press, Oxford, pp 1258–1263

Bormashenko E, Grynyov R (2012) Plasma treatment induced wetting transitions on biological tissue (pigeon feathers). Coll Surf B 92:367–371

Boxall RA, Brice JR, Taylor SJ, Bancroft RD, Golob P, Farrell G, Orchard JE (2002) Crop postharvest, science and technology. Volume 1. Principles and practice. Blackwell Science Ltd, Oxford, pp 141–204

Bradford KJ (1986) Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience 21:1105

Brocklehurst PA, Dearman J, Drew RKL (1984) Effects of osmotic priming on seed germination and seedling growth in leek. Sci Hortic 24:201–210
Castro BA (1998) Evaluation of Gaucho seed treatment and soil insecticides for management of the red imported fire ant on seedling grain sorghum during 1994-1996
Cheah KSE, Osborne DJ (1978) DNA lesions occur with loss of viability in embryos of ageing rye seed. Nature 272:593–599
Chin HF, Roberts EH (1980) Recalcitrant crop seeds. Tropical Press, Kuala Lumpur
Chowdhury ME, Lim H, Bae H (2014) Update on the effects of sound wave on plants. Res Plant Dis 20(1):1–7
Copeland LO, McDonald MB (1995) Principles of seed science and technology. Chapman and Hall, New York, NY, p 409
Copeland LO, McDonald MB (1999) Seed longevity and deterioration. In: Principles of seed science and technology. Springer, Boston, MA, pp 181–220
Copeland LO, McDonald MB (2001) Seed germination. In: Principles of seed science and technology. Springer, Boston, MA, pp 72–123
Dahiya BS, Deswal DP, Singh RK (2005) Pulses seed production and storage. In: Pulses. Agrotech Publishing Academy, Udaipur
Danesh YR, Ghiyasi M, Tajbakhsh M, Najafi S, Amirnia R (2014) Seed health management strategies in organic farming. In: Turkey 5th Seed Congress with International Participation
Dhayal M, Lee SY, Park SU (2006) Using low-pressure plasma for *Charthamus tinctorium L.* seed surface modification. Vacuum 80:499–506
Dillman AC, Toole EH (1937) Effect of age, condition, and temperature on the germination of flaxseed. Am Soc Agron J 29:23–29
Do Rego MM, Araujo ER, Do Rego ER, De Castro JP (2009) In vitro seed germination of mandacaru (*Cereus jamacaru* DC.). Rev Caatinga 22(4):34–38
El-Mohamedy RS, Alla MA (2013) Bio-priming seed treatment for biological control of soil-borne fungi causing root rot of green bean (*Phaseolus vulgaris L.*). J Agric Technol 9:589–599
Filatova I, Azharonok V, Lushkevich V, Zhukovsky A, Gadzhieva G, Spasic K, Zivkovic S, Puac N, Lazovic S, Malovic G, Petrovic ZL (2013) Plasma seeds treatment as a promising technique for seed germination improvement. In: Proceeding of the 31st International Conference on Phenomena in Ionized Gases
Golob P, Farrell G, Orchard JE (eds) (2002) Crop post-harvest: science and technology. Blackwell Science
Goussous SJ, Samarah NH, Alqudah AM, Othman MO (2010) Enhancing seed germination of four crop species using an ultrasonic technique. Exp Agric 46(2):231–242
Gray D (1981) Fluid drilling of vegetable seeds. Hortic Rev 3:1–27
Haferkamp ME, Smith L, Nilan RA (1953) Studies on aged seeds. I. Relation of age of seed to germination and longevity. Agron J 45(9):434–437
Harrington JF (1972) Seed storage and longevity. In: Kozlowski TT (ed) Seed biology, vol 3. Academic Press, New York, NY; London, pp 145–245
Harrington JF, Thompson JJ (1952) Effect of variety and area of production on subsequent germination of lettuce seed at high temperatures. Proc Am Soc Hort Sci 59:445–450
Hartmann HT, Kester DE (1975) Plant propagation: principles and practices. Prentice-Hall, Inc, Englewood Cliffs, NJ
Harty RL (1980) Seed processing, storage, and quality control. Trop Grassl 14(3):169
Hassanien RH, Hou TZ, Li YF, Li BM (2014) Advances in the effects of sound waves on plants. J Integr Agric 13(2):335–348
Hayashi N, Nakahigashi A, Goto M, Kitazaki S, Koga K, Shiratani M (2011) Redox characteristics of thiol compounds using radicals produced by water vapor radio frequency discharge. Japanese J Appl Phys 50(8S1):08JF04
Henselová M, Slováková Ľ, Martinka M, Zahoranová A (2012) Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low-temperature plasma. Biologia 67(3):490–497

Heydecker W (1972) Vigor. In: Roberts EH (ed) Viability of seeds. Syracuse University Press, Syracuse, NY, pp 209–252
Heydecker W, Coolbear P (1977) Seed treatments for improved performance survey and attempted prognosis. Seed Sci Technol 5:353
Horne PA, Page J (2008) Integrated pest management for crops and pastures. Csiro Publishing, Clayton, VIC
Huffaker CB (1984) Natural control of insect populations. Ecol Entomol:359–398
International Seed Testing Association (1985) International rules for seed testing Rules 1985. Seed Sci Technol 13(2):299–513
Janmohammadi M, Dezfuli PM, Sharifzadeh F (2008) Seed invigoration techniques to improve germination and early growth of the inbred line of maize under salinity and drought stress. Gen Appl Plant Physiol 34(3-4):215–226
Jiayun T, Rui HE, Xiaoli Z, Ruoting Z, Weiwen C, Size Y (2014) Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of *Andrographis paniculata*. Plasma Sci Technol 16(3):260
Justice OL, Bass LN (1978) Principles and practices of seed storage. US Government Printing Office, Washington, DC
Kahrl F, Yunju L, Roland-Holst D, Jianchu X, Zilberman D (2010) Toward sustainable use of nitrogen fertilizers in China. ARE Update 14(2):5–7
Karlen DL, Andrews SS, Weinhold BJ, Doran JW (2003) Soil quality: Humankind's foundation for survival a research editorial by conservation professionals. J Soil Water Conserv 58(4):171–179
Khan AA (1992) Preplant physiological seed conditioning. Hortic Rev 13(1):131–181
Khan AA, Tao KL, Knypl JS, Borkowska B, Powell LE (1977) Osmotic conditioning of seeds: physiological and biochemical changes. In: Symposium on seed problems in horticulture, vol 83, pp 267–278
Kiaya V (2014) Post-harvest losses and strategies to reduce them. Technical paper on postharvest losses. Action Contre la Faim (ACF), Paris
Kiong AL, Lai AG, Hussein S, Harun AR (2008) Physiological responses of Orthosiphon stamineus plantlets to gamma irradiation. Am Eur J Sustain Agric 2(2):135–149
Klämpfl TG, Isbary G, Shimizu T, Li YF, Zimmermann JL, Stolz W et al (2012) Cold atmospheric air plasma sterilization against spores and other microorganisms of clinical interest. Appl Environ Microbiol 78:5077–5082
Kubik KK, Eastin JA, Eastin JD, Eskridge KM (1988) Solid matrix priming of tomato and pepper. In: Proc. Intl. Conf. Stand Establishment Hort. Crops, Lancaster, PA, pp 86–96
Kumar D, Mishra DK (2014) Variability in permeability and integrity of cell membrane and depletion of food reserves in neem (*Azadirachta indica*) seeds from trees of different age classes. J For Res 25:147–153
Langan TD, Pendleton JW, Oplinger ES (1986) Peroxide coated seed emergence in water-saturated soil. Agron J 78(5):769–772
Ling L, Jiafeng J, Jiangang L, Minchong S, Xin H, Hanliang S, Yuanhua D (2014) Effects of cold plasma treatment on seed germination and seedling growth of soybean. Sci Rep 4:5859
Liu J, Wang Q, Karagić Đ, Liu X, Cui J, Gui J, Gu M, Gao W (2016) Effects of ultrasonication on increased germination and improved seedling growth of aged grass seeds of tall fescue and Russian wild rye. Sci Rep 6:22403
Mackay DB, Tonkin JH (1967) Investigations in crop seed longevity. I. An analysis of long-term experiments, with special reference to the influence of species, cultivar, provenance and season. J Nat Inst Agric Bot 11:209–225
Malik AU, Ahmad M, Hussain I (2009) Effect of seed rates sown on different dates on wheat under agro-ecological conditions of Dera Ghazi Khan. JAPS, J Anim Plant Sci 19(3):126–129
Marcar N, Crawford D, Leppert P, Jovanovic T, Floyd R, Farrow R (1995) Trees for salt and: a guide to selecting native species for Australia. Csiro Publishing, Clayton, VIC

Martínez G, Reyes G, Falcón R, Núñez V (2015) Effect of seed treatment with chitosan on the growth of rice (*Oryza sativa* L.) seedlings cv. INCA LP-5 in the saline medium. Cultivos Tropicales 36(1):143–150

Mathur SB, Jorgensen J (1998) Different types of damages in seeds caused by seed borne fungi. In: Proceedings of CTA seminar, 20–25 June 1998, Copenhagen, Denmark

Matzk F, Mahn A (1994) Improved techniques for haploid production in wheat using chromosome elimination. Plant Breed 113(2):125–129

McCormack JH (2004) Seed processing and storage: principles and practices of seed harvesting, processing, and storage: an organic seed production manual for seed growers in the Mid-Atlantic and Southern US

Melki M, Sallami D (2008) Studies the effects of a low dose of gamma rays on the behavior of chickpea under various conditions. Pak J Biol Sci 19:2326–2330

Mohammed M (2014) Manual on post-harvest management strategies to reduce losses of perishable crops. In: UWI/CTA/NAMDEVCO Workshop on Strategies to Reduce Postharvesting Techniques and Maintenance of Seed Quality harvest Losses in Perishable Commodities at NAMDEVCO Conference Facility. Piarco, Trinidad

de Moraes CF, Suzin M, Nienow AA, Grando MF, Mantovani N, Calvete EO, Donida BT (2010) In vitro artichoke seed germination. Hortic Bras 28(1):64–69

Morejon LP, Castro Palacio JC, Velazquez Abad L, Govea AP (2007) Stimulation of *Pinus tropicalis M.* seeds by magnetically treated water. Int Agrophys 21(2):173

Murungu FS, Chiduza C, Nyamugafata P, Clark LJ, Whalley WR, Finch-Savage WE (2004) Effects of 'on-farm seed priming' on consecutive daily sowing occasions on the emergence and growth of maize in semi-arid Zimbabwe. Field Crop Res 89(1):49–57

Nalepa S, Hahn T (2013) The value of neonicotinoid seed treatment in the European Union: a socio-economic, technological and environmental review. Humboldt Forum for Food and Agriculture (HFFA), Berlin. 96 p

Ni BR (1997) Seed coating, film coating and pelleting. In: Seed industry and agricultural development. Chinese Association of Agricultural Sciences. DOA, Ministry of Agriculture. China Agriculture Press, Beijing, pp 737–747

Nyland RD (1996) Silviculture concepts and applications. McGraw-Hill, Inc, New York, NY

Ollerenshaw JH (1985) Influence of waterlogging on the emergence and growth of *Lolium perenne L.* shoots from seed coated with calcium peroxide. Plant Soil 85(1):131–141

Oyebanji OB, Nweke O, Odebunmi O, Galadima NB, Idris MS, Nnodi UN, Afolabi AS, Ogbadu GH (2009) Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. Afr J Biotechnol 8(20):5395

Pandey P, Verma MK, De N (2018) Chitosan in agricultural context–a review. Bull Environ Pharmacol Life Sci 7(4):87–96

Pollock BM, Toole VK (1961) After ripening, rest period, and dormancy. USDA Yearbook of Agriculture. GPO, Washington, DC, p 106

Porter RH, Durrell M, Romm HJ (1947) The use of 2, 3, 5-triphenyl-tetrazolium chloride as a measure of seed germinability. Plant Physiol 22(2):149

Potts SE, Lumpkin TA (1997) Cryopreservation of *Wasabia* spp. seeds. Cryo-Letters 18:185

Pratap V, Sharma YK (2010) Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo*). J Environ Biol 31(5):721

Pritchard HW, Nadarajan J (2008) Cryopreservation of orthodox (desiccation-tolerant) seeds. In: Plant cryopreservation: a practical guide. Springer, New York, NY, pp 485–501

Rajjou L, Gallardo K, Debeaujon I, Vandekerckhove J, Job C, Job D (2004) The effect of α-amanitin on the Arabidopsis seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. Plant Physiol 134(4):1598–1613

Rehman HU, Basra SM, Farooq M (2011) Field appraisal of seed priming to improve the growth, yield, and quality of direct-seeded rice. Turk J Agric For 35(4):357–365

Riefler M, Novak O, Strnad M, Schmülling T (2006) Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. Plant Cell 18(1):40–54

Risca IM, Fartais L, Stiuca P (2007) Ultrasound effects contributions on the Norway spruce seeds germination (*Picea abies (L.) Karsten*). Gen Biol Mol 8:87–88

Roberts HA, Feast PM (1972) Fate of seeds of some annual weeds in different depths of cultivated and undisturbed soil. Weed Res 12(4):316–324

Rogozhin VV, Kuriliuk TT, Filippova NP (2000) Change in the reaction of the antioxidant system of wheat sprouts after UV-irradiation of seeds. Biofizika 45(4):730–736

Sacilik K (2007) Effect of drying methods on thin-layer drying characteristics of hull-less seed pumpkin (*Cucurbita pepo L.*). J Food Eng 79(1):23–30

Schmidt L (2000) Guide to the handling of tropical and subtropical forest seed. Danida Forest Seed Centre, Humlebaek

Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Adv Agron 42:43–83

Sera B, Spatenka P, Sery M, Vrchotová N, Hruskova I (2010) Influence of plasma treatment on wheat and oat germination and early growth. IEEE Trans Plasma Sci 38(10):2963–2968

Sharma KK, Singh US, Sharma P, Kumar A, Sharma L (2015) Seed treatments for sustainable agriculture-a review. J Appl Nat Sci 7(1):521–539

Sikhao P, Taylor AG, Marino ET, Catranis CM, Siri B (2015) Development of seed agglomeration technology using lettuce and tomato as model vegetable crop seeds. Sci Hortic 184:85–92

Smith AE, Miller R (1987) Seed pellets for improved seed distribution of small-seeded forage crops. J Seed Technol 11:42–51

Smith DM, Larson BC, Kelty MJ, Ashton PM, Bowersox TW (1997) The practice of silviculture: applied forest ecology. John Wiley & Sons, New York, NY

Sosnin EA, Stoffels E, Erofeev MV, Kieft IE, Kunts SE (2004) The effects of UV irradiation and gas plasma treatment on living mammalian cells and bacteria: a comparative approach. IEEE Trans Plasma Sci 32(4):1544–1550

Sudhakar N, Murugesan K (2011) Induction of systemic resistance in *Lycopersicon esculentum* cv. PKM1 (tomato) against Cucumber mosaic virus by UV-B irradiation on calli. Arch Phytopathol Plant Protect 44(5):460–474

Sudhakar N, Nagendra-Prasad D, Mohan N, Hill B, Gunasekaran M, Murugesan K (2011) Assessing the influence of ozone in tomato seed dormancy alleviation. Am J Plant Sci 2(3):443

Tatipata A (2009) Effect of seed moisture content packaging and storage period on mitochondria inner membrane of soybean seed. J Agric Technol 5(1):51–64

Taylor AG, Harman GE (1990) Concepts and technologies of selected seed treatments. Annu Rev Phytopathol 28(1):321–339

Taylor AG, Klein DE, Whitlow TH (1988) SMP: solid matrix priming of seeds. Sci Hortic 37 (1-2):1–1

Thanos CA, Georghiou K, Passam HC (1989) Osmoconditioning and ageing of pepper seeds during storage. Ann Bot 63(1):65–70

Thomas JMG, Boote KJ, Allen LH, Gallo-Meagher DJM (2003) Elevated temperature and carbon dioxide effects on soybean seed composition and transcript abundance. Crop Sci 43:1548–1557

Thomashow LS, Weller DM (1998) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis var. tritici*. J Bacteriol 170:34993508

Tripp R, Louwaars N, van der Burg WJ, Virk DS, Witcombe JR (1997) Alternatives for seed regulatory reform: an analysis of variety testing, variety regulation and seed quality control. Agricultural Research and Extension Network. Network Paper No. 69. Overseas Development Institute (ODI), London

Van Loon LC, Bakker PA, Pieterse CM (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36(1):453–483

Ventura L, Donà M, Macovei A, Carbonera D, Buttafava A, Mondoni A (2012) Understanding the molecular pathways associated with seed vigour. Plant Physiol Biochem 60:196–206

Vibhuti CS, Bargali K, Bargali SS (2015) Seed germination and seedling growth parameters of rice (*Oryza sativa* L.) varieties as affected by salt and water stress. Indian J Agric Sci 85(1):102–108
Volin JC, Denes FS, Young RA, Park SM (2000) Modification of seed germination performance through cold plasma chemistry technology. Crop Sci 40(6):1706–1718
Warghane RS, Rajkumar E (2017) Review on design and optimization of thresher in crop cutting machine. Int J MechEng Technol 8(11):1020–1028
Warham EJ, Sutton BC, Butler LD (1996) Seed testing of maize and wheat: a laboratory guide. CIMMYT, Mexico
Wharton MJ (1955) The use of tetrazolium test for determining the viability of seeds of the genus Brassica. Proc Int Seed Test Assoc 20:81–88
Wi SG, Chung BY, Kim JS, Kim JH, Baek MH, Lee JW, Kim YS (2007) Effects of gamma irradiation on morphological changes and biological responses in plants. Micron 38(6):553–564
Willan RL (1985) FAO Forestry Paper 20/2-A guide to forest seed handling: with special reference to the tropics. Food and Agricultural Organisation, Rome
Yadav RDS (2018) Influence of seed encrustation on germination, establishment band seed yield in rapeseed mustard. Int J Curr Microbiol App Sci Spec Issue 7:3539–3541
Yilmaz A, Boydak E (2006) The effects of cobalt-60 applications on yield components of cotton (*Gossypium barbadense L.*). Pak J Biol Sci 9:2761–2769
Yousaf Z, Saleh N, Ramazan A, Aftab A (2016) Postharvesting techniques and maintenance of seed quality. In: New challenges in seed biology: basic and translational research driving seed technology. InTech Open, Rijeka, p 113
Zhou Z, Huang Y, Yang S, Chen W (2011) Introduction of a new atmospheric pressure plasma device and application on tomato seeds. Agric Sci 2(1):23–27

Natural Products for Alternative Seed Treatment

18

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Abstract

The demand for products with less impact on the environment and human health has restricted the use of synthetic chemicals in seed treatment against pests and pathogens. Therefore, natural products and antagonistic organisms appear as alternatives. Numerous cases of success in the use of alternative practices have been reported in the seed treatment of different plant species, including equivalent effectiveness to synthetic products used conventionally. Seeds of important agricultural crops such as corn, soybeans, beans, wheat, and sorghum already have alternative products with good results. Among the most efficient products in controlling seed pests, diatomaceous earth and plant powders stand out for the control of weevils and plant extracts and essential oils for the control and repellency of various pests. Regarding the control of microorganisms, it is possible to emphasize the action of plant extracts, essential oils, and natural products of animal origin, such as chitosan and propolis, in the control of many fungal pests of seeds. Inoculation with antagonist organisms, such as *Trichoderma* and *Bacillus*, in addition to being efficient in controlling plant seed pathogens, has stimulating action during the initial plant development. However, as the advancement in alternative seed treatment is relatively recent, research is still needed to complement the existing information. Among the areas of high demand are the identification of action mechanisms on target organisms,

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effects of alternative treatments on physiological seed quality, product stability during seed storage and action spectrum against unwanted organisms in seeds.

Keywords

Seed protection · Diatomaceous earth · Plant powder · Plant extract · Essential oil

18.1 Introduction

The growing concern of society with health and the environment demands changes to the current system of agricultural production to make it more sustainable. The search for food free from pesticides and more attractive prices has resurrected the model of organic production, redeeming social, ecological, and environmental issues of agriculture (Niederle and Almeida 2013).

It is estimated that the market for organic products was approximately $97 billion in 2017, occupying an area of approximately 70 million hectares, which is an expansion of 2.3 times in area cultivated from 10 years ago. Among the countries with the largest areas intended for organic livestock production are Australia, Argentina, and China (Sahota 2019; Willer et al. 2019).

However, despite the demand and expansion of the organic production area, there are bottlenecks that limit the farmers' transition from conventional production systems. One of the main challenges faced is the scarce availability of seeds and seedlings suited to organic production systems. In addition to the lack of material adapted to the conditions of cultivation, most seeds receive treatments with synthetic pesticides before they are marketed, preventing their use in organic agriculture.

The seed is the main agricultural input that the farmer depends on for the success of the activity. It houses genetic characteristics obtained by natural and artificial selection, the latter being performed by farmers and researchers over thousands of years, representing incalculable value for humanity. These changes are fundamental to the development of cultivars or varieties more adapted to the conditions of cultivation. Among the important characteristics for the relationship between the seed and biotic components of the agroecosystem, resistance to pests and diseases stands out.

Low quality seed can reduce seedling emergence speed, increase sensitivity to stress, and give rise to plants with low competitive ability for water, solar radiation, and nutrients. As a result, there is a reduction in plant stand, a condition that usually limits the productive potential of majority of the cultivated species (Marcos-Filho 2015).

Seed quality may be affected by several factors, of which biological contamination demands special attention. This is because in addition to harming the physical and physiological quality, seeds contaminated by pathogens and/or insects can introduce new pests and diseases in the community. As a consequence, farmers need to identify the presence of new unwanted organisms in the agroecosystem (Dalla Pria and Silva 2010).

The use of fungicides and insecticides has been, for decades, the most usual and effective way for seed treatment in conventional agriculture (Vanzolini et al. 2000). However, the prohibition of the use of synthetic products in organic agriculture generates demand for alternative products in seed treatment. In this perspective, extracts, powders, and essential oils of natural origin, as well as antagonist organisms have been highlighted. Regardless of the type of alternative control, efficacy in the inhibition of unwanted organisms without compromising on physiological seed quality is being sought. The following are aspects related to alternative treatment for the control of insect pests and pathogens in seeds of cultivated species.

18.2 Alternative Treatments for the Control and Repellency of Pests in Stored Seeds

The use of natural products with repellent and insecticidal action is not a recent technique. Pyrethrin, rotenone, cevadine, veratridine, and ryanodine are examples of substances widely known and used as botanical insecticides, due to their efficiency and low persistence in the environment and toxicity to mammals (Aguiar-Menezes 2005). However, since the ease of acquisition, implementation, and efficiency of organic synthetic insecticides that originated in the green revolution, the use of natural insecticides has virtually ceased (Fazolin et al. 2016).

Because they are often made by mixing several substances, products of plants may have different action mechanisms in insects and modes of application (Melo et al. 2014; Borsonaro et al. 2013). The action mechanisms are highly dependent on the plant species and the physical and chemical properties of its constituents (Bruneton 1999). The mode of application varies according to the type of action (repellency, deterrence, ovicide, larvicide, and pupacide, insect hormone analogs, and food deterrents) that it is expected from the product and its formulation (Kedia et al. 2015).

Among the most studied products in recent years in the control of seed pests, diatomaceous earth (Lorini 2001; Silva et al. 2012), dried and milled or calcined plant products (Bini and Simonetti 2016; Guimarães 2014), and extracts and essential oils of plant origin stand out (Mateus et al. 2017; Campos et al. 2014).

18.2.1 Diatomaceous Earth in the Management of Seed Pests

Diatomaceous earth is one of the main alternative products for pest storage control today, being effective, especially in the control of coleopterans, such as *Acanthoscelides obtectus* (Say), *Rhyzopertha dominica* (F.), *Sitophilus zeamais* (Motsch.), *S. oryzae* (L.), *S. granarius* (L.), *Tribolium confusum* (J. du Val), *T. Castaneum* (Herbst), *Tenebrio molitor* (L.), *Zabrotes subfasciatus* (Boh.), and *Cryptolestes ferrugineus*.

Diatomaceous earth is an inert powder obtained from fossilized diatoms having carbon dioxide silica as the main ingredient. Silica adheres to the insect body by

electrostatic charge, favoring the loss of water through the removal of epicuticular wax and causing death by dehydration (Korunic 1998; Lorini 2001). Another action is due to obstruction of insect blowholes or penetration into its body through the respiratory system, causing death by asphyxiation in an interval of 1–7 days, depending on the pest species (Lorini et al. 2001). Due to its action mechanism, the physical properties of diatomaceous earth have a direct relationship with insect mortality (Korunic 1997).

Diatomaceous earth action is considered durable, losing little efficiency in the course of seed storage (Lorini 2001). Another advantage compared to most alternative products is the safety of the product for the users, as evidenced by the fact of constituting additives in animal feed and human nutrition in certain countries (Banks and Fields 1995). In addition, it does not react with other substances in the seed or those present in the environment, producing no toxic wastes (Korunic 1998) and not interfering negatively in the seed physiological quality (Oliveira et al. 2018; Smiderle and Cícero, 1999).

Several formulations of diatomaceous earth are marketed in the world, differing in their effectiveness in insect control. In addition to the product characteristics, there is also heterogeneity in susceptibility of target organisms. The efficiency of diatomaceous earth as an insecticide varies according to its morphological, physical, and chemical characteristics and species of insect pest (Korunic 1998; Rosseto et al. 2014) (Table 18.1).

In general, particle size of <12 μm, high content of amorphous SiO_2, and lower density elevate the activity of diatomaceous earth in insects (Korunic 1997) (Table 18.1). However, regardless of the characteristics mentioned above, diatomaceous earth tends to be highly efficient in the control of beetles even at low doses.

So far, there have been no reports in the literature regarding pest insect resistance to diatomaceous earth. By presenting a physical mode of action primarily on insect control to genetic resistance, it is unlikely to occur with this type of product, constituting one more advantage of diatomaceous earth in relation to other products of natural and/or synthetic origin.

18.2.2 Plant Powder and Ash in the Management of Seed Pests

In addition to the crushing of dried plant material to obtain vegetable powder, it is possible to employ calcination for obtaining ash. The latter is perhaps the easiest and most practical and economic way to use alternative products for the management of seed pests. However, high temperature necessary for the calcination process volatilizes a great part of the molecules with repellent and insecticide potential present in the original material. For this reason, the action mechanism of ash in insects is much more physical than chemical, causing suffocation and dehydration.

In addition to vegetable ashes, plant powder also leverages insect mortality by the additional effect of physical order, resembling diatomaceous earth. Thus, in addition to the toxicity of the hazardous constituents of powder, it may also act due to insect

Table 18.1 Use of diatomaceous earth from different geographic locations for the control of S*itophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) in stored wheat

Diatomaceous earth (DE)	Type	DE tapped Density (g L^{-1})	Particle size		SIO_2 content (%)	LC_{90} (ppm)	Insect species
			Mean (μm)	Below (μm)			
Celite 209 (USA)	Marine	222	8.2	65.0	87	565 (395 ± 857)	*S. oryzae*
DE Macedon	Marine	230	9.7	62.8	>80	709 (485 ± 1101)	*S. oryzae*
DE Japan 2	Marine	230	13.1	46.3	>80	762 (554 ± 1105)	*S. oryzae*
DE Japan 3	Marine	230	7.5	75.7	>80	1157 (891 ± 1567)	*S. oryzae*
Celite 209 (USA)	Marine	222	8.2	65.0	87	417 (328 ± 529)	*T. castaneum*
DE Macedon	Marine	230	9.7	62.8	>80	575 (445 ± 745)	*T. castaneum*
DE Japan 2	Marine	230	13.1	46.3	>80	631 (506 ± 787)	*T. castaneum*
DE Japan 3	Marine	230	7.5	75.7	>80	563 (430 ± 737)	*T. castaneum*
DE Australia	Fresh water	220	11.1	57.8	80–90	849 (612 ± 1239)	*S. oryzae*
Dicalite (USA)	Fresh water	218	10.4	57.4	80–90	1013 (766 ± 1402)	*S. oryzae*
DE China 7	Fresh water	234	16.4	34.7	>85	1356 (978 ± 1982)	*S. oryzae*
DE Mexico 2	Fresh water	328	N/A	N/A	N/A	1810 (1191 ± 2945)	*S. oryzae*
DE China 9	Fresh water	325	9.3	61.0	>88	1250 ppm = 74%	*S. oryzae*
DE Japan B	Fresh water	320	31.8	20.8	>80	1250 ppm = 82%	*S. oryzae*
Perma Guard (USA)	Fresh water	286	10.7	62.7	8.0	1475 (972 ± 1394)	*S. oryzae*
Melocide DE 100 (USA)	Fresh water	500	11.1	54.8	7.2	1500 ppm = 62%	*S. oryzae*
DE China 18	Fresh water	679	7.5	67.6	65–70	2000 ppm = 61%	*S. oryzae*
DE China 22	Fresh water	606	8.9	62.7	60–70	1750 ppm = 17%	*S. oryzae*
DE China 16	Fresh water	370	32.3	10.7	>88	1250 ppm = 0%	*S. oryzae*
DE Australia	Fresh water	220	11.1	57.8	80–90	694 (633 ± 1332)	*T. castaneum*
Dicalite (USA)	Fresh water	218	10.4	57.4	80–90	954 (699 ± 1286)	*T. castaneum*
DE China 7	Fresh water	234	16.4	34.7	>85	1500 (1100 ± 2591)	*T. castaneum*
DE Mexico 2	Fresh water	328	N/A	N/A	N/A	1250 ppm = 82%	*T. castaneum*
DE China 9	Fresh water	325	9.3	61.0	70–80	1250 ppm = 50%	*T. castaneum*

(continued)

Table 18.1 (continued)

Diatomaceous earth (DE)	Type	DE tapped Density (g L^{-1})	Particle size		SIO_2 content (%)	LC_{90} (ppm)	Insect species
			Mean (μm)	Below (μm)			
DE Japan B	Fresh water	320	31.8	20.8	>80	1250 ppm = 43%	*T. castaneum*
Perma Guard (USA)	Fresh water	286	10.7	62.7	93	1000 ppm = 43%	*T. castaneum*
Melocide DE 100 (USA)	Fresh water	500	11.1	54.8	83.6	1500 ppm = 47%	*T. castaneum*
DE China 18	Fresh water	679	7.5	67.8	65–70	2000 ppm = 48%	*T. castaneum*
DE China 22	Fresh water	606	8.9	62.7	60–70	1750 ppm = 14%	*T. castaneum*
DE China 16	Fresh water	370	32.3	10.7	>80	1250 ppm = 8%	*T. castaneum*

Source: (Adapted from Korunic 1997)

blowhole obstruction (Denloye 2010) or degradation of the epicuticular wax that covers them.

Ofuya and Dawodu (2002) demonstrated a direct relationship between insect mortality and particle size of plant powder. The thinner granulometry presented better distribution and adhesion to seed surface and the wall of the storage container, increasing the extent of insect contact toxicity. Although they exist, there are still few studies evaluating the influence of the size of the plant powder on target organism mortality.

There is also a shortage of studies assessing the insecticidal and repellent effect of ash from different species and plant organs, making understanding of the influence of these factors on the effectiveness difficult and consequently taking a decision on the choice of the product. Silica and potassium oxide, for example, are considered key elements in insect control using plant ash, and the concentration of both varies depending on the species and plant organ used. In addition, other elements and compounds present in plants can also influence their activity in pest insects.

The effect of the products applied in seed is another area that lacks studies and information. This is due to the priority given to the evaluation of the effect of products on pests, its activity on non-target organisms falling behind. Alternative treatment can negatively influence physiological seed quality, depending on the characteristics of the natural product and dose.

Despite the lack of studies that allow the understanding of important interactions among natural products, seeds, and pests, some of them are already widely used by farmers. Both plant powder and diatomaceous earth, for example, are employed with success in the control of beetle pests of stored seeds and grains, especially in corn and bean crops. A brief relationship of plant species, used in the form of powder, recommended for seed treatment is presented in Table 18.2.

Plant powder formulation for pest control has an advantage over liquid formulation because they are easier to obtain and use. This is due to the fact that the production of plant powder only requires procedures for drying and milling and does not need any solvent or specific equipment employed for obtaining extracts and essential oils.

In addition to being efficient in controlling many pests of stored seeds, plant powders and diatomaceous earth have important insect repellent action, preventing the insect from approximating the seed. However, the persistence of repellency during seed storage depends on the characteristics of the used product (Oliveira et al. 2018).

Repellency has a fundamental importance for the reduction of storage pest infestation. Most of them have cross-infestation characteristics and may have beginning both in the field of cultivation and in deposits of seed storage (Jiménez et al. 2017). Thus, the ability of these products to repel insects contaminating the seeds since cultivation can contribute to the reduction of undesirable population in storage places.

Table 18.2 Plant species used in the form of powder recommended for seed treatment

Culture	Insect pest	Plant species	Part used	Dose	Reference
Zea mays	*Sitophilus zeamais*	*Allium sativum*	Bulbils	5 g/kg seeds	Carvalho et al. (2019)
		Corymbia citriodora	Leaves (Ash)		
Phaseolus vulgaris	*Zabrotes subfasciatus*	*Rosmarinus officinalis*	Aerial part	5 g/kg seeds	Tonini et al. (2010)
		Cinnamomum verum	Aerial part		
		Syzygium aromaticum	Aerial part		
		Ocimum basilicum	Aerial part		
	Acanthoscelides obtectus	*Cinnamomum zeylanicum*	Bark	10 g/kg seeds	Oliveira et al. (2018)
		Corymbia citriodora	Stem (Ash)		
		Piper nigrum	Grains	4 g/kg seeds	
Vigna unguiculata	*Callosobruchus maculatus (Frab.)*	*Lippia sidoides Cham*	Fruits	110 g/kg seeds	Castro et al. (2010)
		Piper tuberculatum Jacq.	Leaves and stalks		
		Sapindus saponaria L.	Leaves and seeds		
	Sitophilus spp.	*Citrus reticulata*	Bark	6 g/kg seeds	Lima et al. (1999)

18.2.3 Plant Powder and Essential Oils in the Management of Seed Pests

Extracts and essential oils are composed of several substances, with numerous physical and chemical characteristics. In studies of seed alternative treatment, the fraction of greater volatility tends to be prioritized, due to its high bioactivity. Among chemical classes, monoterpenes, sesquiterpenes, and phenylpropanoids stand out (Regnault-Roger et al. 2012), which bring together many highly effective substances at insect control and repellency.

Both extracts and essential oils usually are costlier than plant powder. Between the first two, while extracts involve relatively simple techniques such as infusion, grinding, or maceration, essential oils require more complex techniques and equipment. Essential oil of plant origin can be obtained by hydro distillation, steam distillation, dry distillation, extraction with organic solvent, supercritical CO_2, or plant cold pressing (Valentim and Soares 2018; Regnault-Roger et al. 2012). Despite

greater difficulty in production, both extracts and essential oils are easier to apply than powder, allowing better distribution and homogeneity in seed mass.

Another advantage of liquid formulation compared with solid is the ease of the first admit the selection of the fractions containing chemical classes of greater bioactivity. The extraction method can directly interfere in the composition of the products obtained in relation to those present in the matrix (Lawless 2013). The steam destillation, for example, allows the selection of low-molecular-weight metabolites and exclusion of high-molecular-weight molecules (Regnault-Roger et al. 2012). Thus, the fraction obtained by steam destillation may present a higher concentration of active ingredients than oven dried plant powder.

The plant extract can also undergo fractioning, and liquid–liquid extraction may be carried out to select the most bioactive fraction according to its polarity. When the toxic or repellent substances are known, it is possible to employ equivalent polarity solvents to separate them from inert substances or low activity in the target organism. Therefore, this also allows the production of a more concentrated product than powder.

For this reason, care with phytotoxicity in seeds must be redoubled, since extracts and essential oils, especially, tend to have high concentrations of active components. These higher concentrations can cause the solution to impair seed embryonic cells, even after dilution. Several studies have suggested care in applying products due to negative effects on germination and seed vigor (Table 18.3).

The high concentration and diversity of bioactive compounds in extracts and essential oils, compared with plant powder, also results in different modes of action on target insects, including the occurrence of cytotoxic disturbances, deterrents, fumigants, ovicides, larvicides, pupacides, hormonal analogs of insects, food deterrents, and repellent action (Kedia et al. 2015).

However, extracts and plant oils have been more extensively used for the control of fungal and bacterial diseases, and on a smaller scale, for the control of pest insects. This might occur due to the high efficiency of plant powder in insect control and low efficiency in the control of seed plant pathogens.

18.3 Alternative Control of Plant Pathogens in Seeds

Several plant pathogens affect seeds, impairing the germination and vigor as well as interfering in plant growth and development. Fungi are the most numerous and important organisms that affect seed quality, which is an important source of dissemination and survival for the phytopathogen, whose viability is maintained during seed storage.

Seed treatment is important for preserving the genetic potential and preventing the spread of seed pathogens. Chemical control has been the most widely used method to protect seeds from pathogens. However, many synthetic products can negatively influence seed quality (Osman and Abdulrahman 2003), increase production costs, or provide the emergence of resistant isolates. In addition, there is a

Table 18.3 Plant species used in the form of essential oils recommended for seed treatment

Culture	Insect pest	Plant species	Part used	Dose	Physiological quality	Reference
Zea mays	*Sitophilus zeamais*	*Peumus boldus*	Leaves	4% (v/v)	Not impaired	Rodríguez et al. (2015)
		Laurelia sempervirens		4% (v/v)	Not impaired	
		Laureliopsis philippiana		4% (v/v)	Not impaired	
		Ricinus communis	Seeds	40 mL/kg seeds	Impaired	Wale and Assegie (2015)
		Artemisia princeps	Leaves	0.5 mL/kg seeds	Impaired	Liu et al. (2006)
		Cinnamomuncamphora	Seeds	0.5 mL/kg seeds	Impaired	
Sorghum bicolor		*Citrus sinensis*	Orange fruit peel	2.5 mL/kg seeds	Impaired	Gebreegziabiher et al. (2017)
		Azadirachtaindica	Seeds	2.5 mL/kg seeds	Impaired	
Phaseolus vulgaris	*Acanthoscelides obtectus*	*Azadirachtaindica*	Grains	3.0 mL/kg seeds	Not impaired	Oliveira et al. (2018)
	Zabrotes subfasciatus	*Glycine max*	Grains	7.0 mL/kg seeds	Impaired	Garcia et al. (2000)
		Licania rigida	Grains	9.0 mL/kg seeds	Not impaired	Queiroga et al. (2012)
		Ricinus communis	Grains	9.0 mL/kg seeds	Not impaired	
		Glycine max	Grains	9.0 mL/kg seeds	Not impaired	
		Gossypium	Grains	3.0 mL/kg seeds	Not impaired	Funichello and Santos (2017)
		Eucalyptus globulus	Leaf	0.5 mL/kg seeds	–	França et al. (2012)
		Eucalyptus citriodora	Leaf	0.5 mL/kg seeds	–	

demand for more sustainable products and lower environmental impact by restricting the use of synthetic chemicals.

The diversity of active components present in natural products, for example in derivatives from medicinal plants, tends to have lower environmental impact and reduce risks to select isolates of resistant pathogens, as well as opens up new and promising possibilities for seed treatment (Christian 2007). However, there is a paucity of information and alternative products for the treatment of seed pathogens.

18.3.1 Plant Derivatives in the Treatment of Seed Plant Pathogens

Natural products from plants have great potential in phytosanitary control due their antibacterial, antifungal, and insecticidal activities. Thus, the use of plant derivatives may represent an important option for seed treatment and for controlling or reducing the infection caused by pathogens. However, care should be taken to prevent damage to seed physiological quality.

Several studies have demonstrated the biological activity of plant derivatives, including extracts, powders, hydrolates, and essential oils, in the control of plant pathogens in seeds of several cultivated species. The essential oil of thyme (*Thymus vulgaris*), for example, has effect on endophytic fungi *Alternaria alternata*, *Alternaria infectoria*, *Aspergillus flavus*, *Epicocum nigrum*, and *Fusarium poae* (Anžlovar et al. 2017). All these colonizing pathogens were controlled with the essential oil of thyme, without harming the quality of wheat seeds.

The treatment of sorghum seeds (*Sorghum bicolor*) with aqueous extract of *Agave sisalana* showed 75% inhibition of fungi of the genus *Fusarium*in and was more efficient than tebuconazole-, enilconazole-, and fludioxonil-based fungicides. Besides the inhibitory effect on the fungi, the ethanolic extract of *Agave sisalana* had stimulating action on vigor, increasing the dry weight of sorghum seedlings by 35% (Andresen et al. 2015).

The aqueous extract of neem (*Azadirachta indica*) at concentrations between 160 and 200 g L^{-1} reduced the presence of contaminants in tomato seeds, demonstrating its antimicrobial effect. In these initial concentrations, the physiological quality of tomato seeds was maintained and did not reduce seedling germination rate and vigor (Cardozo and Pinhão Neto 2019).

Hydroalcoholic extracts of turmeric (*Curcuma longa* L.) and rosemary (*Rosmarinus officinalis* L.) controlled *Fusarium* spp. and reduced the incidence of *Colletotrichum dematium* in soybean seeds (Coppo et al. 2017). Both extracts showed no side effects on the physiological quality of soybean seeds.

The essential oil of *Cedrus deodora* is effective in inhibiting *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Cladosporium cladosporioides, Curvularia lunata*, and *Paecilomyces variotti* in coriander seeds. The alternative treatment was more effective than synthetic fungicides, with no adverse effect on seed germination and seedling growth (Dikshit et al. 1983).

Aqueous extracts and macerated dried leaves of neem (*Azadirachta indica* A. Juss), chinaberry (*Melia azedarach* L.), and lemongrass (*Cymbopogon*

winterianus Jowitt) inhibited *Penicillium*, whereas garlic (*Allium sativum* L.) inhibited *Fusarium* spp. and *Penicillium* in maize seeds at concentrations of 13.3–20 mL and 2–3 g kg, respectively (Gurgel et al. 2018).

Essential oils of *Caesulia axillaris* and *Mentha arvensis* were efficient at >600 ppm in wheat seed treatment. The application of the alternative treatment provided 96% protection on *Aspergillus flavus* inoculated in wheat seeds stored for up to 12 months (Varma and Dubey 2001).

Studies that employed the use of essential oils and plant extracts for seed treatment reported control or significant reduction of several seed pathogens (Cardozo and Pinhão Neto 2019; Leite et al. 2018; Santos 2018). However, they can cause negative effects on germination and vigor. Lettuce seeds, for example, show sensitivity to *Mentha pulegium* L. (Shiraishi et al. 2002). Common bean (*Phaseolus vulgaris* L.) has seeds sensitive to the essential oils of sweet orange (*Citrus vulgaris*), clove (*Syzygium aromaticum)*, Tahiti lime (*Citrus latifolia)*, and lemongrass (*Cymbopogon citratus*). Similarly, the aqueous extract of cinnamon (*Cinnamomum zeylanicum*) and the essential oil of clove basil (*Ocimum gratissimum*) reduced the viability and vigor of sorghum seeds (Flávio et al. 2014). Regardless of concentration, the mint extract reduced the germination and vigor of *Plantago major* seeds (Bonfim et al. 2011).

Paradoxically, some extracts may have a positive effect on growth and initial development of seedlings. The aqueous extract of coriander (*Coriandrum sativum*), for example, increased the fresh weight of the aerial part and the number of leaves and seedlings of lettuce (Carmello and Cardoso 2018). In Table 18.4, the main plants with known potential are exhibited for seed treatment for the control of plant pathogens, with respective forms of use and species evaluated.

18.3.2 Natural Products of Animal Origin in the Treatment of Seed Plant Pathogens

In addition to products derived from plants, several other natural products have shown potential in seed treatment seeking the plant pathogen control. Some of these products are already very known for the effect and application in other areas, with more recent studies aiming at their use in agriculture. An example is chitosan, which consists of a polymer derived from the deacetylation of chitin—a structural component of crustaceans, insect exoskeletons, and fungal cell walls.

Chitosan acts in plant protection due to its antimicrobial and phytoalexin defense mechanism (Xing et al. 2015). One of the main foci of the use of chitosan in seed treatment has been its application to nanoparticles (Choudhary et al. 2017). Chitosan nanoparticles have proved to be effective in the control of different pathogens common in seeds, such as species belonging to genera *Aspergillus*, *Rhizopus*, and *Colletotrichum* (Sotelo-Boyás et al. 2016). Another potential use of chitosan is biofilm production to the seed coating, which can be combined with agents of biological disease control, such as the *Trichoderma* fungus (Chandrika et al. 2019).

Table 18.4 Treatment with plant derivatives in plant pathogen control in seeds of cultivated species

Bioactive plant	Forms of use	Species treated	Plant pathogen	Reference
Piper nigrum (black pepper)	Essential oil	*Phaseolus vulgaris*	*Macrophomina phaseolina (Tassi) Goid.*	Santos (2018)
Cymbopogon martinii (palmarosa)	Essential oil	*Zea mays; Phaseolus vulgaris; Glycine max*	*Alternaria carthami, Alternaria* sp. *Rhizoctonia solani*	Hillen et al. (2012)
Zingiber officinalie (ginger)	Essential oil	*Glycine max*	*Cladosporim* sp., *Rhizopus* sp., *Fusarium* spp.	Gonçalves et al. (2009)
Allium sativum (garlic)	Hydroalcoholic extract	*Zea mays*	*Fusarium proliferatum*	Souza et al. (2007)
Grass Cymbopogon citratus (lemon grass)	Hydroalcoholic extract	*Zea mays*	*Fusarium proliferatum*	Souza et al. (2007)
Propolis	Aqueous extract	*Cucumis sativus*	*Aspergillus* sp.	Souza et al. (2017)
Cinnamomum zeylanicum (cinnamon)	Essential oil	*Phaseolus vulgaris*	*Aspergillus* sp., *Penicillium* sp.	Leite et al. (2018)
Curcuma longa L. (*Turmeric*) *and Rosmarinus officinalis L.* (rosemary)	Hydroalcoholic extract	*Glycine max*	*Fusarium* sp.	Coppo et al. (2017)
Allium sativum (allho)	Aqueous and dried macerated extract	*Zea mays*	*Fusarium* sp.	Gurgel et al. (2018)
Ocimum gratissimum (clove basil)	Essential oil	*Sorghum bicolor*	*Penicillium, Aspergillus Rhizopus, Alternaria, Curvularia, Fusarium* sp	Flávio et al. (2014)
Mentha arvensis (mint)	Essential oil	*Triticum aestivum*	*Aspergillus flavus*	Varma and Dubey (2001)
Thymus vulgaris (thyme)	Essential oil	*Triticum aestivum*	*Alternaria alternata, Alternaria infectoria, Aspergillus flavus, Epicocum nigrum and Fusarium poae*	Anžlovar et al. (2017)
Ocimum, Artemisia, Rosmarinus and Zingiber	Hydrolate	*Triticum aestivum*	*Helminthosporium sativum, Rhizopus, Aspergillus* e*and Penicillium*	Cruz et al. (1999)

(continued)

Table 18.4 (continued)

Bioactive plant	Forms of use	Species treated	Plant pathogen	Reference
Azadirachta indica (neem)	Aqueous extract	*Solanum lycopersicum* (*Tomato*)	Several	Cardozo and Pinhão Neto (2019)
Agave sisalana (sisal)	Aqueous extract	*Sorghum bicolor*	*Fusarium* spp.	Andresen et al. (2015)
Momordica charantia (bitter gourd)	Aqueous extract	*Pterogyne nitens* (bush peanut)	*Fusarium sp., Cladosporium sp., Curvularia sp. and Alternaria sp.*	Medeiros (2013)
Allium sativum (allho)	Aqueous extract	*Daucus carota* subsp. *sativus* (carrot)	*A. alternata and A. dauci*	Rentschler (2014)
Citrus sinensis L. Osbeck) (orange peel)	Essential oil	*Daucus carota* subsp. *sativus* (carrot)	*A. alternata and A. dauci*	Rentschler (2014)
Ocimum gratissimum (clove basil)	Essential oil and leaf powder	*Triticum aeestivum*	*Bipolaris sorokiniana*	Rodrigues et al. (2006)
Black pepper	Aqueous extract	*Glycine max*	*Aspergillus flavus*	Alves et al. (2015)

Wheat seed coating with chitosan reduces infection by *Fusarium graminearum*. In addition, it increases seedling resistance, stimulating the accumulation of phenolic compounds and lignin (Ruan and Xue 2002). The treatment of sugar beet and tomato with chitosan induces seedling resistance, reducing the incidence of damping off caused by the fungus *Rhizoctonia* sp. (Mazaro et al. 2009).

Another product that has demonstrated potential in seed treatment is propolis, which consists of resin. The material is formed by a complex of substances that bees collect from plants, whose purpose is to protect the colony from diseases. There are several biological activities already reported in the literature for propolis, including cytotoxic, antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant activities (Bankova et al. 2014).

Recent studies have demonstrated the potential of propolis in plant pathogen control, especially due to its antimicrobial effect (Diba et al. 2018; Matny 2015). Although studies of propolis in seed treatment is still in its infancy, promising results have been obtained in the control of fungi, such as *Penicillium* sp. (Souza et al. 2017).

Mixtures of natural products have also demonstrated effect on seed treatment for the control of plant pathogens. One example comes from the synergy between

chitosan and the ethanolic extract of propolis against the fungus *Fusarium circinatum* in seeds and seedlings of *Pinus* spp. (Silva-Castro et al. 2018).

18.3.3 Seed Microbiolization for Plant Pathogen Control

Microbiolization consists of the use of microorganisms or their metabolites in seed protection for the control of diseases and/or to promote plant growth and development (Junges et al. 2014). The inoculation of microorganisms in seeds is very known and studied in agriculture, especially with nitrogen-fixing bacteria, mycorrhizal fungi, and growth promoters. Although microbiolization is employed with success in several cultures of agronomic interest currently, its use in seed treatment for plant pathogen control is less common.

Among organisms used in microbiolization for plant pathogen control in cultivated species are fungi of the genus *Trichoderma* (Junges et al. 2014). This is one of the most studied fungi and used in the biological control of plant diseases, presenting a direct effect on the pathogen and in some cases by increasing the culture efficiency (Xue et al. 2017). The efficiency of the use of *Trichoderma* in seeds has been reported for several cultures and plant pathogens (Swaminathan et al. 2016), among them *Fusarium oxysporum* in bean seeds (Carvalho et al. 2011).

Some bacteria have also shown efficiency in seed treatment for plant pathogen control, with emphasis on the genus *Bacillus* (Bezerra et al. 2013). Isolates of *Bacillus subtilis* controlled *Penicillium digitatum* in citrus, reducing fungal growth (Kupper et al. 2013). Tomato seeds microbiolized with isolates from plant growth-promoting rhizobacteria (*Bacillus* sp.) present a reduction in the severity of bacterial leaf spot caused by *Xanthomonas gardeneri* (Naué et al. 2014). Microbiolized rice seeds with isolates of *Pseudomonas*, *Bacillu*s, and *Stenotrophomonas* showed reduction of contamination and incidence of *Bipolaris oryzae* and *Rhynchosporium secalis* (Ludwig et al. 2009).

Microbiolization of wheat seeds promoted the control of *Bipolaris sorokiniana*, *Pyricularia oryzae*, *Dreschlera tritici-repentis*, and *Stagonospora nodorum*, with results higher to those obtained with synthetic fungicides (Luz 1998). The application of the antagonist *Trichoderma viride* in wheat seed bearer of *Helmintosporium sativum* reduced pathogen incidence, which did not differ from the treatment with the standard fungicide (iprodione + thiram) (Pessoa 1991).

The efficiency obtained by seed microbiolization with different agents and in agricultural cultures as well as its application as a growth promoter and in the control of plant pathogens stimulated advances in research. The obtained results demonstrate its importance as a promising strategy and sustainable development in seed treatment.

18.3.4 Final Considerations

Several natural products have shown potential for seed treatment and protection against pests and diseases, and in many cases without compromising their physiological quality. A large part of the studies involving the application of alternative methods is recent, evidencing the importance of the search for alternative products with lower environmental impact. In most cases, research is still needed to define the parts of plants with higher concentrations of active ingredients, the most efficient way for their insulation, the best way to use spectrum of action, product stability, effect on physiological seed quality and on non-target organisms, and involved action mechanisms. Finally, for several of these products whose efficacy in seed treatment has already been proven, further studies are still needed to facilitate their use by farmers.

References

Aguiar-Menezes EL (2005) Inseticidas botânicos: seus princípios ativos, modos de ação e uso agrícola. Embrapa Agrobiologia, Seropédica, p 58

Alves NMC, Castro LRO, Galle NBC, Silva MIP (2015) Avaliação da qualidade fisiológica e sanitária de sementes de soja tratadas com extrato vegetal. Enciclopédia Biosfera, Centro Científico Conhecer – Goiânia 11:37–20

Andresen M, Wulff EG, Mbega ER, Stokholm MS, Glazowska SE, Zida PE, Mabagala RB, Lund OS (2015) Seed treatment with an aqueous extract of *Agave sisalana* improves seed health and seedling growth of sorghum. Eur J Plant Pathol 141:119–132

Anžlovar S, Likar M, Koce JD (2017) Antifungal potential of thyme essential oil as a preservative for storage of wheat seeds. Acta Bot Croat 76:64–71

Bankova V, Popova M, Trusheva B (2014) Propolis volatite compounds: chemical diversity and biological activity: a review. Chem Cent J 48:1–8

Banks HJ, Fields PG (1995) Physical methods for insect control in stored-grain ecosystems. In: Jayas DS, NDG W, Muir WE (eds) Stored grain ecosystems. Marcell Dekker, New York, pp 353–409

Bezerra AG, Macedo AD, Nascimento OI, Sousa PT, Costa BN, Sousa ARFL (2013) Uso de *Bacillus* spp. no controle de fitopatógenos em sementes de soja variedade BRS valiosa RR. Agroecossistemas 5:68–73

Bini LF, Simonetti APMM (2016) Controle de caruncho do milho com óleo de neem. Revista Cultivando O Saber 1:23–31

Bonfim FPG, Honório ICG, Casali VWD, Fonseca MCM, Mantovani-Alvarenga E, Andrade FMC, Pereira AJ, Gonçalves MG (2011) Potencial alelopático de extratos aquosos de *Melissa officinalis* L. e *Mentha* x *villosa* L. na germinação e vigor de sementes de *Plantago major L.* Revista Brasileira de Plantas Medicinais 13:564–568

Borsonaro MT, Senô KCA, Iamaguti PS, Neves MCT, Silva PT (2013) Extrato aquoso de folhas de neem (*Azadirachta indica* A. Juss) no controle de *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae) em milho armazenado. Nucleus 10:161–168

Bruneton J (1999) Pharmacognosie: phytochimie, plantes medicinales, vol 3. Lavoisier Tech & Doc, Paris, pp 421–499

Campos ACT, Radunz LL, Radunz AL, Mossi AJ, Dionello RG, Ecker SL (2014) Atividade repelente e inseticida do óleo essencial de carqueja doce sobre o caruncho do feijão. Revista Brasileira de Engenharia Agrícola e Ambiental 18:861–865

Cardozo LVF, Pinhão Neto MV (2019) Extrato de neem no tratamento de sementes de tomate. Revista Verde de Agroecologia e Desenvolvimento Sustentável 14:01–04

Carmello CR, Cardoso JC (2018) Effects of plant extracts and sodium hypochlorite on lettuce germination and inhibition of *Cercospora longissima* in vitro. Sci Hortic 234:245–249

Carvalho CDD, Mello SCM, Júnior ML, Silva MC (2011) Controle de *Fusarium oxysporum* f.sp. *phaseoli* in vitro e em sementes, e promoção do crescimento inicial do feijoeiro comum por *Trichoderma harzianum*. Trop Plant Pathol 36:028–034

Carvalho JH, Bonome LTS, Leal IL, Moura GS, Kruppa MF, Martins BR, Portolan IB (2019) Utilização de Derivados Vegetais e Minerais no Controle do Gorgulho do Milho. Cadernos de Agroecologia 14:1–3

Castro MJP, Silva PHS, Santos JR, Silva JAL (2010) Efeito de pós vegetais sobre a ovoposição de *Callosobruchus maculatus* (Frab.) (Coleoptera Bruchidae) em Feijão-caupi. Bioassay 5:1–4

Chandrika KSVP, Prasad RD, Godbole V (2019) Development of chitosan-PEG blended films using Trichoderma: enhancement of antimicrobial activity and seed quality. Int J Biol Macromol 126:282–290

Choudhary RC, Joshi A, Kumari S, Rv K, Saharan V (2017) Preparation of Cu-chitosan nanoparticle and its effect on growth and enzyme activity during seed germination in maize. J Pharmacognosy Phytochem 6:669–673

Christian EJ (2007) Plant extracted essential oils as a contact fungicide seed treatment for organic corn. A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of Master of Science. Iowa State University, Ames, Iowa

Coppo JC, Mioranza TM, Coltro-Roncato S, Stargalin JR, Kuhn OJ, Schwan-Estrada KRF (2017) Sanidade e germinação de sementes de soja tratadas com extratos de plantas e de fungo. Revista de Ciências Agroambientais 15:93–99

Cruz MES, Schwan-Estrada KRF, Inoue MH, Ávila MR, Batista MA, Stangarlin JR (1999) Água aromatizada de plantas medicinais no tratamento de sementes de trigo. Fitopatol Bras 24:278

Dalla Pria M, Silva OC (2010) Antracnose. In: Dalla Pria M, Silva OC (eds) Cultura do feijão: doenças e controle, 2nd edn. Editora UEPG, Ponta Grossa, pp 49–56

Denloye AA (2010) Bioactivity of powder and extracts from garlic, *Allium sativum* L. (Alliaceae) and spring onion, *Allium fistulosum* L. (Alliaceae) against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) on cowpea, *Vigna unguiculata* (L.)Walp (Leguminosae) seeds. Psyche 2010:1–5

Diba K, Mahmoodi M, Hashemi J (2018) In vitro activity of Propolis alcoholic extract on opportunistic pathogenic fungi. Int J Res Appl Basic Med Sci 4:68–73

Dikshit A, Dubey NK, Tripathi NN, Dixit SN (1983) Cedrus oil - a promising storage fungitoxicant. J Stored Prod 19:159–162

Fazolin M, Alécio MR, Estrela JLV, Alves SB (2016) Controle alternativo de pragas para o cultivo e armazenamento de feijões. In: Mattar EPL, Oliveira E, Santos RC, Siviero A (eds) Feijões do vale do Juruá. IFAC, Rio Branco, p 336

Flávio NSD, Sales NLP, Aquino CF, Soares EPS, Aquino LFS, Catão HCRM (2014) Health and physiological quality of sorghum seeds treated with aqueous extracts and essential oils. Semina Ciências Agrárias 35:7–20

França SM, Oliveira JV, Filho ABE, Oliveira CM (2012) Toxicidade e repelência de óleos essenciais a *Zabrotes subfasciatus* (Boheman) (Coleoptera, Chrysomelidae, Bruchinae) em grãos de *Phaseolus vulgaris* L. Acta Amazon 42:381–386

Funichello M, Santos LG (2017) Efeito de óleo vegetal de algodão no controle de *Zabrotes subfasciatus* (Coleoptera: Bruchidae) em sementes de feijão. Colloquium Agrariae 13:76–81

Garcia J, Veloso VRS, Duarte JB, Kamada T (2000) Eficiência de produtos alternativos no controle *Zabrotes subfasciatus*, e seus efeitos sobre a qualidade das sementes de Phaseolus vulgaris. Pesquisa Agropecuária Tropical 2:39–42

Gebreegziabiher K, Wakgari M, Goftishu M (2017) Evaluation of some botanicals oils for the management of maize weevil, *Sithophiluszeamais* Motsch. (Coleoptera: Curculionidae). Int J Life Sci 5:10–20

Gonçalves GG, Mattos LPV, Morais LAS (2009) Óleos essenciais e extratos vegetais no controle de fitopatógenos de grãos de Óleos essenciais e extratos vegetais no controle de fitopatógenos de grãos de soja. Hortic Bras 27:102–107

Guimarães SS (2014) Ação repelente, inseticida e fagoinibidora de extratos de pimenta dedo-de-moça sobre o gorgulho do milho. Agric Entomol 8:322–328

Gurgel LMS, Moura JF, Santos AMG, Rosa RCT, Assis TC (2018) Qualidade Fisiológica e Sanitária de sementes de milho tratadas com produtos alternativos em condições de armazenamento. Anais da Academia Pernambucana de Ciência Agronômica 15:127–136

Hillen T, Schwan-Estrada KRF, Mesquini RM, Cruz MES, Stangarlin JR, Nozaki M (2012) Atividade antimicrobiana de óleos essenciais no controle de alguns fitopatógenos fúngicos in vitro e no tratamento de sementes. Revista Brasileira de Plantas Medicinais 14:439–445

Jiménez JC, La Fuente M, Ordás B, Domínguez LEG, Malvar RA (2017) Resistance categories to *Acanthoscelides obtectus* (Coleoptera: Bruchidae) in terapy bean (*Phaseolus acutifolius*), new sources of resistance for dry bean (*Phaseolus vulgaris*) breeding. Crop Prot 98:255–266

Junges E, Bastos OB, Toebe M, Muller J, Pedroso CD, Muniz BFM (2014) Restrição hídrica e peliculização na microbiolização de sementes de milho com *Trichoderma* spp. Comunicata Scientiae 5:18–25

Kedia A, Prakash B, Mishra PK, Singh P, Dubey NK (2015) Botanical as eco friendly biorational alternatives of synthetic pesticides against *Callosobruchus* spp. (Coleoptera: Bruchidae) – a review. J Food Sci Technol 52:1239–1257

Korunic Z (1997) Rapid assessment of the insecticidal value of diatomaceous earth without conducting. J Stored Prod Res 33:219–229

Korunic Z (1998) Review diatomaceous earths, a group of natural insecticides. J Stored Prod Res 34:87–97

Kupper CK, Cervantes LLA, Klein NM, Silva CA (2013) Avaliação de microrganismos antagônicos, *Saccharomyces cerevisiae* e *Bacillus subtilis* para o controle de *Penicillium digitatum*. Rev Bras Frutic 35:425–436

Lawless J (2013) The encyclopedia of essential oils: the complete guide to the use of aromatic oils in aromatherapy, herbalism, health & well-being. Conari-Press, San Francisco, p 228

Leite K, Bonome LTS, Moura GS, Franzener G (2018) Essential oils in the treatment of *Phaseolus vulgaris* L. seeds during storage. Revista Verde de Agroecologia e Desenvolvimento Sustentável 13:186–199

Lima HF, Bruno RLA, Bruno GB, Bandeira ISA (1999) Avaliação de produtos alternativos no controle de pragas e na qualidade fisiológica de sementes de feijão macassar armazenadas. Revista Brasileira Engenharia Agrícola e Ambiental 3:49–53

Liu CH, Mishra AK, Tan RX, Tang C, Yang H, Shen YF (2006) Repellent and insecticidal activities of essential oils from *Artemisia princeps* and *Cinnamomumcamphora* and their effect onseed germination of wheat and broad bean. Bioresour Technol 97:1969–1973

Lorini I (2001) Manual técnico para o manejo integrado de pragas de grãos armazenados. Embrapa trigo, Passo Fundo, p 80

Lorini I, Ferreira FA, Barbieri I, Demaman NA, Martins RRD (2001) Terra de diatomáceas como alternativa no controle de pragas de milho armazenado em propriedade familiar. Agroecologia e Desenvolvimento Rural Sustentável 2:32–36

Ludwig J, Moura AB, dos Santos AS, Ribeiro AS (2009) Microbiolização de sementes para o controle da mancha-parda e da escaldadura em arroz irrigadoMicrobiolização de sementes para o controle da mancha-parda e da escaldadura em arroz irrigado. Trop Plant Pathol 34:322–328

Luz WC (1998) Microbiolização das sementes: uma comparação com o tratamento químico no controle dos principais patógenos das sementes de trigo. Pesq Agrop Brasileira 33:509–513

Marcos-Filho J (2015) Fisiologia de sementes de plantas cultivadas. ABRATES, Londrina, PR, p 659

Mateus AE, Azevedo FR, Alves ACL, Feitosa JV (2017) Potencial da *Moringa oleifera* como inseticida no controle de adultos de *Sitophilus zeamais* (Coleoptera: Curculionidae) em grãos de milho armazenados. Acta Iguazu 6:112–122

Matny ON (2015) Efficacy evaluation of Iraqi propolis against gray mold of stored orange caused by *Penicillium digitatum*. Plant Pathol J 14:153–157

Mazaro SM, Júnior AW, Santos I, Citadin I, Possenti JC, Gouvêa AD (2009) Controle do tombamento de plântulas de beterraba e tomate pelo tratamento de sementes com quitosana. Pesq Agrop Brasileira 44:1424–1430

Medeiros JGF, Araujo Neto AC, Medeiros DS, Nascimento LC, Alves EU (2013) Extratos vegetais no controle de patógenos em sementes de *Pterogyne nitens* Tul. Floresta e Ambiente 20:384–390

Melo BA, Molina-Rugama AJ, Leite DT, Godoy MS, Araujo EL (2014) Bioatividade de pós de espécies vegetais sobre a reprodução de *Callosobruchus maculatus* (FABR 1775) (Coleoptera: bruchidae). Biosci J 30:346–353

Naué CR, Rocha DJA, Moura AB (2014) Biological control of tomato bacterial spot by seed microbiolization. Trop Plant Pathol 39:413–416

Niederle PA, Almeida L (2013) A nova arquitetura dos mercados para produtos orgânicos: o debate da convencionalização. In: Niederle PA, Almeida L, Vezzani FM (eds) Agroecologia: práticas, mercados e políticas para uma nova agricultura. Kairós, Curitiba, p 393

Ofuya TI, Dawodu EO (2002) Aspects of insecticidal action of *Piper guineese* Schum and Thonn fruit powders against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Nigerian J Entomol 19:40–50

Oliveira MR, Bonome LTS, Bittencourt HVH, Zarowni E, Lefchak LS (2018) Alternative treatments in bean seeds for repelling *Acanthoscelides obtectus* (SAY). J Seed Sci 40:362–369

Osman KA, Abdulrahman HT (2003) Risk assesment of pesticide to human andthe environment. Saudi J Biol Sci 10:81–106

Pessoa MNG (1991) Controle de *Helminthosporium sativum* através do tratamento biológico de sementes de trigo (*Triticum aestivum* L.). Summa Phytopathol 17:22

Queiroga MFC, Gomes JP, Almeida FCA, Pessoa EB, Alves NMC (2012) Aplicação de óleo no controle de *Zabrotes subfasciatus* e na germinação de *Phaseolus vulgaris*. Revista Brasileira de Engenharia Agrícola e Ambiental 16:777–783

Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: low-risk produtcs in a high-stakes world. Annu Rev Entomol 57:405–424

Rentschler, LPA (2014) Qualidade Fisiológica e uso de extratos e óleos essenciais vegetais no controle de *Alternaria* spp. em sementes de cenoura. Dissertação (Mestrado) – Universidade Estadual do Norte do Paraná, Campus Luiz Meneghel, pp 49

Rodrigues EA, Schwan-Estrada KRF, Stangarlin JR, Scapim CA, Fiori-Tudida ACG (2006) Potencial da planta medicinal *Ocimum gratissimum* no controle de *Bipolaris sorokiniana* em sementes de trigo. Acta Sci Agron 28:213–220

Rodríguez CH, Mendoza CR, Morales IB, Aguayo GS, Parra AU, Cares IF, Bolaños LM, Maciel JCR, Tejeda AL, Navarrete EP, Salazar LB (2015) Bioactivity of P*eumus boldus* Molina, *Laurelia sempervirens* (Ruiz & Pav.) Tul. and *Laureliopsis philippiana* (Looser) Schodde (Monimiacea) essential oils against *Sitophilus zeamais* Motschulsky. Chilean J Agric Res 3:334–340

Rosseto C, Lorini I, Ventura MU (2014) Composição química de formulações de terra de diatomáceas usadas no controle de pragas de grão armazenados. In: Anais Conferência Brasileira de Pós-Colheita. Londrina, Maringá, pp 284–289

Ruan SL, Xue QZ (2002) Effects of chitosan coating on seed germination and salt-tolerance of seedlings in hybrid rice (*Oryza sativa* L.). Acta Agron Sin 28:803–808

Sahota A (2019) The global market for organic food & drink. In: Willer H, Lernoud J (eds) The world of organic agriculture. Statistics & emerging trends 2019. FiBL/IFOAM, Frick and Bonn/ Berlin, pp 25–35

Santos PL (2018) Manejo de *Macrophomina phaseolina* (Tassi) Goid. em sementes de feijoeiro (*Phaseolus vulgaris* L.) com óleos essenciais e antagonistas. Tese (Doutorado), Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, 76p

Shiraishi S, Watanabe I, Kuno K, Fujii Y (2002) Allelopathic activity of leaching from dry leaves and exudate from roots of ground cover plants assayed on agar. Weed Biol Manag 2:133–142

Silva DFG, Ahrens DC, Paixão MF, Sokara Neto F, Romel CC, Comiran F, Nazareno NRX, Coelho CJ (2012) Tratamento de milho em grão e espiga com pós inertes no controle do gorgulho do milho *Sitophilus zeamais*. Revista Brasileira de Agroecologia 7:143–151

Silva-Castro I, Diez JJ, Martín-Ramos P, Pinto G, Alves A, Martín-Gil J, Martín-García J (2018) Application of bioactive coatings based on chitosan and propolis for *Pinus* spp. protection against *Fusarium circinatum*. Forests 9:1–14

Smiderle OJ, Cícero SM (1999) Tratamento inseticida e qualidade de sementes de milho durante o armazenamento. Sci Agric 56:1245–1254

Sotelo-Boyás ME, Bautista-Baños S, Correa-Pacheco ZN, Jiménez-Aparicio A, Sivakumar D (2016) Biological activity of chitosan nanoparticles against pathogenic fungi and bacteria. Chitosan Preserv Agric Commod 13:339–349

Souza AEF, Araújo E, Nascimento LC (2007) Atividade antifúngica de extratos de alho e capim santo sobre o desenvolvimento de *Fusarium proliferatum* isolados de grão de milho. Fitopatol Bras 32:465–470

Souza EP, Moscato BS, Perino FHB, Nakada-Freitas PG, Blumer S, Cardoso AII, Bonini CSB (2017) Doses de extrato de própolis no controle do fungo *Aspergillus* sp. e no tratamento de sementes de pepino. Brazilian J Biosyst Eng 11:360–364

Swaminathan J, van Koten C, Henderson HV, Jackson TA, Wilson MJ (2016) Formulations for delivering *Trichoderma atroviridae* spores as seed coatings, effects of temperature and relative humidity on storage stability. J Appl Microbiol 120:425–431

Tonini M, Pacheco FP, Nóbrega PLH, Rosa DM, Mauli MM (2010) Potencial repelente de plantas aromáticas e condimentares no tratamento de feijão carioca armazenado. Associação Brasileira de Pós Colheita 5:468–472

Valentim JA, Soares EC (2018) Extração de óleos essenciais por arraste a vapor: um kit experimental para o ensino de química. Química nova na escola 40:297–301

Vanzolini S, Torres RM, Panizzi RC (2000) Efeito do tamanho, da densidade e do tratamento fungicida sobre a qualidade de sementes de amendoim. Revista Ceres 47:603–612

Varma J, Dubey NK (2001) Efficacy of essential oils of *Caesulia axillaris* and *Mentha arvensis* against some storage pests causing biodeterioration of food commodities. Int J Food Microbiol 68:207–210

Wale M, Assegie H (2015) Efficacy of castor bean oil (*Ricinus communis* L.) against maize weevils (*Sitophilus zeamais* Mots.) in northwestern Ethiopia. J Stored Prod Res 63:38–41

Willer H, Lernoud J, Kemper L (2019) The world of organic agriculture 2019: summary. In: Willer H, Lernoud J (eds) The world of organic agriculture. Statistics & emerging trends 2019. FiBL/IFOAM, Frick and Bonn/Berlin, pp 25–35

Xing K, Zhu X, Peng X, Qin S (2015) Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. Agron Sustain Dev 35:569–588

Xue AG, Guo W, Chen Y, Siddiqui I, Marchand G, Liu J, Ren C (2017) Effect of seed treatment with novel strains of *Trichoderma* spp. on establishment and yield of spring wheat. Crop Prot 96:97–102

Advances in Big Data Analytics and Applications in Seed Technology

19

Isaac O. Daniel

Abstract

Over the last decade, advances in computer science and computational capabilities have enabled the analysis of massive multi-dimensional datasets to implement decision support systems for generating actionable insights in real time. It is the big data revolution. Big data concepts have been impacting the seed value chain as global seed systems move from development to market-driven models. This chapter deals with big data applications in the crop improvement industry and seed technology in particular as it evolves into modern production pipeline seed systems. The objectives of the chapter are to: (1) review the basics of big data in relation to seed technology and (2) analyze a conceptual framework for seed technology workflow scheme to deliver data-driven seed analytics and applications. The review identified possibilities of using unstructured datasets of seeds to draw insights for crop improvement decisions. The analysis of digital images and sensor datasets of seeds with machine learning algorithms to extract patterns and features from the unstructured datasets will enable data assimilation and feature identification for seed phenotyping. The high precision seed phenotype datasets can in turn be used as inputs for data-driven seed analytics. A conceptual framework is used to illustrate this idea for automation and high-throughput data-driven seed applications. At the application level, use case examples of seed analytics relevant to the seed technology workflow are presented. On the side of seed production, the framework can use seed production datasets to generate insights into seed quality traceability. Likewise, integrating seed phenotype datasets from the seed images with data from genomics and environmental domains can be used to derive insights for smart breeding.

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Keywords

Big data technologies · Image-based phenomics · Machine learning · Data-driven seed applications

19.1 Introduction

Big data concepts refer to recent advances in computer science and computational enhancements for capture, analysis, storage, administration and interpretation of large datasets. That makes data an abundant asset in the modern knowledge economy. Holst (2019) estimated global data generation to be 41 zettabytes by the end of 2019 (Fig. 19.1). For agriculture, an example of big data application is installed capabilities in modern models of farm machines for navigation systems, sensors and internet of things (IoT) for data collection and transmission to centralized servers or cloud storage (Xuan and Martin 2018). The possibility of rapidly generating farm-based data that can be integrated and analysed with larger datasets of diversified formats for decision making is the trigger for big data agriculture.

The dependence of modern agriculture on research makes data a necessary input for innovations and growth in the sector. In the last century, the research-driven crop improvement industry has created a thriving global seed industry valued at US$ 69.8 billion in 2019, which at the current growth rate is expected to reach US$ 86 billion in 2023 (Shahbandeh 2019). Seeds had been the commodity of trade in this industry

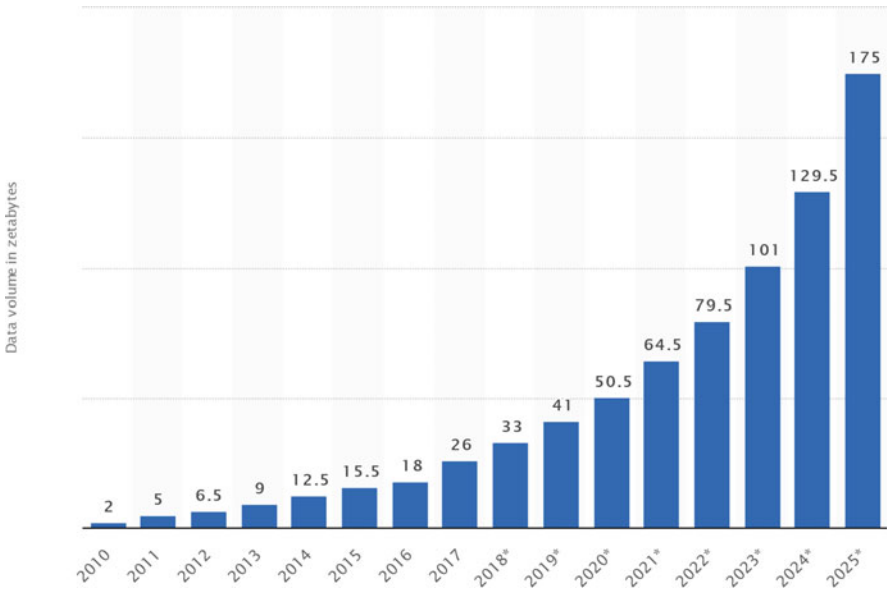

Fig. 19.1 Current growth of big data technology applications over a period of 15 years. (Source: Statista 2019 [Holst (2019). Statista])

because it is the means of delivering improved genetics to end users. In the US alone, farmers' investment in seeds has been around 35% of the seed market since 2010 (Dunn 2019). This is why seed technology gained prominence as a sub-discipline of crop science and agriculture in the last half century.

19.1.1 An Overview of Seed Technology and Data

Seed technology can be defined as a field of science that deals with transferring the genetic quality gained from crop improvement projects to crop producers through seeds that are optimized for physical purity and physiological quality. The singular aim of seed technology is the delivery of quality seeds for crop production. However, seed quality is achieved by drawing from many fields of knowledge that makes up seed technology including plant reproductive systems, seed formation, chemistry, physiology, production, processing, certification, testing, storage, marketing and legislation (Copeland and McDonald 1995). In this chapter, seed technology workflows will be summarized into two frames, namely seed development and seed production based on the OECD (2019) scheme for most crops. Seed development comprises of seed formation, chemistry and physiology while seed production comprises seed testing, storage, marketing and policy (Fig. 19.2; Table 19.1).

There has been demand for data cuts across the seed technology value chain. Gene bank curators generate data from the accessions of seed germplasm that they maintain ex-situ as raw materials for pre-breeding of crops. Researchers and plant breeders work with germplasm resources in the gene banks to design and develop breeder seeds through repeated cycles of evaluations at various levels of sophistication of breeding methods, generating data in the process. Seed analysts conduct seed tests to evaluate and generate data on the genetic, physiological, pathological, analytical and proximate quality traits of the seeds. Seed producers also generate data as they multiply and enhance the improved seed genetics along the seed production pipeline (Table 19.1). The vision of seed technology researchers is to have all the data maintained in a way that they can be integrated for broad-based analytics, hence the quest for big data techniques (Daniel and Ajala 2003; Dell' Aquila 2007, 2009).

The use of big data technologies in various aspects of the seed industry's value chain has generated numerous innovations that are protected by intellectual property rights. However, as data gets increasingly democratized, the availability of more open source tools for seed technology analytics within big data frameworks extends the resources available to the public for seed innovations. This chapter is an effort to leverage big data and seed technology concepts. It serves to (1) review the theoretical concepts of big data as it relates to seed technology and (2) analyze a conceptual framework for seed technology workflow to deliver data-driven seed analytics applications.

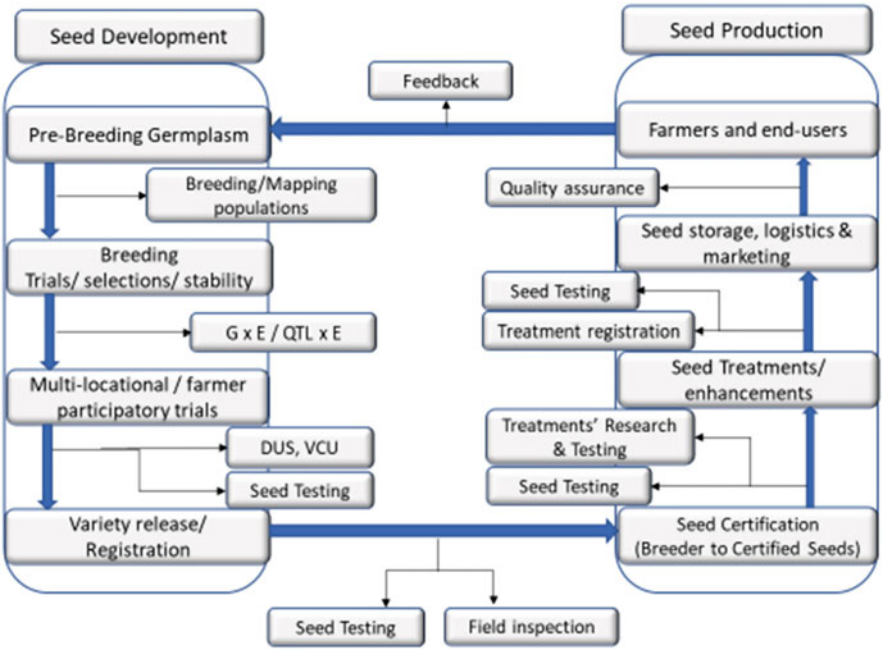

Fig. 19.2 Seed development and production stages in the seed technology workflow. Chart is an expanded form conventional OECD (2019) (Seed Schemes rules and regulations. https://www.oecd.org/agriculture/seeds/documents/oecd-seed-schemes-rules-and-regulations.pdf) seed scheme of pedigreed seed production for most crop types

19.2 Big Data Concepts for Seed Technology

Reviews show that big data does not necessarily refer to data that are difficult to analyse because of size, since some small data are more difficult to handle than larger datasets (MIT 2013; Tavlor and Schroeder 2015). Sivarajah et al. (2017) outlined a normative perspective big data features, namely data dimensions, processing and management. I will discuss big data concepts for seed technology based on these perspectives.

19.2.1 Data Dimensions

Data in big data context relates to three dimensions or the "3 Vs" of big data: volume, variety and velocity (Laney 2001). The veracity and value dimensions were added by Sonka (2014), and variability and visualization features of big data were added by Sivarajah et al. (2017). The first three V's had been used to model agriculture related workflows for big data (Chi et al. 2016; Kamilaris et al. 2017; Chandra Sekhar et al. 2018). Table 19.2 shows a suggestion of data dimensions in

Table 19.1 Modest data requirements and data generation within the seed technology workflow

Seed technology value stage	Activities	Data requirements and big data sources	Data generated for big data analytics
Seed development	Pre-breeding research	1. Germplasm data sets (Historical data, provenance, research and image data) 2. Climate data from weather stations 3. Seed storage data from seed store sensors and camera	1. Curation data (Accession, treatments, storage etc.) 2. Reference phenotype datasets 3. Reference genetic marker datasets 4. Reference genomic annotation datasets 5. Classification datasets 6. Seed quality testing (lab) datasets (genetic, physiological, moisture content, GMO etc.)
	Field evaluation cycles (depending on breeding methods)	1. Phenotype variation data from breeder's databases 2. Remote sensors, digital phenotype of plants data like NDVI 3. Genotyping datasets from breeders' databases, proprietary and open source genomics platforms 4. Environmental datasets from weather stations 5. Trait of interest association datasets from open source plant breeding platforms 6. Open source datasets from big-data platforms e.g. disease, economic advantage etc.	1. G × E datasets 2. Breeding population data 3. Parental material data 4. Trait selection data 5. Seed quality testing (lab) datasets 6. Trait stability datasets (DUS-NN/VCU tests) 7. Variety descriptors and variant estimation data
	Multilocational testing	1. Test location data from GPS, GIS, sensors, etc. 2. Participating growers/ researchers' datasets 3. Variety descriptive data (GIS or remote sensors) 4. Agronomic practices information (Grower's database) 5. Phytosanitary data (seed company/government open source) 6. Open source datasets from big-data platforms	1. Genotype × location × year crop performance data 2. Variety descriptors data for specific locations, NDVI data 3. DUS-NN data sets 4. VCU data sets 5. Seed multiplication data 6. Seed quality testing data

(continued)

Table 19.1 (continued)

Seed technology value stage	Activities	Data requirements and big data sources	Data generated for big data analytics
	Variety release/ registration	1. Variety descriptors (Breeders' databases, regulator's datasets) 2. Data from official registration procedures 3. DUS, novel trait, VCU datasets 4. Seed quality analysis data from seed lab databases	1. Breeders seed multiplication data 2. Seed licensing data 3. Seed market demand data
Seed Production	1. Seed multiplication classes (Certification) – Breeder seeds – Foundations – Registered – Certified	1. Seed source data from breeders and seed company 2. Seed genetics reference data 3. Seed testing (lab) data 4. Variety and pedigree data 5. Variety descriptors 6. Registration data	1. Seed lot number assigned 2. Seed tags and certificates data 3. Treatment, storage and handling data 4. Seed inspection datasets 5. Seed quality testing data (physiology, phytosanitary, moisture content, GMO, etc.)
	Contracts – Regulations – Financing – Agronomics	1. Breeder seed producer license 2. Regulatory information	1. Grower and contract details data 2. Field inspections datasets
		1. Financial plan data 2. Insurance data	1. Bank records 2. Cost of production 3. Profit/loss data 4. Tax/regulation datasets
		1. Field choice and rental data, field history data 2. Field soil fertility data Fertilizer/plant nutrition data 3. Field health	1. Plot land use data and location related datasets 2. Field management data 3. Phyto-sanitary data 4. Contractors/field labour data
	Processing/ Conditioning	Equipment, processing pipeline data	1. Equipment operation logs 2. Seed treatments 3. Storage 4. Seed quality testing data
	Marketing	1. Contract terms data 2. Market analysis data 3. Storage/treatment data 4. Logistics data	1. Sales and delivery data 2. Contract data 3. Seed quality testing data 4. Treatment, storage and handling data 5. Shipping data 6. Marketing data

terms of variety, volume and velocity for datasets derived from typical seed testing results.

In terms of volume, big data includes datasets that would be taking up space in terms of terabytes, petabytes, zettabytes and above. The capacity to gather data with IoT devices like sensors, mobile devices, aerial (remote sensing), software data logs, cameras, radio-frequency identification (RFID) readers and ultrasound sensors enhances large datasets (Barnaghi et al. 2013). For seeds, this is already a reality as many seed traits data are already being captured with Red Green Blue colour model (RGB) (Dell' Aquila 2007), thermal infrared sensors (Men et al. 2017), fluorescence sensors, gas sensors, and multi/hyperspectral sensor devices (El Masry et al. 2019) (Table 19.2). Thus, seed datasets fit big data analytics in terms of data volume dimension.

The variety dimension or heterogeneity of big data relates to the capacity of big data architectures to handle different datatypes of multiple data formats at the same time. This is one of the strengths and one of the management issues for big data (Labrinidis and Jagadish 2012). Tables 19.1 and 19.2 show that seed datasets can either be structured, that is, numbers organized in table formats of columns and rows as in spreadsheets and relational databases or unstructured formats like images, texts and other sensor datasets like noise, smell, etc.

Velocity relates to the speed of inflow of the large and heterogenous datasets into big data analytical structures to create new data or update the existing data (Chen et al. 2013). Velocity dimensions for seed testing datasets is made possible with the use of automated sensors to capture seed-related datasets (Table 19.2). Data velocity is important for seed analytics when the typical data acquisition combines data from multiple digital sensors with environmental data, seed production and storage history data, location data, weather data, and phenotype and genomics datasets to implement Phenome Wide Association Study (PheWAS) and Genome Wide Association Study (GWAS) (Mochida et al. 2018).

Big-data value is finding patterns in big heterogenous datasets to generate knowledge and applications. At the application level, data is trained to analyse future samples with machine learning algorithms (Sivarajah et al. 2017). Value for datasets in seed technology workflow would mean deriving applications to generate smart breeding insights from crop genome databases or applications generating seed quality insights from seed production datasets (Table 19.3).

19.2.2 Big Data Processing Algorithms

Algorithms are a sequence of instructions used to solve a problem. They are developed by human programmers to instruct computers' tasks. The ultimate goal of algorithms that run on big data environments is to process data by training computers to learn from the data so as to do new tasks beyond human cognition unsupervised or through machine learning (ML) (Sonka 2016; Wolfert et al. 2017).

ML models are advanced statistical and analytical tools that allow computers to learn pattens from data using combinations of algorithms. ML algorithms are generally divided into three: classification, clustering and predicting algorithms

Table 19.2 Big data dimensions of datasets from typical seed testing results

Seed testing category	Tests	Data dimensions		
		Data variety	Data volume*	Data velocity
Analytical/physical	Purity	Image (RGB) Sensor (multispectral) Measurements	H H L	H H L
	Sizing	Imaging (RGB) Measurements	H M	H L
	Texture	Image (RGB, SEM)	H	H
	Moisture content	Estimates Sensor measurement Image [near infrared (NIR)]	L H H	L H H
	1000 grain weight	Balance measurement	L	L
	Nutritional quality	Estimates (proximate) Image (NIR)	L H	L H
Germination	Viability	Image Thermal/ infrared (IR) Estimates	H H L	H H L
	Seedling	Image	H	H
	Conductivity	Measurement	M	M
Vigour	Cold test	Estimates	L	L
	Accelerated Ageing	Estimates	L	L
	Tetrazolium	Image (RGB, NIR)	H	H
Genetic	DUS	Estimates	L	L
	VCU	Estimates	L	L
	Molecular markers	Estimates	H	H
	Bioassays/GMOs	Measurements, Fluorescence	H M	M M
	Novel traits	Estimates	H	M
Seed health	Ergot etc.	Image	H	H
General	Scanners	Labels/barcodes/NIR	H	H

FAO (2018). Module 3 Seed Toolkit: Seed quality Assurance. FAO/Africa Seed. ISBN 978-92-5-130951-3 (FAO)

**H* high, *M* medium, *L* low

(Fahad et al. 2014). Singh et al. (2016) presented a four-category ML algorithm scheme to phenotype plants in the field, including identification, classification, quantification and prediction. The capacity of ML to process unstructured datasets to produce visualised analytics or new inputs for another ML model makes it the

Table 19.3 Data value in terms of insights derivable

Seed technology value stage	Data	Value in terms of seed technology insights
Seed Development	*Germplasm data* Seed storage, Seed quality, Sensor data, Seed morphometric image datasets	Vegetation distribution insights Prediction of seed germination and vigour Decision support for gene bank management Classification for breeding, trait selection, and varietal verification decisions
	Breeding Phenotyping (PheWAS) Genomics (GWAS) Multi-locational testing datasets	Breeding decisions. Trait development and integration designing tools when data is integrated with genomics and environment datasets Searches for gene function, regulatory pathways and expression in cloud databases Seed and trait pipeline development tools
	Environment (climate, soil)	Phenotype, performance and yield prediction tools
	Variety release datasets	Data supported decision for variety descriptors and registration decisions
Seed production	Seed Class	Data-driven seed quality test
	Contracts/ regulations Financing Agronomics	Data-supported business decisions Data-driven financial decisions Data-driven farm management decision
	Processing	Data-driven processing pipeline optimization and maintenance
	Marketing	Data for market decisions and delivery logistics

model of choice for analysing crop big data (Galford et al. 2008; Mucherino et al. 2009; Vibhute and Bodhe 2012). Various ML algorithms had been used to implement analytics on seed technology datasets in various combinations, mostly involving principal component analysis (PCA), linear discriminant analysis (LDA), support vector machine (SVM), convolutional neural network (CNN) classification analysis, genomic best linear unbiased prediction (G-BLUP) and regression prediction analysis (Table 19.4).

The quest for high-throughput and automation of crop analytics has led to exploration of an advanced ML technique called deep learning. Deep learning works with neural network algorithms to provide for pattern recognition, which makes it an advanced artificial intelligence (AI) tool (Liu et al. 2005; Pound et al. 2017; Mochida et al. 2018). It uses a hierarchical learning process to extract high-level, complex abstractions as data representations. Moreover, deep learning provides capabilities for automating unsupervised learning processes, thus enabling high throughput analytics (Desarkar and Das 2017). There are neural networks for recognizing simple patterns like SVM, and there are neural networks with many layers of hidden nodes for complex pattern recognition like CNN. Various

Table 19.4 Big data algorithms and functionalities in the seed technology workflows

Seed technology workflow		Big data algorithm	Functionality	References
Seed development	Plant phenotyping	Python-based machine learning libraries (Scikit-image, OpenCV, SciPy and Scikit-learn)	Plant growth imaging with RGB cameras	Zhou et al. (2017)
	Seed phenotyping	Various classification models depending on seed analytics desired. Mostly, SVM and CNN	Multispectral imaging of physicochemical quality traits, predicting physiological parameters, detection of defect, pest infestation and seed health	El Masry et al. (2019)
	Phenotyping/ genomic prediction pipelining	Genomic best linear unbiased prediction (G-BLUP)	Plant phenotyping with RGB and UAV mounted NIR-GB cameras	Watanabe et al. (2017)
	Integration of environmental data	Hadoop DB, MapReduce, Cloudera etc., CNN, SVM, K- means	Remote sensing images and root imaging with hyperspectral cameras under drought	Chi et al. (2016) Cai et al. (2015)
Seed Production	Seed viability	Matrix Laboratory (MATLAB), SVM	Thermal/IR image analysis	Men et al. (2017)
	Seed germination	2-way analysis of variance (ANOVA) with Co-Stat.	Quantified LED vs Fluorescent light scheme effects	Sanoubar et al. (2018)
	Seed vigour prediction	PCA	Image analysis of seed vigour based on seed colour	Dell' Aquila (2007, 2009)
	Seed vigour prediction	LDA, SVM	E-nose sensor to detect germination and estimate vigour	Zhang et al. (2017a, b)
	Seed colour identification	MATLAB, PCA, CNN	Genotype discrimination by seed image metrics	Liu et al. (2005)
	Seed biochemistry	Regression analysis and Standard error of cross validation	Near infrared reflectance spectroscopy of seeds	Fassio et al. (2015)
	Seed morphometry	Machine learning with Python libraries (SciPy)/MATLAB	X-ray/μCT of grains and spike	Hughes et al. (2017)
	Historical data	Cloudera, Hadoop DB, MapReduce, Blockchain Etherium	Seed quality traceability	Salah et al. (2019)

combinations of both schemes of algorithms for pattern recognition have been engaged in analysing images and sensor datasets (Mochida et al. 2018). For seed technology, neural network algorithms have been used for identification of seeds for cultivar verification (Liu et al. 2005; Hughes et al. 2017; Lin et al. 2018).

19.2.3 Data Management

Data management is concerned with issues of balancing big data architecture for data governance (accuracy of maintaining data value), cost, security, sharing and ownership (Wolfert et al. 2017; Bronson and Knezevic 2016).

A typical big data architecture is designed for the efficient storage and management of large volumes of heterogenous data, which demands investment in hardware infrastructure support for running advanced algorithm software. Hardware support for big data are massive parallel storage systems, which may be distributed file systems, cluster file systems and parallel file systems. Examples include Lustre[1] and Hadoop Distributed File System (HDFS).[2] On top of that, we need framework for user-specific solutions where several tools have been developed. Apache Hive[3] is a distributed data warehouse framework for analysing data stored in HDFS and compatible systems using SQL-like language called HiveQL. Apache Pig[4] further simplifies complex data analysis using simpler scripting language targeting domain experts. Traditional relational database management systems handle structured datasets very well and often have difficulty handling big data because of their deficiency in horizontal scalability, which requires hard consistency and become very complex when dealing with large volume of heterogeneous data. Big data manages these conditions in datasets with non-relational databases (NoSQL), an alternative to SQL on big data platforms because they enable scalability and flexibility. The popular NoSQL database management systems include key-value stores, columnar databases, graph databases and document-oriented databases.

19.3 Conceptual Framework

The conceptual framework presented in this chapter is based on neural network classification of seed image datasets for a number of reasons. First, the drive of plant science researchers to automate and implement high throughput plant phenotyping systems has led to significant advances in image-based plant phenotype analytics (Singh et al. 2016). Moreover, the availability of repositories of pretrained image

[1]The OpenSFS and Lustre Community Portal. http://lustre.opensfs.org.

[2]The Apache Hadoop Project. http://hadoop.apache.org.

[3]The Apache Hive data warehouse software. http://hive.apache.org.

[4]The Apache Pig platform. http://pig.apache.org.

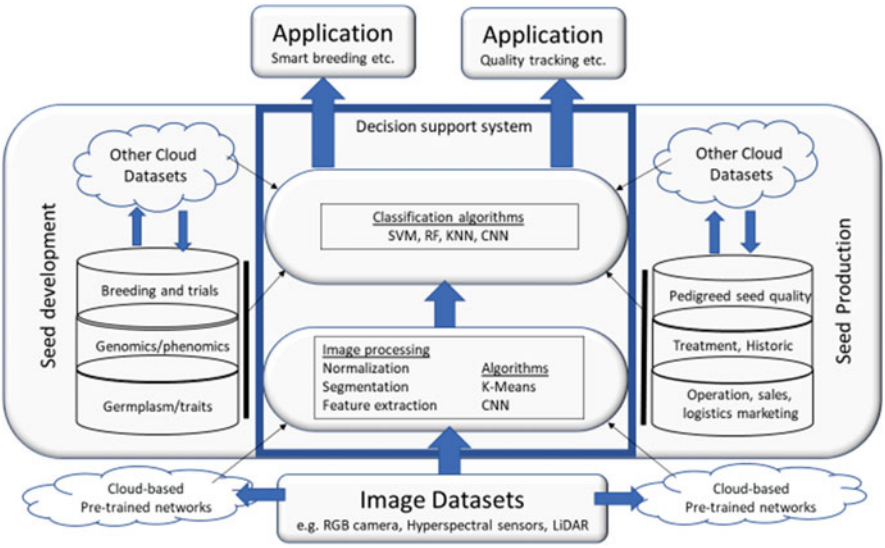

Fig. 19.3 A conceptual framework for big data application to seed technology

models for plant identification and CNN-based algorithms afford new tools for machine learning-based plant phenotyping for advancements in plant recognition and taxonomic classification (Mochida et al. 2018). Lastly, the robustness of CNN for unsupervised image analysis above other algorithms like SVM makes it the algorithm of choice for automation and high-throughput phenotyping (Lin et al. 2018).

The framework presents a decision support system driven by seed phenotype data to generate prediction applications (Fig. 19.3). The inputs into the system are seed digital image datasets derivable through algorithmic feature extraction processing of images of seed samples. CNN models are known to be capable of learning from images through complex matrix calculations of the features extracted to create datasets as output. In the big-data ecosystem, the seed phenotype data interacts with cloud data sources and data domains from seed development and seed production sectors.

A typical order of image-based analysis consists of preprocessing, segmentation, feature extraction and classification with various machine learning algorithms. The framework consists of mainly three layers: (1) source of seed image data from image sensor devices or high-capacity cameras, (2) decision support systems running various machine learning and neural network algorithms for feature extraction, pattern recognition and classification and (3) application layer where knowledge discovery is turned into insights and interpretations delivered to end users with visualisation (Fig. 19.3).

The methodology for the conceptual framework involves a seed image-driven approach adapted from typical plant phenotyping models (Mochida et al. 2018). The key steps in the preprocessing layer are segmentation and feature extraction, which is

done by quantifying pixel intensity with various matrix algebra algorithms depending on the previous preprocessing of the image. Researchers use various strategies to achieve high percentage of segmentation accuracy and feature extraction for various plant organs. For seeds, Hughes et al. (2017) achieved a high level of segmented image of wheat grains by setting adaptive thresholds on the images with MATLAB. For feature extraction, CNN deep learning algorithm is the most frequently used state-of-the-art tool when automated image processing of multiple samples is a requirement (Lin et al. 2018; Pound et al. 2017).

The classification module of the decision support system represents case-control phenotypes of the seed image taxonomic classification. This is where phenotype datasets and labelled pretrained image datasets integrate with other datasets in cloud networks. Exploration of associations among classification results will generate knowledge for the identification of samples. At the application level, outputs from classification analysis can be integrated with datasets from other data domains of the seed technology workflow like genomic datasets to conduct genome-wide association studies (GWAS) to generate insights for smart breeding when the seed phenotype data has been used to pretrain genomic datasets (Watanabe et al. 2017). For seed production insights, classification of the seed image with seed quality datasets in the production stage of the workflow can generate knowledge for quality tracking (Fig. 19.3).

19.4 Big Data Applications to Seed Technology

Integrating seed development with production datasets produces a highly heterogenous data dimension within the conceptual framework of the seed technology workflow, making it ideal for data-driven seed analytics. Examples of used cases of big data-driven applications based on seed image data within the conceptual framework will be discussed from three perspectives: (a) seed quality analytics and traceability, (b) digital imaging and high throughput phenotyping and (c) seed image data integration.

19.4.1 Seed Quality Analytics and Traceability

Wireless sensor nodes have enabled the IoT technology and increased the pace of building big data concepts for agricultural applications (Díaz et al. 2011; Jawhar et al. 2014; Brinis and AzouzSaidane 2016; Jawad et al. 2017). Perez-Sanz et al. (2017) provides a list of sensor-based whole plant datasets complete with camera types and software tools for segmentation, feature extraction and classification. Generally, RGB imaging is a fast means of gathering image data, although the classification in the colour spectrum may be limited when compared with other imaging schemes like infrared or ultraviolet light schemes.

Image sensing tools have provided opportunities for data-driven seed analytics as they are used to capture and analyse seed images for analysing systems at the

research level (Dell'Aquila 2006, 2007, 2009; Marcos-Filho et al. 2006). Image analysis was used by Dell'Aquila (2006, 2007) to confirm the possibility of estimating seed vigour and colour using the RGB colour scheme and PCA to classify seed deterioration state. More recently, Silva and Cicero (2014), using tomato germination sensor, reported that the parameters obtained by the computerised seedling analysis (seedling length and indexes of vigour and seedling growth) with software SVIS® are efficient to detect differences between tomato seed lots of high and low vigour. Zhang et al. (2017a) optimised a bionic nose to sense gases released during germination, which was confirmed by LDA and SVM to predict seed vigour.

Multispectral imaging techniques, integrating spectroscopy and imaging technologies with different multivariate chemometric analytics has been successfully implemented to assess seed proximate properties. They provide fast, accurate and non-destructive seed analysis depending basically on the resolution of the sensors and mathematical chemometric model. Moreover, due to their ability to explore internal properties of biological materials, several multispectral imaging techniques have become useful seed testing tools for evaluating chemical and nutritional compositions (Fassio et al. 2015; Silva-Perez et al. 2017). Besides biochemical and nutritional evaluation, spectrometric technologies have been employed in determining many other relevant seed quality traits for applications in seed phenotyping, quality monitoring, predicting physiological parameters like viability and vigour and detecting defective seeds, pest infestation and seed health (El Masry et al. 2019).

Classical spectroscopic techniques (fluorescence, visible and infrared and near infrared) are known to be generally quick and inexpensive to implement. They can be used in seed evaluation pipelining in automation and high-throughput phenotyping systems, and thus have been upscaled in proprietary state-of-the-art farm and laboratory equipment and seed analysis platforms. Qualysense™ introduced a seed image-driven system implementing deep neural network classification algorithms to create an industrial grade seed sorter which could inspect billions of seeds in grain lots in a matter of minutes and integrate the data to other datasets for seed quality analytics.[5] Moreover, proprietary high-throughput seed phenotyping platforms are being developed on big data algorithms; an example was Syngenta's SeedGerm™, a project to automate seed phenotyping to assess seed performance on a reliable, scalable and cost-effective platform.

Seed quality tracking is another data-driven application to seed technology. There are platforms enabling supply chain dealers to trace product quality and processes, enabling crops to be integrated to the international supply chain (Sawant et al. 2016). A recent paper on seed quality traceability proposes a blockchain peer to peer network with Etherium contracts without human supervision for tracing the quality of soybeans in the quality supply chain (Salah et al. 2019). Other workers evaluated neural network models for seed quality traceability. For example, scanning seed images creates reference datasets that can be used to train neural networks to verify

[5] https://www.qualysense.com/?production.

the genetic quality of seeds and tracking quality of seed products for verification, thus creating the potential to enhance product traceability through the supply chain (Armbruster and MacDonell 2014) and increasing food safety (Jóźwiaka et al. 2016). In this regard, big data-driven seed production streamlines seed production decisions, targeting the precise volume and precise genetics of seed to produce and supply to minimize carryover stock and maintain seed physiological quality.

19.4.2 Digital Imaging and High-Throughput Seed Phenotyping

Digital imaging is a veritable source of big data for the seed technology workflow according to the conceptual framework going by the availability of various sensor-based data-capturing devices and methods already available for phenotyping (Table 19.2). In the literature, digital phenotyping methodologies are significantly extending the throughput of data capturing of plant parts above manual trait measurements, which are slower and prone to errors (Fahlgren et al. 2015; Araus et al. 2018). One of the reasons being that image-based techniques are fast clicks of cameras to capture images for analysis based on the camera's configuration, capacity and processing software. Digital imaging therefore has potential to vastly increase the scale and throughput of plant phenotyping activities. Image-based plant phenotyping also involves non-invasive methodologies of capturing datasets, which is another reason it is gaining advantage over traditional invasive methods (Dell' Aquila 2007; Ubbens and Stavness 2017). Moreover, digital phenotyping aids automation of image collection and chain of actions when controlled by software-driven sensors. The combination of the new imaging technologies with AI and ML schemes has enabled robotic and conveyer-belt systems in greenhouses, drone-based imaging and ground-based and aerial imaging platforms in fields for plant phenotyping (Fahlgren et al. 2015; Mochida et al. 2018). As a result, high-throughput phenotyping platforms have been implemented; an example is PhenoArch, a phenotyping study platform capturing differences in daily growth among 254 maize hybrids in different soil and water conditions to locate the genetic loci affecting stomatal conductance through GWAS (Prado et al. 2018).

Sensor-based seed testing and phenotyping scale up seed technology for big data analytical frameworks in many ways that promote the possibilities of transfer, storage, management, analysis, and visualization of seed datasets. Zhang et al. (2018) developed a high-throughput seed phenotyping system that can estimate seed phenotypic traits and generate datasets from seed images acquired with commercial-grade cameras. Plant images taken with smartphones had also been investigated and used to generate plant phenotype datasets (Aquino et al. 2017; Confalonieri et al. 2017). Several classification algorithms have been used to discriminate seeds based on seed images, enabling possibilities of using machine vision to estimate various seed parameters within the seed technology workflow (Tables 19.2 and 19.4). For instance, Liu et al. (2005) segmented rice seed images with MATLAB and classified them with PCA and CNN networks in order to discriminate the genotypes. In another study, Hughes et al. (2017) used X-ray images

segmented with image thresholding strategies and modelled with python machine learning libraries to capture the spikes and grain morphometric parameters of wheat plants exposed to high temperatures under two different water treatments. The segmentation was based on the combinatorial use of adaptive thresholds and morphology algorithms applied to examine the spike and the grains. The technique has also been used for seed image analysis in foxtail millet, oats, darnel and ryegrass. Jahnke et al. (2016) demonstrated that automated seed phenotyping on a single-seed basis can contribute valuable information for applications in a wide range of wild or crop species, including seed classification, seed sorting and assessment of seed quality.

19.4.3 Seed Technology and Smart Plant Breeding

Since the development of full genomes of many food crops, the quantum of datasets that the ~omics technologies have made available to plant breeders on various open source and proprietary platforms can only be explored for breeding by the big data explosion associated with high-throughput phenotyping (Ubbens and Stavness 2017). Consequently, the reality of modern smart breeding is matching plant phenotype datasets with genomic datasets on almost 200 platforms of omics libraries available as open source databases on NCBI and other databases (Chen et al. 2017). Machine learning models are now used to analyse integrated high-throughput phenomic datasets with genomic datasets for genomic selection and other plant genetics analytics within the seed technology workflow. Watanabe et al. (2017) used integrated high-throughput phenotype data collected with mounted NIR-GB cameras on UAV with genomic datasets to predict sorghum varieties with G-BLUP (Table 19.4).

The inception of smart breeding with marker-assisted selection birthed non-invasive alternatives to genetic engineering methods (Vogel 2009). Since then, the breeding paradigm that follows factory production line system or pipeline breeding was being used for breeding, especially in the private sector (Collard and Mackill 2008). Pipeline breeding process involved transforming traditional breeding to a product-oriented and data-driven variety development pipelines. A typical breeding pipeline will integrate data from phenotyping and genotyping domains to predict new cultivars that respond to market demand; hence, they are often protected by intellectual property rights. However, new pipeline breeding based on open source databases is now emerging. An example of a project called "Transforming Rice Breeding" at IRRI, which involved implementing the pipeline breeding integrated marker-assisted breeding with throughput phenotyping to reduce breeding cycle by 2–3 years, thereby increasing breeding genetic gain. The strategy uses big data analytics of genomic selection integrated with trained phenotype data to implement genome-wide prediction of phenotypes (Collard Bertrand et al. 2019). Integrating seed phenotype with genotype datasets from omics databases can also provide a platform for smart breeding based on seed phenotype datasets. In this regard, new innovations in big data phenotyping are exploring designs of data-driven devices that scan bags or trailers of seeds individually and integrate the seed phenotype data with other datasets to derive analytics of interest. Examples of new innovation concepts are systems that capture images of every single seed in a

lot, generating their phenotype datasets in the process and integrating the data with datasets from other domains in real time. The extent of analytics derivable from the process depends on the trait of interest and the understanding of the mechanisms. New platform-based analytics for seed quality integrate datasets from seed technology databases with datasets from other domains like genomics, disease datasets and environmental datasets. For example, GeNee™ platform implementing AI algorithms and big data architecture deliver seed quality analysis that can integrate seed morphometric data with cloud data to genomics and seed health information.[6]

19.5 Conclusion

This paper is a review of advances in big data for seed technology. The characteristics of big data was discussed in the context of a seed technology workflow that identified two stages in the seed value chain: seed development and seed production stages. The different sources of big data within the activities of the workflow were discussed. A conceptual framework for seed technology big data involving seed image data input that produces analytics for seed applications was presented. Cases of use of the conceptual framework to develop applications for the seed technology workflow were discussed. Applications for seed quality evaluation and traceability, digital imaging, high-throughput phenotyping, phenotype genomic integration and smart breeding were discussed. Altogether, the review has demonstrated that seed technology workflow concept is a key to the automation and high-throughput seed analytics.

References

Aquino A et al (2017) A new methodology for estimating the grapevine-berry number per cluster using image analysis. Biosyst Eng 156:80–95

Araus JL, Kefauver SC, Zaman-Allah M, Olsen MS, Cairns JE (2018) Translating high-throughput phenotyping into genetic gain. Trends Plant Sci 23(5):450–466. https://doi.org/10.1016/j.tplants.2018.02.001

Armbruster WJ, MacDonell MM (2014) Informatics to Support International Food Safety. s.l., s.n., pp 127–134

Barnaghi P, Sheth A, Henson C (2013) From data to actionable knowledge: big data challenges in the web of things. IEEE Intell Syst 28(6):6–11

Brinis N, AzouzSaidane L (2016) Context aware wireless sensor network suitable for precision agriculture. Wirel Sens Netw 2016(8):1–12

Bronson K, Knezevic I (2016) Big Data in food and agriculture. Big Data Soc 2016:1–5. https://doi.org/10.1177/2053951716648174

Cai J, Zeng Z, Connor JN, Huang CY, Melino V, Kumar P, Miklavcic SJ (2015) RootGraph: a graphic optimization tool for automated image analysis of plant roots. J Exp Bot 66 (21):6551–6562. https://doi.org/10.1093/jxb/erv359

[6] https://www.seed-x.com/.

Chandra Sekhar C, Uday Kumar J, Kishor Kumar B, Sekha C (2018) Effective use of Big Data analytics in crop planning to increase agriculture production in India. Int J Adv Sci Technol 113:31–40. https://doi.org/10.14257/ijast.2018.113.04

Chen J, Chen Y, Du X, Li C, Lu J, Zhao S, Zhou X (2013) Big data challenge: a data management perspective. Front Comp Sci 7(2):157–164

Chen C, Huang H, Wu CH (2017) Protein bioinformatics databases and resources. Methods Mol Biol 1558:3–39. https://doi.org/10.1007/978-1-4939-6783-4_1

Chi M, Plaza A, Benediktsson JA, Sun Z, Shen J, Zhu Y (2016) Big data for remote sensing: challenges and opportunities. Proc IEEE 104(11):2207–2219

Collard Bertrand CY, Gregorio GB, Thomson MJ, Islam MR, Vergara GV, Laborte AG, Nissila E, Kretzschmar T, Cobb JN (2019) Transforming rice breeding: re-designing the irrigated breeding pipeline at the International Rice Research Institute (IRRI). Crop Breed Genet Genom 1: e190008. https://doi.org/10.20900/cbgg20190008

Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. Philos Trans R Soc B Biol Sci 363(1491):557–572. https://doi.org/10.1098/rstb.2007.2170

Confalonieri R et al (2017) PocketPlant3D: analysing canopy structure using a smartphone. Development of smart apps to use phones as measuring tools agro-environmental research. Biosyst Eng 164:1–12

Copeland LO, McDonald MB (1995) Principles of seed science and technology, 3rd edn. Chapman and Hall, New York, 409 p

Daniel IO, Ajala MO (2003) Developing a database for maize variety in Nigeria. Moor J Agric Res 4(1):78–84

Dell' Aquila A (2007) Pepper seed germination assessed by combined X-radiography and computer-aided imaging analysis. Biologia Plantarum 51(4):777–781

Dell' Aquila A (2009) Digital imaging information technology applied to seed germination testing. A review. Agron Sustain Dev 29(1):213–221

Desarkar A, Das A (2017) Big-data analytics, machine learning algorithms and scalable/parallel/distributed algorithms. In: Bhatt C et al (eds) Internet of things and big data. Springer

Díaz SE, Pérez JC, Mateos AC, Marinescu M-C, Guerra BB (2011) A novel methodology for the monitoring of the agricultural production process based on wireless sensor networks. Comput Electron Agric 76:252–265. https://doi.org/10.1016/j.compag.2011.02.004

Dunn EG (2019) The startup taking on Bayer with cheaper, non-GMO seeds. Bloomberg Businessweek. Retrieved 03 July 2019

El Masry G, Mandour N, Al-Rejaie S, Belin E, Rousseau D (2019) Recent Applications of Multispectral Imaging in Seed Phenotyping and Quality Monitoring-An Overview. Sensors (Basel, Switzerland) 19(5):1090. https://doi.org/10.3390/s19051090

Fahad A, Alshatri N, Tari Z, Alamri A, Khalil I, Zomaya AY, Foufou S, Bouras A (2014) A survey of clustering algorithms for big data: taxonomy and empirical analysis. IEEE Trans Emerg Topics Comput 2(3):267–279

Fahlgren N, Feldman M, Gehan MA, Wilson MS, Shyu C, Bryant DW, Hill ST, McEntee CJ, Warnasooriya SN, Kumar I, Ficor T, Turnipseed S, Gilbert KB, Brutnell TP, Carrington JC, Mockler TC, Baxter I (2015) A versatile phenotyping system and analytics platform reveals diverse temporal responses to water availability in Setaria. Mol Plant 8:1520–1535

FAO (2018) Module 3 seed toolkit: seed quality assurance. FAO/Africa seed. ISBN 978-92-5-130951-3 (FAO)

Fassio A, Restaino E, Cozzolino D (2015) Determination of oil content in whole corn (Zea mays L.) seeds bymeans of near infrared reflectance spectroscopy. Comput Electron Agric 110:171–175

Galford GL, Mustard JF, Melillo J, Gendrin A, Cerri CC (2008) Wavelet analysis of MODIS time series to detect expansion and intensification of row-crop agriculture in Brazil. Remote Sens Environ 112(2):576–587

Holst A (2019) Volume of data/information created worldwide from 2010 to 2025. Statista Software 2019. https://www.statista.com/statistics/871513/worldwide-data-created/

Hughes N, Askew K, Scotson CP, Williams K, Sauze C, Corke F, Doonan JH, Nibau C (2017) Non-destructive, high-content analysis of wheat grain traits using X-ray micro computed tomography. Plant Method 13:76

Jahnke S, Roussel J, Hombach T, Kochs J, Fischbach A, Huber G, Scharr H (2016) phenoSeeder - a robot system for automated handling and phenotyping of individual seeds. Plant Physiol 172:1358–1370

Jawad HM, Nordin R, Gharghan SK, Jawad AM, Ismail M (2017) Energy-efficient wireless sensor networks for precision agriculture: a review. Sensors (Basel, Switzerland) 17(8):1781. https://doi.org/10.3390/s17081781

Jawhar I, Mohamed N, Al-Jaroodi J, Zhang S (2014) A framework for using unmanned aerial vehicles for data collection in linear wireless sensor networks. J Intell Robot Syst 74:437–453. https://doi.org/10.1007/s10846-013-9965-9

Jóźwiaka Á, Milkovics M, Lakne Z (2016) A network-science support system for food chain safety: a case from Hungarian cattle production. Int Food Agribusiness Manage Rev 19(A):17–42

Kamilaris A, Kartakoullis A, Prenafeta-Boldú FX (2017) A review on the practice of big data analysis in agriculture. Comput Electron Agric 143. https://doi.org/10.1016/j.compag.2017.09.037

Labrinidis A, Jagadish HV (2012) Challenges and opportunities with big data. Proc VLDB Endowment 5(12):2032–2033

Laney D (2001) 3D data management: Controlling data volume, velocity and variety, Gartner, Tech. Rep., Feb. 2001

Lin P, Li XL, Chen YM, He Y (2018) A deep convolutional neural network architecture for boosting image discrimination accuracy of rice species. Food Bioprocess Technol 11 (4):765–773

Liu Z-y, Cheng F, Yi-bin Y, Xiu-qin R (2005) Identification of rice seed varieties using neural network. J Zhejiang Univ Sci 6B(11):1095–1100

Marcos-Filho J, Bennett MA, McDonald MB, Evans AF, Grassbaugh EM (2006) Assessment of melon seed vigour by an automated computer imaging system compared to traditional procedures. Seed Sci Technol 34(2):485–497. https://doi.org/10.15258/sst.2006.34.2.23

Men S, Yan L, Liu J, Qian H, Luo Q (2017) A classification method for seed viability assessment with infrared thermography. Sensors 17:845. https://doi.org/10.3390/s17040845

MIT Technology Review (2013) The big data conundrum: how to define it? https://www.technologyreview.com/s/519851/the-big-data-conundrumhow-to-define-it/

Mochida K, Koda S, Inoue K, Hirayama T, Tanaka S, Nishii R, Melgani F (2018) Computer vision-based phenotyping for improvement of plant productivity: a machine learning perspective. GigaScience 8(2018):1–12. https://doi.org/10.1093/gigascience/giy153

Mucherino A, Papajorgji P, Pardalos P (2009) Data mining in agriculture, vol 34. Springer Science & Business Media

OECD (2019) Part II: rules and regulations of seed schemes. Seed schemes rules and regulations, pp 20–57. https://www.oecd.org/agriculture/seeds/documents/oecd-seedschemes-rules-and-regulations.pdf

Perez-Sanz F, Navarro PJ, Egea-Cortines M (2017) Plant phenomics: an overview of image acquisition technologies and image data analysis algorithms. GigaScience 6(11):1–18. https://doi.org/10.1093/gigascience/gix092

Pound MP, Atkinson JA, Townsend AJ, Wilson MH, Griffiths M, Jackson AS, Bulat A, Tzimiropoulos G, Wells DM, Murchie EH, Pridmore TP, French AP (2017) Deep machinelearning provides state-of-the-art performance in image-basedplant phenotyping. GigaScience 6(10):1–10

Prado SA, Cabrera-Bosquet L, Grau A et al (2018) Phenomics allows identification of genomic regions affecting maizestomatal conductance with conditional effects of waterdeficit and evaporative demand. Plant Cell Environ 41(2):314–326

Salah K, Nizamuddin N, Jayaraman R, Omar M (2019) Blockchain-based soybean traceability in agricultural supply chain. IEEE Access 7:73295–73305. https://doi.org/10.1109/ACCESS.2019.2918000

Sanoubar R, Calone R, Noli E, Barbanti L (2018) Data on seed germination using LED versus fluorescent light under growth chamber conditions. Data Brief 19:594–600. https://doi.org/10.1016/j.dib.2018.05.040

Sawant M, Urkude R, Jawale S (2016) Organized data and information for efficacious. Agriculture using PRIDE model. Int Food Agribusiness Manage Rev 19(A)

Shahbandeh M (2019) Value of the global seed market 2017-2023. Statista agriculture 2019. https://www.statista.com/statistics/262286/global-seeds-market-value/

Silva NV, Cicero SM (2014) Image seedling analysis to evaluate tomato seed physiological potential. Revista Ciência Agronômica 45(2):327–334

Silva-Perez V et al (2017) Hyperspectral reflectance as a tool to measure biochemical and physiological traits in wheat. J Exp Bot 69:483–496

Singh A, Ganapathysubramanian B, Singh AK, Sarkar S (2016) Machine learning for high-throughput stress phenotyping in plants. Trends Plant Sci 21(2):110–124

Sivarajah U, Kamal MM, Irani Z, VishanthWeerakkody (2017) Critical analysis of Big Data challenges and analytical methods. J Bus Res 70:263–286

Sonka S (2014) Big Data and the Ag sector: more than lots of numbers. Int Food Agribusiness Manage Assoc 17(1):1–20

Sonka S (2016) Big Data: fueling the next evolution of agricultural innovation. J Innov Manage 4 (1):114–136

Tavlor L, Schroeder R (2015) Is bigger better? Emergence of big data as a tool for international development policy. Geo J 30(4):503–518

Ubbens JR, Stavness I (2017) Deep plant phenomics: a deep learning platform for complex plant phenotyping tasks. Front Plant Sci 8:1190. https://doi.org/10.3389/fpls.2017.01190

Vibhute A, Bodhe SK (2012) Applications of image processing in agriculture: a survey. Int J Comput Appl 52(2)

Vogel B (2009) In: Erwood S, Truchi N (eds) Marker-assisted selection: a noninvasive biotechnology alternative to genetic engineering of plant varieties. Greenpeace International, August 2009. greenpeace.org

Watanabe K, Guo W, Arai K, Takanashi H, Kajiya-Kanegae H, Kobayashi M, Yano K, Tokunaga T, Fujiwara T, Tsutsumi N, Iwata H (2017) High-throughput phenotyping of sorghum plant height using an unmanned aerial vehicle and its application to genomic prediction modeling. Front Plant Sci 8:421

Wolfert S, Ge L, Verdouw C, Bogaardt M (2017) Big Data in smart farming–a review. Agric Syst 153:69–80

Xuan P, Martin S (2018) How data analytics is transforming agriculture. ScienceDirect 2018:128–130

Zhang T, Qun S, Lei Y, Liming Y, Jianhua W (2017a) Vigor detection of sweet corn seeds by optimal sensor array based on electronic nose. Trans Chin Soc Agric Eng 33(21):275–281

Zhang X, Huang C, Wu D et al (2017b) High-throughput phenotyping and QTL mapping reveals the genetic architecture of maize plant growth. Plant Physiol 173(3):1554–1564

Zhang C, Si Y, Lamkey J, Boydston RA, Garland-Campbell KA, Sankaran S (2018) High-throughput phenotyping of seed/seedling evaluation using digital image analysis. Agronomy 8(5):63. https://doi.org/10.3390/agronomy8050063

Zhou J, Applegate C, Alonso AD, Reynolds D, Orford S, Michal M, Griffiths S, Penfield S, Pullen N (2017) Leaf-GP: an open and automated soft leaf-GP: an open and automated software application for measuring growth phenotypes for arabidopsis and wheat. Plant Methods 13:117

Seed Micro-Morphological Characteristics in Local Landraces of Finger Millet [*Eleusine coracana* (L.) Gaertn.]

20

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Abstract

The objective of this study was to document the seed micro-morphological characterization of potential under-utilized cereal finger millet using scanning electron microscopy to evaluate variations in local landraces. The testa layer is composed of tissue that resembles the interlocking pattern of cells in landraces FM/ST/01, FM/ST/02, FM/ST/03, FM/KP/01, FM/KP/02, FM/SD/01, and FM/RG/01. The landraces FM/RT/01, FM/RT/02, FM/RT/03, FM/RT/04, and FM/RT/05 showed hexagonal cells. The landraces with interlocking patterns of testa cells had raised micro-papillae, whereas those with hexagonal testa cells had

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slightly raised micro-papillae. Dendrogram constructed based on micro-morphological characteristics grouped all the 12 local landraces of finger millet into three distinct clusters. The seed coat pattern was found to be a significant character for delimitation of local landraces and further utilization for the improvement of finger millet.

Keywords

Finger millet · Local landraces · Seed coat pattern · SEM · Testa cells

20.1 Introduction

To large segments of populations, millets are the major source of staple food and supplies a major portion of nutrition in Africa and Asia (O'Kennedy et al. 2006; Vinoth and Ravindhran 2017). For farmers in Asia and Africa having arid, infertile, marginal, and poor lands, millets serve as critical plant genetic resource to cover food security (Gupta et al. 2017). Generally, there are 7 crops referred to as millets including barnyard millet (*Echinochloa* spp.), finger millet [*Eleusine coracana* (L.) Gaertn], foxtail millet [*Setaria italica* (L.) Beauv], kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrense* Roth. ex Roem. & Schult.), pearl millet [*Pennisetum glaucum* (L.) R.Br.], and proso millet (*Panicum miliaceum* L.). Out of these seven millet crops, pearl millet conquers first position with about 95% of the total millet production followed by foxtail millet (Yadav and Rai 2013; Nedumaran et al. 2014; Vinoth and Ravindhran 2017).

Finger millet [*Eleusine coracana* (L.) Gaertn.] belongs to the family Poaceae, commonly known as Ragi, Nachani, or Nagali. It is the sixth most important cereal crop with tremendous potential but is more under-utilized than other consumed cereals. In India, finger millet stands at sixth position after important cereal crops viz. wheat, rice, maize, sorghum, and bajra and is an important staple food for low income groups. Globally, India is the largest producer of finger millet (Ramakrishnan et al. 2015). More than 34,160 finger millet genotypes are available throughout the world and about 22,583 genotypes are found in India alone (Ramakrishnan et al. 2016). Nutritionally, the grains are a rich source of minerals, especially calcium, vitamins, dietary fibers, proteins, and energy as compared to other cereals (Vadivoo et al. 1998; Devi et al. 2014). It also contains a useful amount of certain minerals such as copper, iron, manganese, and phosphorus (Shashi et al. 2007; Tripathi and Platel 2010). It is an important crop in the diets as "Nachani Satva" for children, pregnant women, and lactating mothers (Gupta et al. 2017). It showed health beneficial pharmacological activities, like anti-diabetic, antimicrobial, antioxidant, atherosclerogenic, and anti-tumerogenic activities (Devi et al. 2014). It is also used in folk medicine as well as in beer production and is valuable as fodder for cattle (Kumar et al. 2016).

In developing countries, malnutrition is becoming a severe problem due to nutrient deficiency (Datta et al. 2006; Renuka et al. 2016). In developing countries, micronutrient deficiencies in pregnant women result in health consequences to the

fetus (Gernand et al. 2016). Finger millet has all the quantitative and qualitative traits required to serve as a model for nutritional supplement. It has better nutritional qualities than rice, wheat, and other prominent cereal crops (Latha et al. 2005; Chandrashekar 2010). To combat malnutrition, conventional plant breeding and genetic engineering are the best ways to improve seed production and nutritional composition (Stein 2010). Understanding inheritance and heritability are the most important factors for designing any breeding program (Grant et al. 2008). For any successful crop improvement program, it is a pre-requisite to have the knowledge of variations among the germplasm. The micro-morphological characterization of seed coat patterns offered distinctive and consistent data for the demarcation of genotypes (Umdale et al. 2019).

Therefore, in the present study, attempts were made to evaluate the micro-morphological characterization of seeds to detect the polymorphism of 12 local landraces of finger millet.

20.2 Materials and Methods

20.2.1 Plant Material

Seeds of local landraces of finger millet were collected from the local farmers from different localities in western regions of Maharashtra, India (Mundada et al. 2019) (Table 20.1). The seeds were cleaned manually to remove debris and stored in air tight plastic containers at room temperature for further analysis.

20.2.2 Seed Micro-Morphological Characterization

Twenty-five seeds of each genotype were used to observe the micro-morphological characters including length-width of testa cell and micro-papillae characteristics

Table 20.1 List of population locations of local cultivars of finger millet (Mundada et al. 2019)

Sr. No.	Accession code	Locality	Latitude	Longitude	Altitude (m)
1	FM/ST/01	Jambhe, Satara	17°56′	73°86′	1067
2	FM/ST/02	Saigaon, Satara	17°79′	73°94′	719
3	FM/ST/03	Borane, Satara	17°60′	73°88′	1063
4	FM/KP/01	Radhanagri, Kolhapur	16°41′	73°99′	571
5	FM/KP/02	Radhanagri, Kolhapur	16°41′	73°99′	571
6	FM/SD/01	Malvan, Sindhudurga	16°06′	73°47′	17
7	FM/RT/01	Gudal, Ratnagiri	16°45′	74°05′	565
8	FM/RT/02	Gudal, Ratnagiri	16°45′	74°05′	565
9	FM/RT/03	Wada Tiwar, Ratnagiri	16°65′	73°51′	31
10	FM/RT/04	Dabhol, Ratnagiri	17°58′	73°17′	22
11	FM/RT/05	Dabhol, Ratnagiri	17°58′	73°19′	22
12	FM/RG/01	Alibag, Raigad	18°51′	73°18′	25

Table 20.2 Micro-morphological characterization of different landraces of finger millet

Landrace code	Length of testa cell (μm)	Width of testa cell (μm)	Area of testa cell (μm)	Length of micro-papillae (μm)	Width of micro-papillae (μm)	Area of micro-papillae (μm)
FM/ST/01	48.53 ± 1.04	49.82 ± 0.79	2403.24 ± 74.86	22.66 ± 1.47	24.31 ± 1.56	355.46 ± 03.75
FM/ST/02	43.08 ± 0.39	37.13 ± 1.33	1374.60 ± 11.59	27.66 ± 0.55	23.58 ± 1.89	636.29 ± 39.74
FM/ST/03	35.31 ± 0.30	41.41 ± 1.49	1200.37 ± 61.91	26.68 ± 1.63	26.01 ± 0.37	544.16 ± 29.38
FM/KP/01	35.36 ± 1.22	37.70 ± 2.15	1185.75 ± 61.17	21.77 ± 0.32	29.11 ± 1.74	385.87 ± 26.53
FM/KP/02	39.45 ± 2.18	46.27 ± 1.51	1472.77 ± 50.67	22.30 ± 1.90	21.71 ± 0.69	376.87 ± 27.88
FM/SD/01	46.89 ± 1.89	46.11 ± 3.25	1764.61 ± 57.76	26.35 ± 0.87	22.12 ± 0.82	520.66 ± 34.51
FM/RT/01	47.59 ± 0.76	36.61 ± 1.95	1515.14 ± 77.48	23.41 ± 0.62	24.02 ± 0.62	479.43 ± 16.42
FM/RT/02	50.34 ± 1.47	37.81 ± 0.80	1562.73 ± 67.48	25.62 ± 0.51	24.59 ± 0.31	468.23 ± 27.69
FM/RT/03	44.44 ± 1.45	48.25 ± 1.44	1886.03 ± 60.93	25.62 ± 0.51	28.52 ± 0.58	637.97 ± 22.25
FM/RT/04	63.81 ± 1.48	52.22 ± 0.33	2466.87 ± 28.97	32.95 ± 0.35	31.42 ± 1.60	970.27 ± 20.32
FM/RT/05	41.29 ± 2.30	47.90 ± 1.86	1496.32 ± 48.69	25.34 ± 0.37	22.52 ± 0.60	444.54 ± 20.16
FM/RG/01	51.73 ± 1.87	51.05 ± 2.54	2016.60 ± 55.69	25.16 ± 0.45	25.75 ± 0.84	443.57 ± 30.69

Data is represented as mean ± SD

(Table 20.2). Initially, 2–4 seeds of each landrace were washed thoroughly with running tap water followed by cleaning with 70% ethanol. Then the seeds were air-dried under shade conditions. Then, the air-dried seeds were placed on aluminum stubs for coating. The seeds were uniformly coated with a thin layer of gold using an ion sputter. Then, the coated seeds were observed using a scanning electron microscope (NOVA NANOSEMNPEP303) at high vacuum mode with 10 keV of operating power source. The seeds were consistently scanned at the surface to study the cellular and intercellular testa patterns. The terminologies used to describe the seed coat pattern were used as proposed by Barthlott (1981, 1990). Randomly selected 15 mature seeds of each landrace were used to measure the parameters related to size (testa cell length-width, area, and micro-papillae length-width and area). The variation in testa cell length-width and micro-papillae length-width among the different landraces were validated as boxplots using R package ggplot2.

20.2.3 Data Analysis

The descriptive analysis including mean ± SD was carried out using MS-Excel. The UPGMA dendrogram based on similarity matrix was constructed using Past software version 3.01. Principal component analysis was performed on data obtained from micro-morphological characters of seed coat using Past software version 3.01.

20.3 Results and Discussion

Figure 20.1 shows the SEM observation of texture of the seed coats of 12 local landraces of finger millet. The seed surface looked smooth on observation with the naked eye. The external appearance of the finger millet testa was quite prominent and different from other cereals (McDonough et al. 1986). The testa layer was composed of a tissue that resembled the interlocking pattern of cells in landraces FM/ST/01, FM/ST/02, FM/ST/03, FM/KP/01, FM/KP/02, FM/SD/01, and FM/RG/01 (Fig. 20.1). Each section was composed of some dimpled mounds and consisted of open spaces underneath some of the mounds. Similarly, McDonough et al. (1986) observed that the first layer was composed of sections of tissue that "interlocked"like the pieces of a jigsaw puzzle. The landraces FM/RT/01, FM/RT/02, FM/RT/03, FM/RT/04, and FM/RT/05 showed hexagonal cells (Fig. 20.1) without any dimpled mounds. Considerable variation in dimensions was observed in the testa cell length, width, and area (Table 20.2, Figs. 20.1 and 20.2a–c). Among the 12 local landraces, length, width, and area of the testa cells were maximum in the landrace FM/RT/04 (Table 20.2) and minimum in the landrace FM/KP/01 (Fig. 20.3). Similar variations in micro-papillae were also observed in dimensions of micro-papillae length, width, and area (Table 20.2, Figs. 20.1 and 20.2a–c). Among the 12 landraces, the landraces FM/ST/01, FM/ST/02, FM/ST/03, FM/KP/01, FM/KP/02, FM/SD/01, and FM/RG/01 displayed a raised appearance of the micro-papillae (Fig. 20.1). The appearance was not much raised in the landraces FM/RT/01, FM/RT/02, FM/RT/03, FM/RT/04,

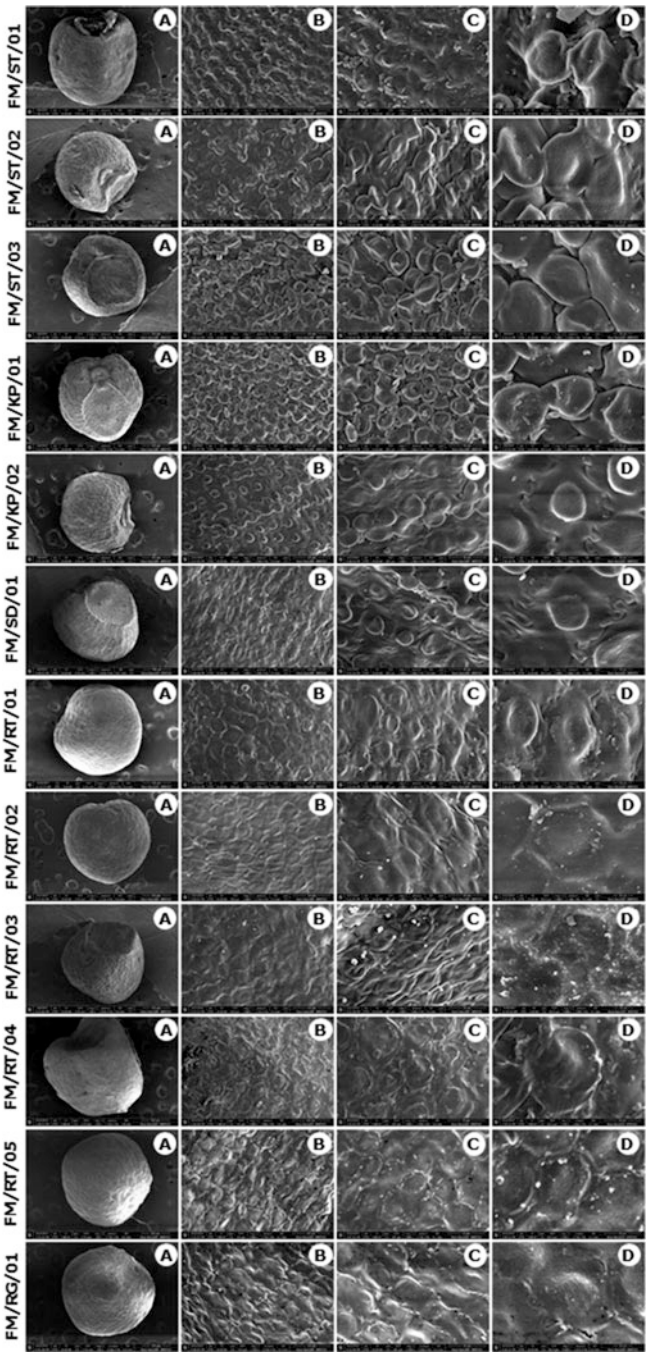

Fig. 20.1 Scanning electron micrographs of seeds of local landraces of finger millet

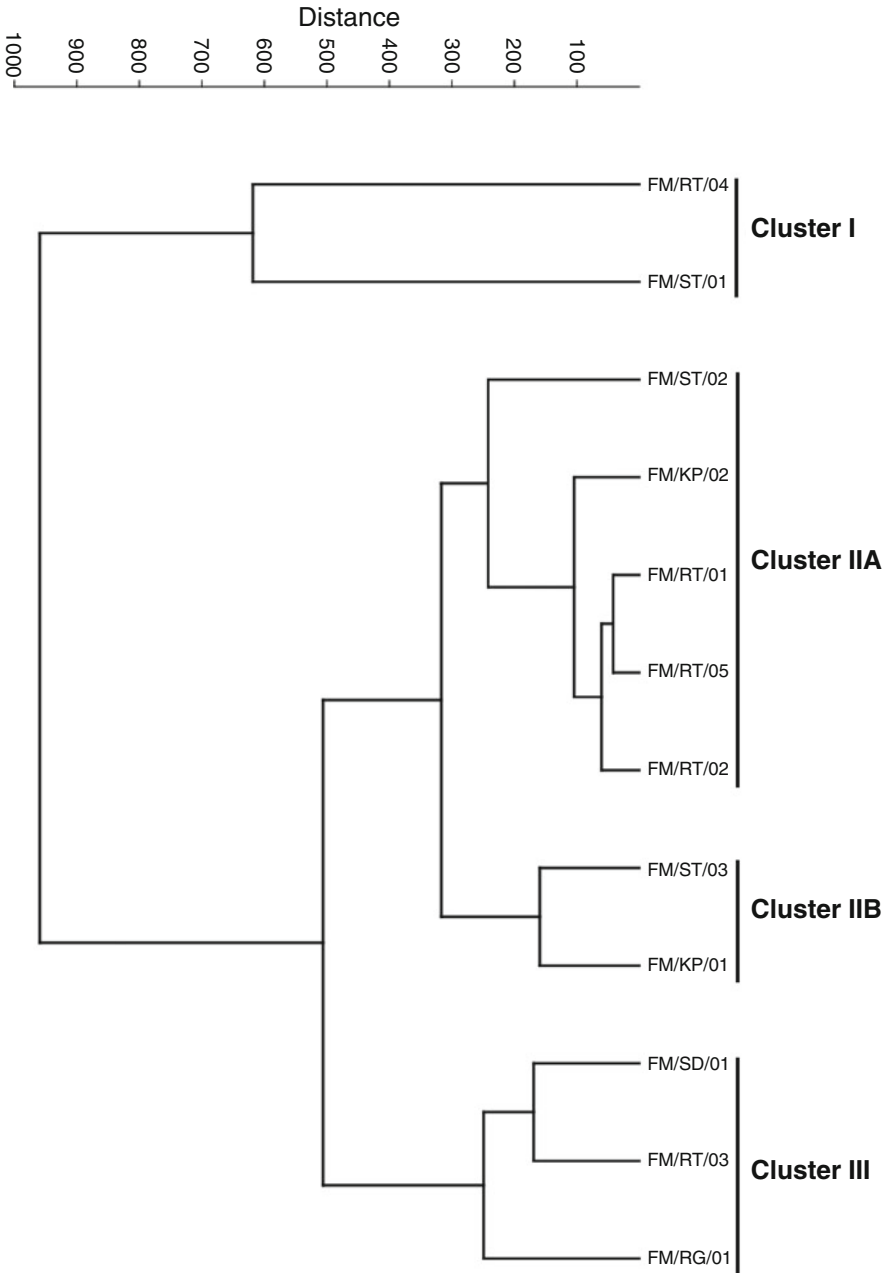

Fig. 20.2 UPGMA dendrogram showing similarity distance of the 12 local landraces of finger millet

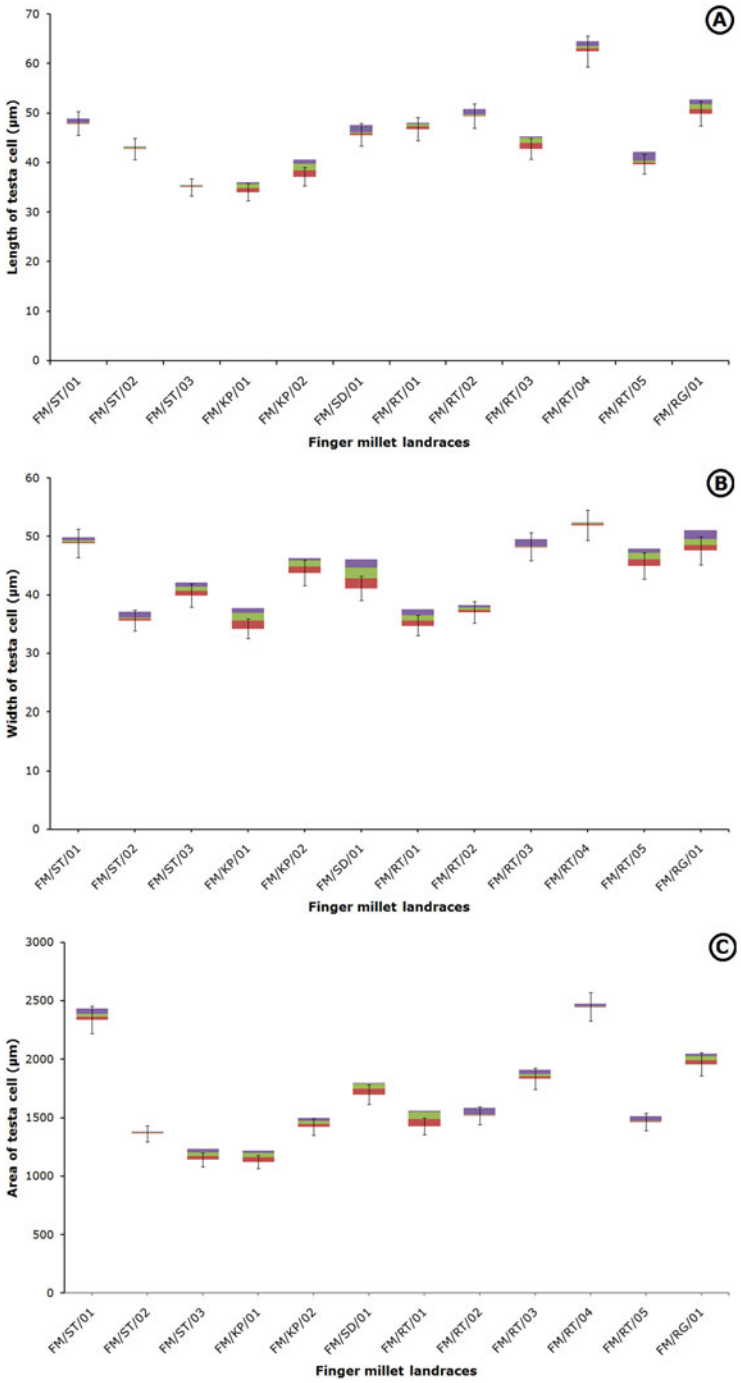

Fig. 20.3 Variation in dimensions of testa cells among the 12 local landraces of finger millet (**a**) cell length, (**b**) cell width, and (**c**) cell area

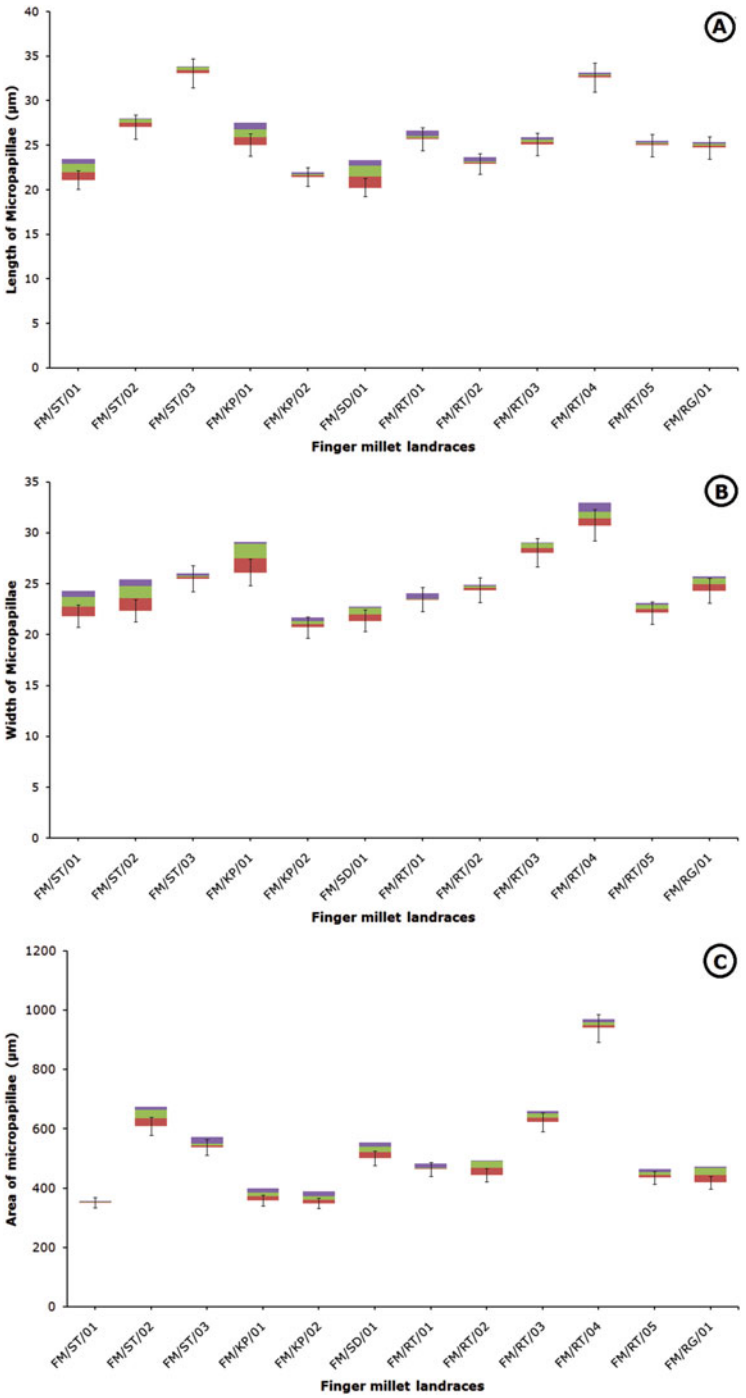

Fig. 20.4 Variation in dimensions of micro-papillae among the 12 local landraces of finger millet (**a**) length, (**b**) width, and (**c**) area of micro-papillae

and FM/RT/05 (Fig. 20.1). The presence of dimpled mounds and micro-papillae might be helpful to imbibe water during seed germination.

Dendrogram constructed based on micro-morphological characteristics grouped all the 12 local landraces of finger millet into three distinct clusters across all the geographic regions (Fig. 20.2). Cluster I comprised of two accessions FM/RT/04 and FM/ST/01 from Ratnagiri and Satara districts, respectively. The cluster was influenced by the character testa cell area. Cluster II was further differentiated into sub-clusters IIA and IIB. Cluster IIA consisted of 5 landraces, namely FM/ST/02, FM/KP/02, FM/RT/01, FM/RT/02, and FM/RT/05. Cluster IIB comprised 2 landraces, namely FM/ST/03 and FM/KP/01. Cluster II was mostly influenced by micro-papillae characteristics (Fig. 20.4). Cluster III consisted of 3 landraces from Konkan region, namely FM/SD/01, FM/RT/03, and FM/RG/01. Cluster III was influenced by the character testa cell length.

20.4 Conclusion

In conclusion, the testa cell size and ornamentation discloses the existence of unique features for the evaluation and management of local finger millet landraces. All finger millet landraces showed significant variations in seed coat pattern. The study provides an opportunity to plant breeders to improve the important cereal crop plant finger millet.

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References

Barthlott W (1981) Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. Nord J Bot 1:345–355

Barthlott W (1990) Scanning electron microscopy of the epidermal surface in plants. In: Claugher D (ed) Scanning electron microscopy in taxonomy and functional morphology. Clarendon, Oxford, pp 69–94

Chandrashekar A (2010) Finger Millet: *Eleusine coracana*. In: Taylor SL (ed) Advances in food and nutrition research. Academic Press, Burlington, pp 215–262

Datta K, Rai M, Parkhi V, Oliva N, Tan J, Datta SK (2006) Improved 'golden' indica rice and post-transgeneration enhancement of metabolic target products of carotenoids (b-carotene) in transgenic elite cultivars (IR64 and BR29). Curr Sci 91:935–939

Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB (2014) Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. J Food Sci Technol 51:1021–1040

Gernand AD, Schulze KJ, Stewart CP, West KP Jr, Christian P (2016) Micronutrient deficiencies in pregnancy worldwide: health effects and prevention. Nat Rev Endocrinol 12:274–289

Grant CA, Clarke JM, Duguid S, Chaney RL (2008) Selection and breeding of plant cultivars to minimize cadmium accumulation. Sci Total Environ 390:301–310

Gupta SM, Arora S, Mirza N, Pande A, Lata C, Puranik S, Kumar J, Kumar A (2017) Finger millet: a "certain" crop for an "uncertain" future and a solution to food insecurity and hidden hunger under stressful environments. Front Plant Sci 8:643

Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S, Singh M, Gupta S, Babu BK, Sood S, Yadav R (2016) Nutraceutical value of finger millet [*Eleusine coracana* (L.) Gaertn.], and their improvement using omics approaches. Front Plant Sci 7:934

Latha AM, Rao KV, Reddy VD (2005) Production of transgenic plants resistant to leaf blast disease in finger millet (*Eleusine coracana* (L.) Gaertn.). Plant Sci 169:657–667

McDonough CM, Rooney LW, Earp CF (1986) Structural characteristics of *Eleusine corocana* (finger millet) using scanning electron and fluorescence microscopy. Food Microstruct 5:247–256

Mundada PS, Umdale SD, Nikam TD, Ahire ML (2019) Genetic diversity using RAPD markers, mineral composition and their correlation in selected local landraces of finger millet (*Eleusine coracana* (L.) Gaertn.). Vegetos 32:1–10

Nedumaran S, Bantilan MCS, Gupta SK, Irshad A, Davis JS (2014) Potential welfare benefit of millets improvement research at ICRISAT: multi country-economic surplus model approach, socioeconomics discussion paper series number 15. ICRISAT, Hyderabad

O'Kennedy MM, Grootboom A, Shewry PR (2006) Harnessing sorghum and millet biotechnology for food and health. J Cereal Sci 44:224–235

Ramakrishnan M, Ceasar SA, Duraipandiyan V, Al-Dhabi NA, Ignacimuthu S (2015) Using molecular markers to assess the genetic diversity and population structure of finger millet (*Eleusine coracana* (L.) Gaertn.) from various geographical regions. Genet Resour Crop Evol 63:361–376

Ramakrishnan M, Ceasar SA, Duraipandiyan V, Al-Dhabi NA, Ignacimuthu S (2016) Assessment of genetic diversity, population structure and relationships in Indian and non-Indian genotypes of finger millet (*Eleusine coracana* (L.) Gaertn.) using genomic SSR markers. SpringerPlus 5:120

Renuka N, Mathure SV, Zanan RL, Thengane RJ, Nadaf AB (2016) Determination of some minerals and b-carotene contents in aromatic indica rice (*Oryza sativa* L.) germplasm. Food Chem 191:2–6

Shashi BK, Sunanda S, Shailaja H, Shankar AG, Nagarathna TK (2007) Micronutrient composition, antinutritional factors and bioaccessibility of iron in different finger millet (*Eleusine coracana*) genotypes. Karnataka J Agric Sci 20:583–585

Stein AJ (2010) Global impacts of human malnutrition. Plant Soil 335:133–154

Tripathi B, Platel K (2010) Finger millet (*Eleucine coracana*) flour as a vehicle for fortification with zinc. J Trace Elem Med Biol 24:46–51

Umdale SD, Gaikwad NB, Malik SK, Gore PG, Yadav SR, Bhat KV (2019) Seed coat polymorphism in *Vigna* section Aconitifoliae in India. Flora 259:151458

Vadivoo AS, Joseph R, Ganesan NM (1998) Genetic variability and diversity for protein and calcium contents in finger millet (*Eleusine coracana* (L.) Gaertn) in relation to grain color. Plant Foods Hum Nutr 52:353–364

Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security. Front Plant Sci 8:29

Yadav OP, Rai KN (2013) Genetic improvement of pearl millet in India. Agric Res 2:275–292

Seed Storage and Longevity: Mechanism, Types and Management

21

Muhammad Amir Bakhtavar and Irfan Afzal

Abstract

About one-third of the food produced in the world is never consumed due to loss or waste, which adversely affects agricultural productivity and food security for the rising population. The primary cause of such losses is poor storage due to high seed moisture content at harvest and damp storage conditions. Thus, maintenance of seed quality during storage is imperative to the propagation of food plants as seed is the first link in the food chain and the ultimate symbol of food security. In storage, seeds are preserved under regulated environmental conditions to maintain their viability but initial seed quality and seed moisture contents are major contributing factors of seed longevity. The purpose of seed storage may vary from seasonal storage to long-term germplasm conservation. Seed deterioration during storage is an inevitable process and can be delayed by controlling different abiotic (relative humidity, temperature and oxygen) and biotic factors (insects, fungi and rodents) that affect viability. Rate of seed deterioration is accelerated with increased initial seed moisture content, temperature and relative humidity of the storage environment. In this chapter, we discuss possible mechanisms of seed deterioration various factors that are related to seed deterioration and management strategies for the preservation of seeds during storage.

Keywords

Seed quality · Seed deterioration · Orthodox seeds · Hermetic storage · Seed longevity

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21.1 Introduction

Maintenance of seed quality during storage is important for further propagation of food plants as seed is the first step in the food chain and the ultimate symbol of food security (Vanangamudi et al. 2017). Safeguarding seeds under regulated environmental conditions to maintain its viability for longer period of time is termed as seed storage (Willan 1986). In its simplest form, under storage, mature seeds are kept for a short duration to wait for the ideal environmental conditions that allows seed planting, its emergence and proper growth (Bonner 2008). The significance of seed storage was highlighted for the very first time when human started to domesticate plants.

Poor storage practices are the main reasons for huge storage losses (50–60%) in cereals worldwide (Kumar and Kalita 2017). Storage losses vary according to prevailing climatic conditions with 10% losses in temperate regions, whereas humid tropical regions face higher storage losses amounting to almost 50% (Wijayaratne et al. 2018). These estimates of storage losses are based on the quantity that is physically lost during storage while losses in seed quality far exceed these figures. Deterioration of seeds starts immediately after their detachment from the mother plants and ideal seed storage practices prolong seed viability by slowing down the deterioration process (Doijode 2006). The main objective behind seed storage of economically important crops is to ensure the availability of planting stock for the next growing season. Accumulation of carryover seed supplies of desired crops for several years is also one of the key purpose of seed storage to meet seed demands during periods of low production (FAO 2018). Advancement of plant breeding knowledge and genetic technologies has also highlighted the importance of seed storage for conservation of germplasm to be used in breeding program.

Normally, seeds are stored for a short period between collection and sowing in the next season to overwinter unfavourable weather conditions. Long-term storage for several years (up to 10 years) makes sure the availability of seeds in the absence of annual crops. Seed storage for germplasm conservation is done for 10 to more than 50 years. Different seed storage techniques have been adapted depending upon the purpose of storage. These include open storage in conventional jute and polypropylene sacs, cryopreservation, modified atmosphere storage, hermetic storage, conditioned storage and low temperature storage. The purpose of any storage technique is to reduce the deteriorative changes occurring within the seeds to prolong seed longevity. Lipid peroxidation due to free radicals, enzyme inactivation, degradation of cell membrane and DNA disintegration is the major cause of seed ageing (Murthy et al. 2003; McDonald 1999).

Storage duration and objectives of the storage provide guidelines needed to devise procedure and strategies of safe storage (Bonner 2008). Factors that influence seed quality during storage also have a major role in deciding storage procedures and strategies. The amount of seed and storage duration must be considered before establishing storage facilities to make it economical. Improper storage leads to the death of 90% seeds before the next growing season and is equally wasteful as it spoils all the efforts made during harvesting and collection process (Willan 1986).

This chapter covers seed storage behaviour, its deterioration mechanism and management strategies for preserving seed quality during storage.

21.2 Seed Storage Behaviour

Before going to storage, it is very important to remember that the seed is a miniature plant that cannot live indefinitely and will die after a certain storage period. There are two major classes of seeds based on their drying and storage behaviour i.e. orthodox and recalcitrant seeds (Roberts 1973). Orthodox seeds are desiccation tolerant and undergo maturation drying. They have long storage life and their longevity can be increased by reducing the moisture content and temperature of storage environment. Cereals and legume crops are mainly orthodox seeds. Contrary to orthodox seeds, recalcitrant seeds are sensitive to drying and freezing and have short storage life (FAO 2018). An intermediate category between orthodox and recalcitrant seeds has also been proposed (Ellis et al. 1990).

21.3 Factors Affecting Seed Quality during Storage

21.3.1 Factors Related to Seeds

Seeds are hygroscopic and absorb and desorb water depending upon external relative humidity. Variation in seed moisture at the same relative humidity level is due to difference in seed composition. Oily seeds have low moisture and storage life at the same relative humidity compared to starchy seeds (Copeland and McDonald 2001; McDonald 2007). Initial seed viability, seed moisture content, seed maturity, seed composition, seed size, mechanical damage and dormancy are factors that are related to seeds, and seed quality after any storage period is strongly linked with these factors.

21.3.1.1 Initial Seed Viability

Seeds with higher values of initial seed viability have more storage life or higher longevity after any storage period as compared to the seed lot having low initial viability. Germination test should be performed for every seed lot before taking it into storage to know about the expected storage life of seed. Sometimes, dormancy-breaking treatments are necessary for the freshly harvested seeds before their germination assessment. Seed lot longevity is highly associated with percentage of seeds that germinate in initial germination test, and it may vary from 50% to 90% for two seed lots of the same species. Storage of seed lot with 50% initial seed germination is wastage of storage space and resources, as this lot will lose its viability more quickly during storage than the seed lot having 90% initial viability. For short-term storage i.e. for few weeks or a couple of months, deterioration in seed quality is not a serious concern but for germplasm conservation, no seed lot should

be accepted which has <85% initial viability depending upon the species or variety in question (Copeland and McDonald 2001).

21.3.1.2 Seed Moisture

Seed moisture content is the most important factor that affects seed longevity in storage. Seed is hygroscopic and absorbs moisture from the surrounding; so, any change in temperature and relative humidity affects its quality. This relationship between prevailing relative humidity and seed moisture content at a given temperature is known as seed moisture isotherm or absorption isotherm (Copeland and McDonald 2001). The importance of seed moisture content has been highlighted by Harrington who stated that in normal range of temperature and moisture, the storage life of seed could be doubled by each reduction in 1% seed moisture content (Harrington 1972). Seed quality is at the greatest risk at high moisture content in storage (Bewley et al. 2013). However, there is lack of awareness that moisture is the culprit for the low quality of seeds in developing countries. Rate of seed respiration increased when moisture content increases from 18% to 20%. Insect infestation is minimal below 8–9% moisture content. A non-dormant seed may germinate above 30% seed moisture content. Fungi cannot grow below 13% seed moisture content on starchy seeds and 7–8% in oily seeds which are in equilibrium with ambient RH value that nearly equals 68% (Bradford et al. 2018). Moisture contents of maize seed stored in conventional polypropylene bags increased due to higher ambient relative humidity, which resulted in poor seed germination and higher storage losses due to stored grain pests and aflatoxin contamination (Afzal et al. 2017). Seed moisture also mediates the seed mechanical damage by harvesting and threshing machinery, thus affecting the storage life of seeds.

21.3.1.3 Seed Maturity

Mature seeds have longer shelf life and longevity than seeds that were harvested before maturity. Immature orthodox seeds deteriorate faster and lose viability quickly compared to mature seeds under same storage conditions. Tomato seeds extracted from early ripe fruits have low vigour compared to mature seeds (Tetteh et al. 2018). Some tree seeds have the ability to complete maturation after they have been removed from the mother tree. Storage of immature cones of pines and other conifers for many weeks before seed extraction can enhance the longevity of the seeds (Bonner 1991).

Indeterminate pattern of flowering has been observed in many crop plants having most immature flower at the top of inflorescence and fully mature flower at the base. Also, younger branches have more immature flowers; thus, on the same plants, seeds of different maturity levels are found. This difference in seed maturity is reflected into seed storability. Some of the biochemical substances are accumulated into seeds during the final stages of seed maturity. These may be dormancy-inducing hormones or proteins related to desiccation tolerance. Seeds harvested before full maturity lack these essential compounds and perform poorly in storage.

Immature seeds are normally green in colour due to the presence of chlorophyll that is not degraded properly due to early harvest. A decrease in chlorophyll

florescence is linked with increased germination of tomato seeds, indicating that mature seeds having less chlorophyll will have more germination potential and high quality (Jalink et al. 1999). Fractions of soybean seeds sorted on the bases of chlorophyll florescence showed a great difference in seed quality (Cicero et al. 2009). Seed fraction with low florescence values gave maximum percentage of normal seedlings, indicating that immature seeds having more chlorophyll florescence have low quality.

21.3.1.4 Seed Composition

Chemical composition is one of the most important factors for seed longevity. For example, oily seeds do not store well as compared to starchy seeds (Table 21.1). One can find support for this concept with the relatively poor storage performance of soybean (Shelar et al. 2008) and sunflower seeds (Abreu et al. 2013). Total seed oil compositional analysis is misleading because the oil contents in the embryo which is responsible for germination are more important for deciding the shelf life of seeds. *Glycine max* seeds have lower oil contents (21%) compared to *Brassica napus* seeds (36.4%), but the storage life of *Brassica napus* seeds is higher than that of *Glycine max* seeds. Seed storage life based of their oil contents and seed moisture contents can be predicted from seed viability equation (Royal Botanic Garden Kew 2019).

21.3.1.5 Seed Size

Seed size is one of the parameters of seed quality. Plant growth, yield and harvest efficiency is mostly related to seed size. Several studies have revealed the superiority of heavy, mature seeds over light, immature seeds in germination, vigour and yield

Table 21.1 Oil contents of some crop species and storage period after which they lose 50% germination at 25 °C and different seed moisture contents [9]

Crop species	Seed oil content (%)	Days to lose 50% germination at 14% SMC	Days to lose 50% germination at 8% SMC
Oryza sativa	2.2	100	1661
Cicer arietinum	4.1	251	3294
Zea mays	5	111	1737
Triticum aestivum	10	63	1674
Allium cepa	20	33	853
Glycine max	21	47	435
Brassica napus	36.40	41	516
Lactuca sativa	33.8	10	196
Gossypium hirsutum	38.4	108	1975
Linum usitatissimum	39.5	19	292
Brassica rapa	41.5	43	556
Arachis hypogaea	47.3	13	132
Sesamum indicum	50	34	322

95% initial germination; *SMC* seed moisture content

tests. Relatively few exceptions have been noted. Larger seeds of finger millet have shown higher germination and vigour index after 8 months of storage as compared to smaller seeds (Lokesh et al. 2000). Seed quality of French bean with larger seed size (5 mm) was superior in terms of germination, seedling vigour index, seedling length, activity of α-amylase and dehydrogenase enzyme (Vishwanath et al. 2011). Bold wheat seeds of >2.2 mm give higher emergence rate and grain yield (Akinci et al. 2008). Larger soybean seeds give higher emergence and yield compared to smaller seeds (Morrison and Xue 2007).

21.3.1.6 Mechanical Damage

Seeds damaged mechanically by harvesting, threshing and cleaning equipment result in loss of viability. Mechanical damage does not affect seed quality immediately but for storage purpose, these effects are very serious and can cause much economic losses (McDonald 1985). Process of seed deterioration cannot be avoided and operates continuously. Mechanical damage to seeds, especially in dry season, can be very damaging as embryo is exposed to environmental factors (Linda 2019). Small damage to seed embryo is very harmful for seed life as compared to large damage to seeds that are not on embryonic tissue. Chances of mechanical damage exist more for seeds having a very thin or papery seed coat. Storage fungi and insects can easily attack damaged seeds as compared to non-damaged seeds, mainly by penetration into seeds through damaged seed coats. X-Ray analysis of sweet corn seeds showed that seeds with damaged embryonic axis resulted in abnormal seedlings and reduced germination (Gomes Junior and Cicero 2012). Inappropriate handling in cotton can damage the seed mechanically and thus accelerate seed deterioration during seed processing and storage (Jyoti and Malik 2013). The extent of mechanical damage mainly depends on seed moisture contents. With increase in seed moisture contents (>12%), seed coat is damaged during processing which exposes the embryo to ambient temperature and humidity, thus triggering seed deterioration (Black et al. 2006).

21.3.2 Factors Related to Storage Environment

Storage environment has a critical role in enhancing the shelf life of seed. The main objective is to minimize the rate of metabolic activities and to prevent seeds from attack of microorganisms and rodents. The reduced metabolic rate and respiratory activities help maintain the integrity of embryos by conserving food reserves within seeds. The following biotic and abiotic factors have major contribution in the longevity of seeds after harvest.

21.3.2.1 Relative Humidity and Storage Temperature

Temperature and relative humidity (RH) are related to each other and both factors collectively influence the seed moisture content (Copeland and McDonald 2001). Moisture-holding capacity of the air is influenced by temperature. Any change in temperature results in a change in air humidity, which ultimately affects the seed

moisture content. This relation between temperature and RH is the base of seed storage principle which states that the aggregate of both temperature (°F) and RH should be <100 (Harrington 1973). High RH is the enemy for storage life of commodities such as cereal grains and oilseeds (Bradford et al. 2018). Seed storage at low RH is recommended as viability and vigour in seeds stored at high RH are significantly reduced (Suma et al. 2013). Temperature determines the speed of seed deterioration in both field and storehouse. Seed deterioration rate is accelerated if initial seed moisture content, temperature and RH of the storage environment is high (Goel et al. 2003). According to seed storage thumb rule, the life span of seeds can be doubled by a 10 °F reduction in temperature (Harrington 1972). Membrane integrity and structure are disrupted due to formation of intracellular ice crystals at low temperature and high (above 14%) seed moisture contents (Copeland and McDonald 2001). Okra seeds died completely at high temperature (45 °C) (Sahib 2014). Wheat seeds stored at a temperature of 40 °C and 45% RH lost 35–85% germination in 1 year, and losses in vigour was recorded to a range of 55–94%. Contrary to this, there were only 10–20% losses in germination and 15–22% vigour losses were recorded when wheat seeds were stored at 25 °C with 45% RH for a period of 1 year (Strelec et al. 2010).

21.3.2.2 Gases

Active oxygen species are produced from molecular oxygen during the transfer of electron through electron transport chain and are very damaging to the cellular structure (Bailly 2004). Free radicals cause peroxidation of lipids and other essential cellular constituents. Cellular lipid contents decreased due to series of harmful chemical reactions. Complete elimination of oxygen from the storage atmosphere seems beneficial to most dry orthodox seeds. However, it is indicated that some oxygen is required for recalcitrant seeds. Adequate ventilation supply (i.e. adequate oxygen) is essential for successful storage of recalcitrant seeds at relatively high moisture contents, as well as for storage of imbibed seeds of orthodox species (King and Roberts 1979).

Insect larvae can be killed using higher concentration of CO_2. For routine killing of insect larvae, orthodox seeds can be stored at modified atmosphere having more than 60% CO_2. Seed is safe from insects and fungal damage if it is dried at 8% seed moisture contents and stored in hermetic bags (Bakhtavar et al. 2019b) Rate of seed respiration and insect activities can be reduced by eliminating the available oxygen from the storage environment. The available oxygen of the storage environment can be replaced with nitrogen gas or CO_2 or by creating vacuum. Storage in hermetic Super Bags preserves seed quality by maintaining seed dryness and restricting oxygen entry into sealed bags (Bakhtavar et al. 2019a). Storage of seeds at high-pressure oxygen increased the rate of seed ageing during storage, suggesting a major role of oxygen in seed deterioration (Groot et al. 2012).

21.3.3 Biological Factors

21.3.3.1 Insects

Stored products are destroyed mainly by the direct feeding of insects. Seed embryo is the preferred food for some insect species. They feed on the embryo of seeds and cause complete loss of germination of damaged seeds. Insects feeding on the endosperm or food storage organs of seeds also cause huge quality and weight losses in stored grains. Mostly, insects prefer to attack the germ of seeds, as the germ is softer than the rest of the seed structures; thus, seed germination is lost readily (Bakhtavar et al. 2019b). Insect species including *Sitotroga cerealella* and *Rhizopertha dominca* are notorious for causing invisible losses to seed germination as their larvae feed on germ without leaving any sign of damage on the seeds (Prakash and Rao 1995). Stored grain pest attack on maize seeds resulted in 35% storage losses within 4 months of storage in conventional polypropylene bags (Afzal et al. 2017).

Seed germination is most sensitive to storage at high humidity/moisture. Fungal growth starts only at RH above 65% and insect activity is higher at 32–65% relative humidity (Fig. 21.1). Seed storage below 20–30% RH will reduce insect and fungal attack and thus, seed quality and longevity can be maintained (Bewley et al. 2013; Bradford et al. 2018).

21.3.3.2 Fungi

Invasion of storage fungi is promoted by several factors including storage temperature, grain moisture contents, RH and initial contamination in the storehouse. Mechanically damaged seeds are easily attacked by the microflora and thus are very prone to fungal invasion (Shelar et al. 2008). Occurrence of aflatoxin on grains

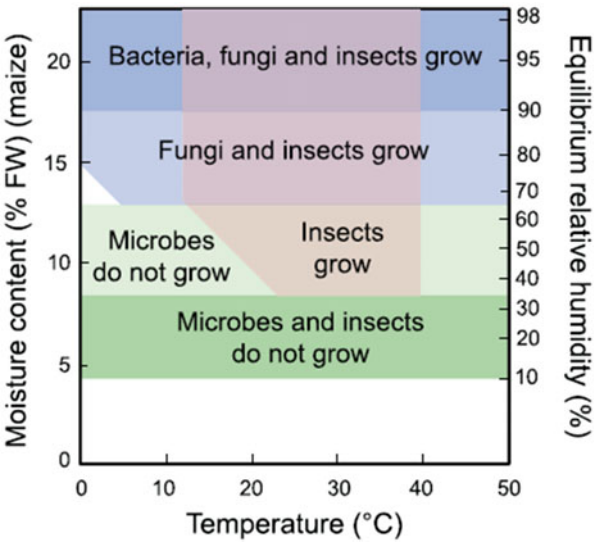

Fig. 21.1 Limits of seed moisture contests and temperature for storage of maize (adapted from Bradford et al. 2018)

can compound seed quality losses. Aflatoxin may develop due to storage at high moisture contents (Bewley et al. 2013). Consumption of grains contaminated with aflatoxin can impair the immune system as aflatoxin is a potent source of carcinogen and causes impaired uptake of nutrients present in food (Wu et al. 2014). Moulds responsible for the production of aflatoxin can grow on seeds only above 85% equilibrium RH (Abdel-Hadi et al. 2012). Such conditions are mostly prevailing in grain storehouses in developing countries (Ahsan et al. 2010).

21.3.3.3 Rodents

Thousands of tons of seeds are destroyed by rats, squirrels and other rodents each year. These losses are not only due to their feeding but most of the losses are by scattering and mixing of seed. Storage losses in cereals due to rodents, mainly rats, are >1%, while in developing countries, these losses are around 3–5% (IRRI 2019). Apart from storage losses, quality issue arises from rodent attack. Around 50 diseases have been reported in the world that is known to be transmitted to human by rodents. The best method to control rodents is to keep them isolated from the storage house. The most successful strategy to achieve this objective is to build such storehouses, which are free from rodent entryways like holes and cracks in the walls. Maintaining cleanliness in the storehouses is also a very good way to control the rodent's entry. Rodent's traps, fumigation and poison baits can also effectively control rodent infestation. Electric and modern zapper traps are getting very popular nowadays due to ease of use and its safety.

21.4 Mechanism of Seed Deterioration during Storage

Seeds have the unique ability to survive as living regenerative organisms, and like all other organisms, they cannot survive indefinitely and hence deteriorate and eventually die (Copeland and McDonald 2001). Basically, seed deterioration is linked with harmful process going on within seeds which make them vulnerable to environmental factors, thus reducing their storage life (Fig. 21.2). General concepts about seed deterioration are that seed deterioration is an unstoppable phenomenon and can be delayed by altering storage conditions. Moreover, seed deterioration is an irreversible process and rate of seed deterioration varies greatly among different species. Seed longevity parameters vary notably even for different accessions of indica rice (Lee et al. 2019).

Seed deterioration is a complex process and does not occur uniformly throughout seeds (Copeland and McDonald 2001). Seeds with high oil concentration, like cottonseeds, are more susceptible to deterioration, especially under poor storage conditions (Iqbal et al. 2002).

Degradation of cell membrane is the major cause of deteriorative changes occurring in seeds. This results in altered physical shapes and normal activities of cells. Increased free radical production and free fatty acid levels due to lipid peroxidation are the major causes of membrane disruption (Grilli et al. 1995). Lipid peroxidation due to free radicals, enzyme inactivation, degradation of cell membrane and DNA

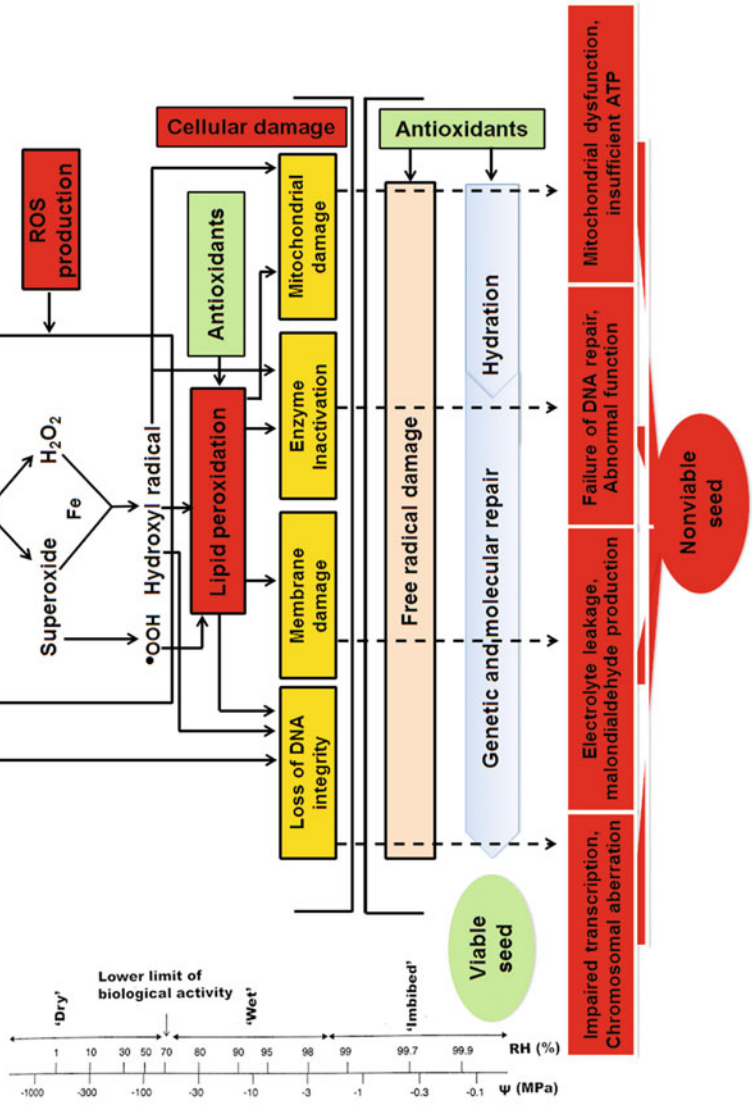

Fig. 21.2 Illustration of pathways and processes involved in seed deterioration. Graph at the left side represents the hydration level of seeds both in terms of water potential and equilibrium relative humidity; 70% RH is the lower limit of biological activity. (Adapted and modified from Osborne (1980), McDonald (1999), Walters et al. (2002), Bewley et al. (2013))

disintegration are the major causes of seed ageing (Murthy et al. 2003; McDonald 1999). Seed ageing is the result of enzyme degradation, which in turn is the result of altered macromolecular structure (Lehner et al. 2008; Bailly 2004). Electron transport chain operating in mitochondria is one of the major sources of ROS in the respiring seeds (Bailly 2004). Structural changes linked with oxidation are less fluidity of membrane, changes in DNA folding, reduced protein elasticity and augmented brittleness of cellular constituents (Walters et al. 2010).

Biochemical aspects of deterioration of seeds are chemical alterations which include degraded DNA, chromosomal aberrations and impaired protein synthesis due to RNA damage, altered membrane structure and reduced food reserves (Kibinza et al. 2006). Loss of protein functions induced by oxidation of protein is also one of the reasons of seed ageing (Rajjou et al. 2008).

Reduced contents of protein, total sugars, oil contents and increased reducing sugars and free fatty acids contents have been observed in aged seeds. Decreased oligosaccharides have been observed in deteriorated seeds, which are known to be involved in stabilizing membranes. In aged reddish seeds, the activity of peroxidase decreased as compared to fresh seeds (Scialabba et al. 2002). There was a sharp decline in the activity of peroxidase enzyme during sunflower ageing (Pallavi et al. 2003). Viability losses in sunflower seeds are linked with increased malondialdehyde content, confirming a strong association between lipid peroxidation and seed deterioration due to reduced efficiency of antioxidant defence system (Kibinza et al. 2006).

21.5 Types of Storage

21.5.1 Open Storage

Open storage has remained one of the basic and widely used storage methods and is still prevalent in developing countries. In traditional storage, maize cobs and sorghum panicles were tied in bundles and hanged with tree branches or post or tight lines within the house. This does not provide much protection against weather, fungus and insects. Seeds are stored in open containers, traditional porous bags, in piles and single layers of sacs and earthen or metal silos under the shelter against rain and protected from rodents (FAO 2018). Open storage gives best results only seeds are stored in a cool and dry place. Seeds of cereals and trees can be stored for long periods in such conditions without any significant reduction in seed longevity.

21.5.2 Conditioned Storage

In conditioned storage, seed viability can be maintained for many years by controlling RH and temperature of the storage environment. Continuous maintenance of such controlled conditions may be uneconomical for most crop seeds but considering the value of germplasm and high value seed stock, that cost could

be justified. In tropical regions, seed viability from harvesting to planting can only be maintained through conditioned storage (Harrington 1973). Factors that should be considered while making choice of seed storage structure are

- Initial seed quality.
- Type of seed to be stored.
- Length of storage period.
- Reduction of seed weight during storage.
- Prerequisites of conditioned storage.

21.5.3 Storage in Controlled or Modified Atmosphere

Controlled atmosphere or modified atmosphere storage is a type of storage in which seeds are stored in an environment having considerably different concentration of CO_2 and O_2 as compared to normal air. Modified atmosphere needs continuous observation and manipulation of CO_2 and O_2 concentrations inside the hermetic containers or storage structure. The main goal of controlled atmosphere storage is to preserve grain and seed by controlling insects and fungal growth (Fleurat-Lessard et al. 1994) and to preserve seed quality under anoxic conditions (Groot et al. 2015). The concentration of gases inside the store varies continuously by the respiration of seeds and leakage of gases through the open spaces of doors and walls of storage structure. Therefore, it is necessary to regularly monitor gas concentration and it should be adjusted to predetermined levels by fresh air, nitrogen gas or any other chemical that can remove CO_2.

Within storage, the respiration of living organisms including (seed, insects and fungi) reduced the concentration of oxygen from 21% (normal air concentration) to 1–2% and increased the CO_2 concentration from 0.035% to nearly 2% (Chakraverty et al. 2003). Modified atmosphere can be created using combustible gases (propane and butane) to produce low oxygen and high CO_2 concentration at 13–15%. Ozone can also be used as an alternative to create modified atmosphere for storage (McDonough et al. 2010). Use of nitrogen gas and high pressure CO_2 treatment has also been reported for modifying storage environment (Navarro et al. 2012).

21.5.4 Hermetic Storage

Hermetic storage is a seed storage technique in which oxygen is reduced and replaced by increasing CO_2 that helps control stored grain insect pest without using any fumigant (Villers et al. 2008; Guenha et al. 2014; Walsh et al. 2014; Afzal et al. 2017). Hermetic storage can be used to store products without using any fumigation chemical or refrigeration practice with high quality. Plastic containers designed especially for hermetic storage can be used to store cereals and other food items. Hermetic storage is based on the principle of generation of interstitial atmosphere of sufficiently low oxygen and elevated CO_2, caused by the respiration of

living organisms in the ecological system of a sealed storage (Jonfia-Essien et al. 2010). Hermetic storage technique has been used worldwide for the storage of cereal seeds including wheat, rice, barley and corn.

Packaging seeds in hermetically sealed containers or moisture-resistant bags during storage and marketing periods has been tested. The basic objective of this exercise is to keep the seeds dry and maintain a specific level that is safe for long-term storage. Traditional cloth or paper bags are least effective in maintaining seed viability, while different polyethylene and laminated bags are moderately effective. The effectiveness of a packaging material is related to its capacity to resist moisture. For hermetic storage, seeds should have 2–3% less moisture as compared to the moisture levels when they are stored in normal containers which are not completely airtight. Seed storage in hermetic bags at higher moisture contents is equally damaging as storage in traditional porous bag cause seed viability losses and aflatoxin contamination due to high seed moisture contents (Bakhtavar et al. 2019b). Complete control over humidity in seed storeroom needs a large investment for creation of such environment using dehumidifying equipment. Not everyone has such special storage facilities, so there is a need to find an alternative to this method. RH can be maintained in closed containers of small size by using desiccants that can maintain values of equilibrium moisture contents according to our own desire. Saturated salt solutions or solution of acids can be used to develop equilibrium moisture contents (Greenspan 1977). A small fan can be mounted inside the container to distribute relative humidity evenly inside the container. Silica gel is a very common desiccant and can be placed in the container along with seeds. Silica gel is coated with cobalt chloride that serves as an indicator of humidity and turns from blue to pink at RH higher than 45%. Along with this, small balls of aluminium silicate ceramics material are being marketed as seed 'Drying Beads®' and have been used to store tomato seeds (Nassari et al. 2014). Drying beads are produced from zeolite clay having microcrystalline pore structure that can tightly hold the water (Van Asbrouck and Taridno 2009; Hay et al. 2012). Rice seeds can be dried using drying beads for small scale gene bank storage (Hay and Timple 2013).

Hermetic PICS bags (Purdue Improved Cowpea Storage) are tipple layer hermetic storage bags that have been used successfully for storage of maize seed with high quality and free from insects and fungal growth (Afzal et al. 2017). The Cocoon™ of Grin Pro is also used as large scale portable hermetic storage system for rice, coca, corn, coffee sorghum, groundnut, beans, and spices in different countries (Rickman and Aquino 2004; Villers 2006; Villers et al. 2008; Jonfia-Essien et al. 2010). The GrainProSuperGrainbag II ZTM is a portable, Ultra Hermetic™ (gastight), water resistant storage option for a wide range of dry food and agricultural produce. Super bags have all the characteristics of hermetic storage system (Donahaye et al. 2001). For storage of rice seeds, hermetically sealed Super Bags have been found very effective in tropical climate by checking humid air movement. Storage in Super bags is a very successful method of seed storage to preserve quality of crop seeds (Bakhtavar et al. 2019a). Maize seed storage in hermetic Super Bag at 8–10% seed moisture contents prevents storage losses and deterioration of seed quality with respect to loss of germination, food reserves and aflatoxin contamination (Bakhtavar et al. 2019b).

21.5.5 Cryogenic Storage

Routine operation and mechanical breakdown of conditioned storage facilities is a costly practice and can be avoided by adopting cryogenic storage technique. In this type of storage, seeds are stored in liquid nitrogen at −196 °C. The main benefit of this strategy is that seeds are stored at such low temperature where there are negligible deteriorative physiological activities are occurring in the seeds (Copeland and McDonald 2001). Practically, the cost of liquid nitrogen is much less as compared to the establishment and maintenance cost of conditioned storage. One drawback of cryogenic storage is that it cannot be implemented for large scale commercial storage of crop seeds but can be successfully utilized for storage of germplasm in the seed bank. Storage of lettuce seeds in liquid nitrogen maintained the seed viability for a longer period (Walters et al. 2004). Cryopreservation can be successfully used for the conservation of medicinal legumes without any harmful effect on germination (Kholina and Voronkova 2012).

Most agronomic and cereal crop seeds can be successfully stored in liquid nitrogen, but not all seeds can bear liquid nitrogen possibly due to freezable water inside the seeds at higher moisture contents that will damage the seeds. Sesame seeds can tolerate liquid nitrogen freezing below 12% seed moisture content; above this limit, their viability decreases substantially (Copeland and McDonald 2001).

21.5.6 Cold Storage

Temperature controls the rate of enzymatic and metabolic reactions occurring within the seeds, and high temperature enhances the rate of these deteriorative reactions. According to Harrington (Harrington 1972), each 5 °C reduction in storage temperature can double the life span of the seed. This basic rule of thumb is the base of cold storage. In most cold storage, high RH is present if not properly controlled, and seeds equilibrate themselves with that high RH and its moisture content increases. So, there should be proper arrangement for humidity control, or seeds should be placed in hermetic containers to control humidity. Facilities of cold storage vary with seed volume and proposed storage period.

Seed storage at low temperature has been reported to retain maximum viability (Pradhan and Badola 2012; Liu et al. 2011). Storage temperature of recalcitrant species must be no lower than −3 °C. It is usually convenient to store recalcitrant seeds in the same facility used for stratification and seedling storage.

21.6 Conclusions

Seed longevity is a complex trait determined by a succession of events through storage that are influenced by environmental conditions. The process of seed deterioration due to free radicle attack on cell membranes and mtDNA can be delayed by controlling storage conditions such as RH and temperature that ultimately affects

seed moisture contents. Seed moisture content is a critical factor in maintaining high quality throughout the supply chain. Hermetic sealed storage at low moisture contents extends seed quality and storage life through protection and repair from oxidative damage. Consequently, quality seed is critical for farmer prosperity and food security.

References

Abdel-Hadi A, Schmidt-Heydt M, Parra R, Geisen R, Magan N (2012) A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by Aspergillus flavus. J R Soc Interface 9:757–767

Abreu LAS, Carvalho CAG, Pinto VY, Kataoka MLM, Silva TTA (2013) Deterioration of sunflower seeds during storage. J Seed Sci 35:240–247

Afzal I, Bakhtavar MA, Ishfaq M, Sagheer M, Baributsa D (2017) Maintaining dryness during storage contributes to higher maize seed quality. J Stored Prod Res 72:49–53

Ahsan S, Bhatti MR, Asi HN, Bhatti IA, Sheikh MA (2010) Occur Aflatoxins Maize Grains from Cent Areas Punjab Pakistan. Int J Agric Biol 12:571–575

Akinci C, Yildirim M, Bahar B (2008) The effects of seed size on emergence and yield of durum wheat. J Food Agric Environ 6:234–237

Bailly C (2004) Active oxygen species and antioxidants in seed biology. Seed Sci Res 14:93–107

Bakhtavar MA, Afzal I, Basra SMA (2019a) Moisture adsorption isotherms and quality of seeds stored in conventional packaging materials and hermetic Super Bag. PLoS One 14:e0207569

Bakhtavar MA, Afzal I, Basra SMA, Wahid A (2019b) Implementing the 'dry chain' during storage reduces losses and maintains quality of maize grain. Food Secur 11(2):345–357. https://doi.org/10.1007/s12571-019-00905-2

Bewley J, Bradford K, Hilhorst H, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy, 3rd edn. Springer, New York

Black M, Bewley JD, Halmer P (2006) The encyclopedia of seeds: science, technology and uses. CABI, Cambridge, MA

Bonner FT (1991) Effect of cone storage on pine seed storage potential. South J Appl For 15:216–221

Bonner FT (2008) Storage of seeds. In: Bonner Karrfalt RPFT (ed) Woody plant seed manual. US Department of Agriculture, Forest Service, Washington, DC, pp 85–96

Bradford KJ, Dahal P, Van Asbrouck J, Kunusoth K, Bello P, Thompson J, Wu F (2018) The dry chain: reducing postharvest losses and improving food safety in humid climates. Trends Food Sci Technol 71:84–93

Chakraverty A, Mujumdar AS, Ramaswamy HS (2003) Handbook of postharvest technology: cereals, fruits, vegetables, tea, and spices. CRC Press, Boca Raton

Cicero SM, Van Der Schoor R, Jalink H (2009) Use of chlrophyll fluorescence sorting to improve soybean seed quality. Rev Bras Sementes 31:145–151

Copeland LO, McDonald MB (2001) Principles of seed science and technology. Springer US, Cham

Doijode SD (2006) Seed quality in vegetable crops. In: Basra AS (ed) Handbook of seed science and technology. Scientific Publishers, India, pp 723–748

Donahaye J, Navarro S, Andales S, del Mundo A, Caliboso F, Sabio G, Felix A, Rindner M, Azrieli A, Dias R (2001) Quality preservation of moist paddy under hermetic conditions. In: Donahaye EJ, Navarro S, Leesch JG (eds) International conference on controlled atmosphere and fumigation in stored products. Executive Printing Services, Fresno, CA, pp 209–225

Ellis RH, Hong TD, Roberts EH (1990) An intermediate category of seed storage behaviour? I. Coffee. J Exp Bot 41:1167–1174

FAO (2018) Seeds toolkit - module 6: seed storage. Rome, Italy

Fleurat-Lessard F, Just P, Barrieu JM, Torch P, Highley E, Wright HJ, Le Raymond D, Saglio P (1994) Eff modif atmos storage wheat seed germination vigour physiol criteria ageing process. In: EJ Banks, BR Champ (eds) Proceedings of sixth international working conference on stored-product protection, vol 2, Ca 0–17

Goel A, Goel AK, Sheoran IS (2003) Changes in oxidative stress enzymes during artificial ageing in cotton (Gossypium hirsutum L.) seeds. J Plant Physiol 160:1093–1100

Gomes Junior FG, Cicero SM (2012) X-ray analysis to assess mechanical damage in sweet corn seeds. Rev Bras Sementes 34:78–85

Greenspan L (1977) Humidity fixed points of binary saturated aqueous solutions (Lewis Greenspan 1976). J Res Natl Inst Stand Technol 81A:89–96

Grilli I, Bacci E, Lombardi T, Spano C, Floris C (1995) Natural ageing: poly(a) polymerase in germinating embryos of Triticum durum wheat. Ann Bot 76:15–21

Groot SPC, Surki AA, de Vos RCH, Kodde J (2012) Seed storage at elevated partial pressure of oxygen, a fast method for analysing seed ageing under dry conditions. Ann Bot 110:1149–1159

Groot SPC, de Groot L, Kodde J, van Treuren R (2015) Prolonging the longevity of *ex situ* conserved seeds by storage under anoxia. Plant Genet Resour 13:18–26

Guenha R, Salvador J, Rickman LF, Goulao IM, Muocha BV, Carvalho MO (2014) Hermet storage with Plast seal to reduce insect infest Secur paddy seed Qual a powerful Strateg rice farmers Mozambique. J Stored Prod Res 59:275–281

Harrington JF (1972) Seed storage and longevity. In: Kozlowski TT (ed) Seed biology. Academic Press, New York, pp 145–245

Harrington J (1973) Problems of seed storage. In: Heydecker W (ed) Seed ecology. Pennsylvania State University Press, University Park, pp 251–263

Hay FR, Timple S (2013) Optimum ratios of zeolite seed drying beads® to dry rice seeds for genebank storage. Seed Sci Technol 41:407–419

Hay FR, Thavong P, Taridno P, Timple S (2012) Evaluation of zeolite seed drying beads for drying rice seeds to low moisture content prior to longterm storage. Seed Sci Technol 40:374–395

Iqbal N, Basra MA, Khalil-Ur-Rehman A (2002) Evaluation of vigour and oil quality in cottonseed during accelerated ageing. Int J Agric Biol 4:318–322

IRRI (2019) Rodents - IRRI Rice Knowledge Bank. http://www.knowledgebank.irri.org/index.php?option=com_zoo&view=item&layout=item&Itemid=687. Accessed 10 June 2019

Jalink H, van der Schoor R, Birnbaum YE, Bino RJ (1999) Seed chlorophyll content as an indicator for seed maturity and seed quality. Acta Hortic 504:219–228

Jonfia-Essien W, Varro S, Villers P (2010) Hermetic storage: a novel approach to the protection of cocoa beans. African Crop Sci J 18:59–68

Jyoti, Malik CP (2013) Seed deterioration: a review. Int J Life Sci Biotechnol Pharma Res 2:374–385

Kholina AB, Voronkova NM (2012) Seed cryopreservation of some medicinal legumes. J Bot 2012:1–7

Kibinza S, Vinel D, Côme D, Bailly C, Corbineau F (2006) Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. Physiol Plant 128:496–506

King MW, Roberts EH (1979) The storage of recalcitrant seeds: achievements and possible approaches: a report on a literature review carried out for the International Board for Plant Genetic Resources. FAO/IBPGR

Kumar D, Kalita P (2017) Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods 6:1–22

Lee J-S, Velasco-Punzalan M, Pacleb M, Valdez R, Kretzschmar T, McNally KL, Ismail AM, Cruz PCS, Sackville Hamilton NR, Hay FR (2019) Variation in seed longevity among diverse Indica rice varieties. Ann Bot 124:447–460

Lehner A, Mamadou N, Poels P, Côme D, Bailly C, Corbineau F (2008) Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. J Cereal Sci 47:555–565

Linda M (2019) Seed quality. https://www.niab.com/pages/id/24/Seed_Quality. Accessed 13 Sept 2019
Liu W, Liu CH, Chinese J, Zhang K, Du GH (2011) Eff accumulated temp seed germination a case study 12 compos species East QinghaiTibet plateau China. Plant Ecol 35:751–758
Lokesh K, Reddy N, Krishnappa K, Chandrakanthappa DMV, Swamy SN (2000) Eff seed size seed storability finger millet Var. Curr Res Agric Sci Bangalore 29:122–126
McDonald M (1985) Physical seed quality of soybean. Seed Sci Technol 13:601–628
McDonald MB (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Technol 27:177–217
McDonald MB (2007) Seed moisture and the equilibrium seed moisture content curve. Seed Technol 29:7–18
McDonough MX, Mason LJ, Woloshuk C, Campabadal C (2010) Ozone technology in the post-harvest storage environment-a comparison of efficacy of high doses of ozone to insects treated under laboratory conditions and field conditions. In: 10th international working conference on stored products protection. Julius-Kühn-Archiv, 425, Estoril, Portugal, pp 386–388
Morrison MJ, Xue AG (2007) The influence of seed size on soybean yield in short-season regions. Can J Plant Sci 87:89–91
Murthy UMN, Kumar PP, Sun WQ (2003) Mechanisms of seed ageing under different storage conditions for Vigna radiata (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. J Exp Bot 54:1057–1067
Nassari PJ, Rao M, Chandra Shekar Reddy K, Raheem A (2014) Post harvest drying of tomato (Lycopersicon esculentum mill) seeds to ultra low moisture safe for storage using desiccant (zeolite) beads and their effects on seed quality. Am J Res Commun 2:75
Navarro S, Timlick B, Demianyk C, White N (2012) Controlled or modified atmospheres. In: Phillips T, Cuperus G (eds) Stored product protection, vol S-156. Kansas State University, KSRE Publications, Manhattan, KS, pp 191–202
Osborne DJ (1980) Review of seed aging. In: Thimann KV (ed) Senescence in plants. CRC Press, Boca Raton, pp 1–13
Pallavi M, Sudheer KS, Annus L, Dangi SK, Reddy AV (2003) Eff seed ageing physiol biochem yield attrib sunflower helianthus cv. Morden Seed Res 31:161–168
Pradhan B, Badola H (2012) Effects of microhabitat, light and temperature onseed germination of a critically endangered Himalayan medicinal herb, Swertia chirayita: conservation implications. PlantBiosystems 146:345–351
Prakash A, Rao J (1995) Insect pest management in stored-rice ecosystems. Marcel Dekker, New York
Rajjou L, Lovigny Y, Groot SPC, Belghazi M, Job C, Job D (2008) Proteome-wide characterization of seed aging in Arabidopsis: a comparison between artificial and natural aging protocols. Plant Physiol 148:620–641
Rickman JF, Aquino E (2004) Appropriate technology for maintaining grain quality in small-scale storage. In: Donahaye E, Navarro S, Bell C, Jayas D, Noyes R, Phillips T (eds) Proceedings of the international conference on controlled atmosphere and fumigation in stored products, Gold-coast Australia. FTIC Ltd. Publishing, Israel, pp 149–157
Roberts E (1973) Predicting the storage life of seeds. Seed Sci Technol 1:499–514
Royal Botanic Garden Kew (2019) Royal Botanic Garden Kew
Sahib MM (2014) Changes in viability, vigor and relative growth rate (RGR) in okra (Abelmoschus esculentus L.) seeds during accelerated aging technique. Mag AlKufa Univ Biol 6:1–9
Scialabba A, Bellani LM, Dell'Aquila A (2002) Effects of ageing on peroxidase activity and localization in radish (Raphanus sativus L.) seeds. Eur J Histochem 46:351–358
Shelar VR, Shaikh RS, Nikam AS (2008) Soybean seed quality during storage: a review. Agric Rev 29:125–131
Strelec I, Popovich R, Ivanisic I, Jurcovic V, Jurcovic Z, Hardi Z, Sabo M (2010) Influence of temperature and relative humidity on grain moisture, germination and vigour of three wheat cultivars during one year storage. Poljoprivreda 16:20–24

Suma A, Sreenivasan K, Singh AK, Radhamani J (2013) Role of relative humidity in processing and storage of seeds and assessment of variability in storage behaviour in brassica spp and Eruca sativa. Sci World J 2013:504141

Tetteh R, Aboagye LM, Darko R, Osafo EA (2018) Effect of maturity stages on seed quality of two tomato accessions. African Crop Sci J 26:237–244

Van Asbrouck J, Taridno P (2009) Fast field drying as a method to maintain quality, increase shelf life and prevent post harvest infections on Cucumis sativum L. Asian J Food Agric Ind 2(Special Issue):S133–S137

Vanangamudi K, Kalaivani S, Vanangamudi M, Sasthri G, Selvakumari A, Srimathi P (2017) Seed quality enhancement: principles and practices. Scientific Publishers, India

Villers P (2006) Safe storage of grain in the tropics. Feed Technol Updat 1(1):2–7

Villers P, Navarro S, DeBruin T (2008) Development of hermetic storage technology in sealed flexible storage structures. In: Daolin G, Navarro S, Jian Y, Cheng T, Zuxun J, Yue L, Haipeng W (eds) Proceedings of the 8th international conference on controlled atmosphere and fumigation in stored products. Sichuan Publishing Group, Sichuan, pp 21–26

Vishwanath K, Pallavi H, Devraju P, Prashanth Y (2011) Prediction of storability of different seed size grades of French bean varieties through accelerated ageing response. Res J Agric Sci 2:213–216

Walsh S, Baribusta D, Remington T, Sperling L (2014) Seed storage brief #2: hermetic seed storage technology: principles, use, and economics – a practitioner's guide. Catholic Relief Services, Nairobi

Walters C, Farrant JM, Pammenter NW, Berjak P (2002) Desiccation stress and damage. In: Black M, Pritchard H (eds) Desiccation and survival in plants: drying without dying. CABI Publishing, New York, pp 263–291

Walters C, Wheeler L, Stanwood PC (2004) Longevity of cryogenically stored seeds. Cryobiology 48:229–244

Walters C, Ballesteros D, Vertucci VA (2010) Structural mechanics of seed deterioration: standing test time. Plant Sci 179:565–573

Wijayaratne LKW, Arthur FH, Whyard S (2018) Methoprene and control of stored-product insects. J Stored Prod Res 76:161–169

Willan RL (1986) Seed storage. In: A guide to forest seed handling. Food and Agriculture Organization of the United Nations, Rome

Wu F, Groopman JD, Pestka JJ (2014) Public health impacts of foodborne Mycotoxins. Annu Rev Food Sci Technol 5:351–372

Modeling Seed Germination Response to Salinity at Different Accelerated Aging Period in Canola

22

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Abstract

Canola is the second largely produced oilseed crop behind soybeans which is known as an important source of vegetable oil, being used in the food, feed, and biofuel feedstock. Seed aging is a serious problem in this plant particularly if the seeds are exposed to salinity stress during their germination stage. In this work, therefore, we applied both halotime and aging models to quantify the effect of the accelerated aging test period (AATP) and salinity (NaCl) on seed germination (SG) response of this plant. The seed moisture content (SMC) and electrical conductivity (EC) in response to AATP were also investigated. Based on our results, SG characteristics (i.e., germination percentage (GP) and germination rate (GR)) are significantly affected by AATP, NaCl, and their interactions ($p < 0.01$). These models successfully described the SG response of canola seeds ($R^2 > 0.87$). Based on the model parameters, the SG of canola was totally inhibited at 82.1 h AATP and 341.1 mM NaCl, respectively. Both SMC and EC increased significantly with the AATP, reflecting the loss of membrane integrity which is directly related to a decrease in SG and vigor. The parameters estimated in this study can be used for simulating canola SG as well as.

Keywords

Aging model · Canola · Electrolyte leakage · Halotime model · Salt stress

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22.1 Introduction

Canola (*Brassica napus*, Brassicaceae) is one of the most important sources of vegetable oil, being used in the food (oil production, mainly in Asia), animal feed, and biofuel feedstock (Carruthers et al. 2017), and accounts for about 20% of the world production. Vegetative parts of this plant are rich in flavonoids, glucosinolates, phytoalexins, and phenylpropanoids (Pedras et al. 2008; Siger et al. 2008) that have beneficial effects on human health, including antioxidant and antitumor properties (Mazza 2007) and prevention and treatment of many diseases such as diabetes, hypertension, neurodegenerative diseases (Alzheimer's and Parkinson's), as well as autoimmune disorder (Szydłowska-Czerniak et al. 2010; Szydłowska-Czerniak et al. 2011). Canola production has continually increased over the past 20 years (1997–2017). The average production/yield quantities have increased from 34.7 (1997) to 76.2 million tones (2017) for an area of 23.3 and 34.9 million ha, respectively. Actually, it is now considered the second largely produced oilseed behind soybeans (FAO 2016). According to the same reference, Asia is the top producer (36.4%) followed by Europe (35.6%), America (23.7%), Oceania (4.1%), and Africa (0.2%) for the same period.

Successful seed germination (SG) is a crucial phase for plants to begin their life cycle and to proliferate, which is largely dependent on temperature (T), moisture, light, nutrient availability (Gilbertson et al. 2014; Wang et al. 2016), and seed vigor (Holdsworth et al. 2008; Rajjou et al. 2008). Relative humidity (RH) and T are the main factors influencing seed deterioration and viability loss during harvest conditions, transport, and prolonged storage (Garza-Caligaris et al. 2012; Radha et al. 2014). The storage of crop seeds in uncontrolled conditions (often in high T and RH) causes poor germination percentage (GP) and negatively affects the growth of seedlings and ultimately the yield (Khaliliaqdam et al. 2013).

Seed aging is a serious problem in oilseed crops particularly in developing countries due to maintaining seeds in an inappropriate condition during storage and harvest (Bakhshandeh and Gholamhossieni 2018). This phenomenon decreases GP and germination rate (GR) and causes even a totality loss of seed viability and vigor (Priestley 1986). Seed aging or seed deterioration initiates diverse biochemical and metabolic alterations that cause loss of membrane integrity (Powell 1986), reduce of primary metabolism, degradation of DNA, etc. (El-Maarouf-Bouteau et al. 2011). The increase in reactive oxygen species (ROS) and lipid peroxidation is generally well thought-out as the major factor causing seed deterioration particularly in oilseed crops such as canola (Bailly 2004; Corbineau et al. 2000; Ebadie et al. 2016; Elias and Copeland 1997; Merritt et al. 2003; Priestley 1986). Considering all these facts, optimizing storage conditions that have been proved successful in postponing the rate of seed aging and eventually increasing the seed viability and application of seeds with high vigor can help farmers to achieve higher yields in annual crops (e.g., canola) through higher germination uniformity, faster SG, and plant density in field conditions (Bakhshandeh and Gholamhossieni 2018; Hatzig et al. 2015).

To achieve such goals, it is important to provide information about the physiological quality of a seed lot using vigor tests such as the accelerated aging test (Elias and Copeland 1997). This test is simple, rapid, and inexpensive and was at first used to characterize a seed lot based on seed vigor and seed breakdown in commercial storage (Copeland and McDonald 2001; Delouche and Baskin 2016). It was effectively correlated to field emergence and establishment for many species, such as *Arachis hypogaea*, *Brassica oleracea*, *Brassica rapa*, *Capsicum* spp., *Lolium perenne*, and *Zea mays*. (Hampton and Tekrony 1995). The accelerated aging test is a reliable way of subjecting seeds to high T and RH for a short-time period causing rapid seed deterioration (Baalbaki et al. 2009; Bakhshandeh and Gholamhossieni 2018). Seed germination parameters are negatively affected by increasing accelerated aging test period (AATP) especially in oilseed crops (Bakhshandeh and Gholamhossieni 2018; Odoba et al. 2016). In fact, the alteration of membranes of aged seeds would cause electrolyte leakage during seed imbibition, especially in oil-rich seeds having linoleic and linolenic acid content (Balešević-Tubić et al. 2007; Chang and Sung 1998; Sung and Jeng 1994). Therefore, special attention to oilseeds storage should be given to prevent the oxidative processes that may occur too soon leading to loss of germination capacity and seed viability.

Abiotic stresses, especially soil salinity, is a severe problem in agriculture worldwide causing a reduction in growth, distribution, and production of crops all over the world (Munns and Tester 2008). Salinity is the most important factor that influences SG and seedling establishment interactively in field and/or natural habitat (Taherkhani et al. 2013). The estimated consequences of climate change (e.g., increase in mean T, irregularity of precipitation, increase in scarcity of irrigation water, etc.) will certainly aggravate this problem in the future especially in arid and semiarid regions. Recent studies showed that 1128 million ha of global land is affected by salinity and sodicity (Akhtar et al. 2015). The germination process is affected by salinity through a decrease in osmotic potential, nutritional disorders, oxidative stress, ion toxicity [accumulating Na^+ and Cl^- ions], alteration of metabolic processes, increase in lipid peroxidation, etc. (Muscolo et al. 2013; Sidari et al. 2008; Gul et al. 2013; Hameed et al. 2014; Khajeh-Hosseini et al. 2003). All these stresses imposed by salinity were reported to delay and decrease SG in different crops such as melon (*Cucumis melo*) (Botía et al. 1998), tomato (*Solanum lycopersicum*) (Cuartero and Fernández-Muñoz 1998), wheat (*Treticum aestivum* L.) (Egamberdieva and Kucharova 2009), canola (Jalili et al. 2009), groundnut (*Arachis hypogaea*) (Saravanakumar and Samiyappan 2007), sunflower (*Helianthus annuus* L.) (Luan et al. 2014; Wu et al. 2015), wild mustard (*Sinapis arvensis* L.) (Kayacetin et al. 2018), chicory (*Cichorium intybus* L.) (Vahabinia et al. 2019), and eruca (*Eruca sativa*) (Bakhshandeh et al. 2019).

Population-based threshold (PBT) models computing the timing of radicle emergence in seed populations are well thought-out helpful methods in depicting and determining the effect of external and/or internal factors on SG such as priming (Bradford and Haigh 1994), development (Still and Bradford 1998), hormones (Bradford and Somasco 1994; Ni and Bradford 1993), aging (Bakhshandeh and Gholamhossieni 2018; Bradford et al. 1993), oxygen (Bradford et al. 2008), light

(Bradford 2005), temperature (Alvarado and Bradford 2002; Bakhshandeh et al. 2019; Garcia-Huidobro et al. 1982), seed respiration rates (Bello and Bradford 2016), water potential (Bakhshandeh et al. 2019; Bradford 2002; Gummerson 1986), and even salinity (Seal et al. 2018; Bakhshandeh et al. 2019). According to Bradford (2018), a general PBT model that could be applied for any internal or/and external factor affecting SG parameters can be expressed as:

$$\theta X = (X - X_{\mathrm{b}}(i))\, t_{\mathrm{i}} \tag{22.1}$$

where θX, X, $X_b(i)$, and t_{i} are the time constant for responses to factor X, the measured quantity of factor X, the threshold distribution of the population for a given phenotype or response, and the time at which fraction i of the population exhibits the response due to factor amount X, respectively. Therefore, main objectives of this study were to (a) study the effects of salinity, AATP, and their interactions on SG characteristics (i.e., GP and GR) of canola; (b) quantify the SG response of this plant to sodium chloride (NaCl) at each AATP using the halotime model; (c) compute the SG response to AATP using the aging model at each NaCl concentration; and (d) investigate the seed moisture content (SMC) and electrical conductivity (EC) in canola seeds in response to AATP.

22.2 Materials and Methods

Canola seeds (*Brassica napus* L.; Brassicaceae) var. "Dalgan" was used in this study is classified as a spring canola cultivar. The seeds were provided by Seed and Plant Improvement Institute, Karaj, Iran, in 2018, and were stored in a plastic bag and kept in a refrigerator at 5 °C before use.

To obtain sub-lots with different seed vigor, an accelerated aging test was applied on ~8 g of canola seeds (initial viability >97%), which were uniformly distributed on a stainless wire screen and put in a plastic container with ~10% of its total volume filled with distilled water. The containers were then covered with lids, sealed, and placed in an incubator at a constant T of 42 ± 1 °C in the dark (Elias and Copeland 1997) and a RH of ~100% during the aging period. After 24, 48, and 72 h of AATP, the seeds were removed from the containers, and the SMC was immediately measured according to ISTA method (ISTA 2009). Then, 40 seeds with three replicates per treatment were spread uniformly within 8 cm Petri dishes on two layers of germination paper (Whatman No. 1) containing 7 mL distilled water or each salinity solution, supplemented with 0.1% thiram fungicide. In all experiments, distilled water was used as a control, and NaCl was used for preparing the following salinity levels: 50, 100, 150, 200, 250, and 300 mM. The Petri dishes were placed in a plastic bag to reduce water evaporation and then were randomly incubated in different positions in an incubator at 25 ± 0.5 °C (Elias and Copeland 1997) in the dark, except during recording germination.

The seeds were counted several times daily, depending on the AATP and salinity level, and were considered to have germinated when the emerged radicle was at least

2 mm long (germinated seeds were removed from the Petri dishes in each recording to avoid an error). The experiments were ended when we have no further germination after two consecutive days in each Petri dish. An electrical conductivity (EC) test was done to determine the effect of AATP on membrane damage of seed by measuring the change in conductivity leached from canola seeds into the distilled water. Briefly, 100 untreated seeds (control, AATP = 0) and each AATP were weighted and soaked in 50 mL of distilled water at 20 °C with three replicates. After 24 h soaking, the EC of each replicate was measured by an EC-meter apparatus.

A repeated probit regression method (Alvarado and Bradford 2002) was used to analyze and determine the model parameters. GR (h^{-1}) was calculated for the 50th percentile according to the following model [$GR_{50} = 1/t_{50}$], where GR_{50} is the GR to reach 50% of germination (h^{-1}) and t_{50} is the time to reach 50% germination, which was calculated by interpolation by curves fit to the time course data.

The halotime model was developed by substituting X in the general PBT model by NaCl concentration (NaCl), so Eq. (22.1) will be written as:

$$\theta_{\text{Halo}} = (\text{NaCl} - \text{NaCl}_{\text{b}}(g))\, t_{\text{g}} \tag{22.2}$$

where θ_{Halo}, NaCl, $\text{NaCl}_{\text{b}}(g)$, and t_{g} are the halotime constant (mM h or mM d), the actual NaCl concentration of the medium (mM), the base value of NaCl inhibiting radicle emergence of percentage g (mM), and the actual time to germination percentage g (h or d), respectively. Thus, the $\text{NaCl}_{\text{b}}(50)$ is the median base NaCl concentration just inhibiting germination of the 50th percentile of the seed population. Since $(\text{NaCl} - \text{NaCl}_{\text{b}}(g))$ will be a negative number, as the magnitude of NaCl is smaller than $\text{NaCl}_{\text{b}}(50)$ at any NaCl permitting germination, we have reversed the order of $\text{NaCl}_{\text{b}}(g)$ and NaCl in Eq. (22.2) to give positive values. So the equation will be:

$$\theta_{\text{Halo}} = (\text{NaCl}_{\text{b}}(g) - \text{NaCl})\, t_{\text{g}} \tag{22.3}$$

A PBT model suggested by Bello and Bradford (2016) was also used to quantify the germination time courses of seeds during aging.

$$\theta_{\text{age}} = (p - p_{\max}(g))\, t_{\text{g}} \tag{22.4}$$

where θ_{age}, p, and $p_{\max}(g)$ are the aging time constant (h or d h), the actual aging period (h or d), and the midpoint of the regression line, respectively, which refers to the aging period at which germination is reduced to specific % (often 50th percentile) of the seed population (h or d). Based on this model, variation in GR over storage (or AATP) is estimated to determine the maximum lifespan of seeds under the storage conditions tested. According to Bello and Bradford (2016), "this model presume that seeds in the population display a distribution of maximum potential lifetimes under the storage conditions ($p_{\max}(g)$), and that the delay in germination with increasing aging period is proportional to the fraction of that potential maximum storage lifetime that has already passed for each seed."

Data analysis was realized using the statistical analysis system ver. 9.4 software (SAS Institute 2013). To study the effect of AATP, salinity, and their interactions on SG characteristics, a two-way ANOVA was carried out at $p < 0.05$. All figures were also constructed using the Sigma Plot ver. 11 software (Systat Software Inc., San Jose CA, USA).

22.3 Results

22.3.1 Effects of Salt and Accelerated Aging Period on GP and GR_{50}

NaCl, AATP, and their interactions (AATP×NaCl) significantly affected GP and GR_{50} ($p < 0.001$). Both GP and GR_{50} decreased with increasing AATP and NaCl concentrations in the medium (Table 22.1). In general, GP declined by 45.2, 66.9, and 91.6% at 24, 48, and 72 h of AATP (average all salinity levels) as compared to the control, respectively. Moreover, more concentrated medium in NaCl from 0 to 50, 100, 150, 200, 250, and 300 mM (corresponding to −0.22, −0.45, −0.67, −0.89, −1.11, and − 1.34 MPa based on the Van't Hoff equation, respectively) decreased GP by 14.6, 25.5, 34.9, 54.6, 69.9, and 88.1% (average all AATP) in comparison with the control, respectively (Table 22.1). Similarly, GR_{50} decreased by 62.5, 82.0, and 95.0% with increasing AATP from 0 to 24, 48, and 72 h, respectively (Table 22.1). Also, increased concentration of NaCl in the medium from 0 to 50, 100, 150, 200, 250, and 300 mM decreased GR_{50} by 26.3, 46.7, 52.1, 50.9, 83.2, and 92.8% compared to the control, respectively (Table 22.1). Therefore, GR_{50} was more sensitive to AATP and salinity levels than GP in canola. The germination time courses of canola seeds at the various AATP and at each of the different concentrations of NaCl are presented in Fig. 22.1a–d. The germination of unaged seeds was recorded at all levels of salt stress with the maximum (~95.8%) of GP in the control seeds (0 mM) and the minimum (~29.2%) when seeds were treated with 300 mM NaCl. With increasing AATP, GP was totally inhibited (GP = 0) for seeds treated with 300, 250, and 150 mM NaCl at 24, 48, and 78 h of AATP as compared to the control, respectively (Fig. 22.1a–d). However, expressing the germination time courses of canola seeds under different salt concentrations at each AATP showed that GP decreased significantly for NaCl>150 mM for control (0 h AATP) and was totally inhibited for treated seeds (24, 48, and 72 h of AATP) at 300 mM NaCl (Fig. 22.2a–g).

22.3.2 Halotime Model

To study the effect of AATP on canola SG under different concentrations of NaCl in the medium and to delimit the SG threshold in each AATP, the halotime model (Eq. (22.3)) was fitted. Our results showed that the coefficient of determination (R^2) ranged from 0.87 to 0.95, reflecting a good fit between the actual and the predicted data (Table 22.2; Fig. 22.1a–d). The θ_{Halo} increased linearly (with the rate of

Table 22.1 Results of analysis of variance and means comparison for germination percentage (GP) and median germination rate (GR_{50}) of canola as affected by accelerated aging test period (AATP) and salt stress (NaCl)

Source of variation	df		GP	GR_{50}
AATP	3		***	***
NaCl	6		***	***
AATP ×NaCl	18		***	***
Coefficient of variation (%)			10.0	16.8
Means comparison[a]		*n*	GP (%)	$GR_{50}(h^{-1})$
AATP (h)	0	21	73.8a	0.0204a
	24	21	40.4b	0.0075b
	48	21	24.4c	0.0036c
	72	21	6.2d	0.0010d
		n	GP (%)	$GR_{50}(h^{-1})$
NaCl (mM)	0	12	61.5a	0.0167a
	50	12	52.5b	0.0123b
	100	12	45.8c	0.0089c
	150	12	40.0d	0.0082c
	200	12	27.9e	0.0063d
	250	12	18.5f	0.0028e
	300	12	7.29 g	0.0012f

df degrees of freedom. The number of total data points is $n = 84$ (AP = 4, NaCl = 7, and replications = 3)

The NaCl concentrations used corresponded to −0.22, −0.45, −0.67, −0.89, −1.11, and −1.34 MPa based on the Van't Hoff equation (Van't Hoff 1887), respectively, which was verified by an osmometer measurement

[a]Means with the same letter are not significantly different based on the Duncan's multiple range test

*** Highly significant at 0.001 probability level

47.2 mM h per h AATP) with increasing AATP from 10238.9 (control) to 13585.0 mM h (72 h of AATP); this increase could be explained by the decrease in GR_{50}. In fact, the GR_{50} decreased significantly with AATP from 0.0333 (control) to 0.0036 at 72 h of AATP (Table 22.2). The rate of decrease was 0.0004 h^{-1} per h increase in AATP. However, the relative frequencies of $NaCl_b(g)$ values in seed population at each AATP using the normal distribution showed that median values or the $NaCl_b(50)$ decreased significantly with increasing AATP from 341.1 mM in the control to 50.19 mM after 72 h of AATP (Fig. 22.1e–h and Table 22.2). The amount of decrease in $NaCl_b(50)$ per h increase in AATP was 4.15 mM, which will totally be inhibited at 82.1 h AATP (Fig. 22.3a). Our findings showed that, with longer AATP, $\psi_b(50)$ value [converted NaClb(50) (mM) to $\psi_b(50)$ (MPa) values using Van't Hoff equation] of canola increased (became more positive) from −1.52 MPa in the control to −0.22 MPa at 72 h AATP (about 85.5% higher than the control) (Table 22.2).

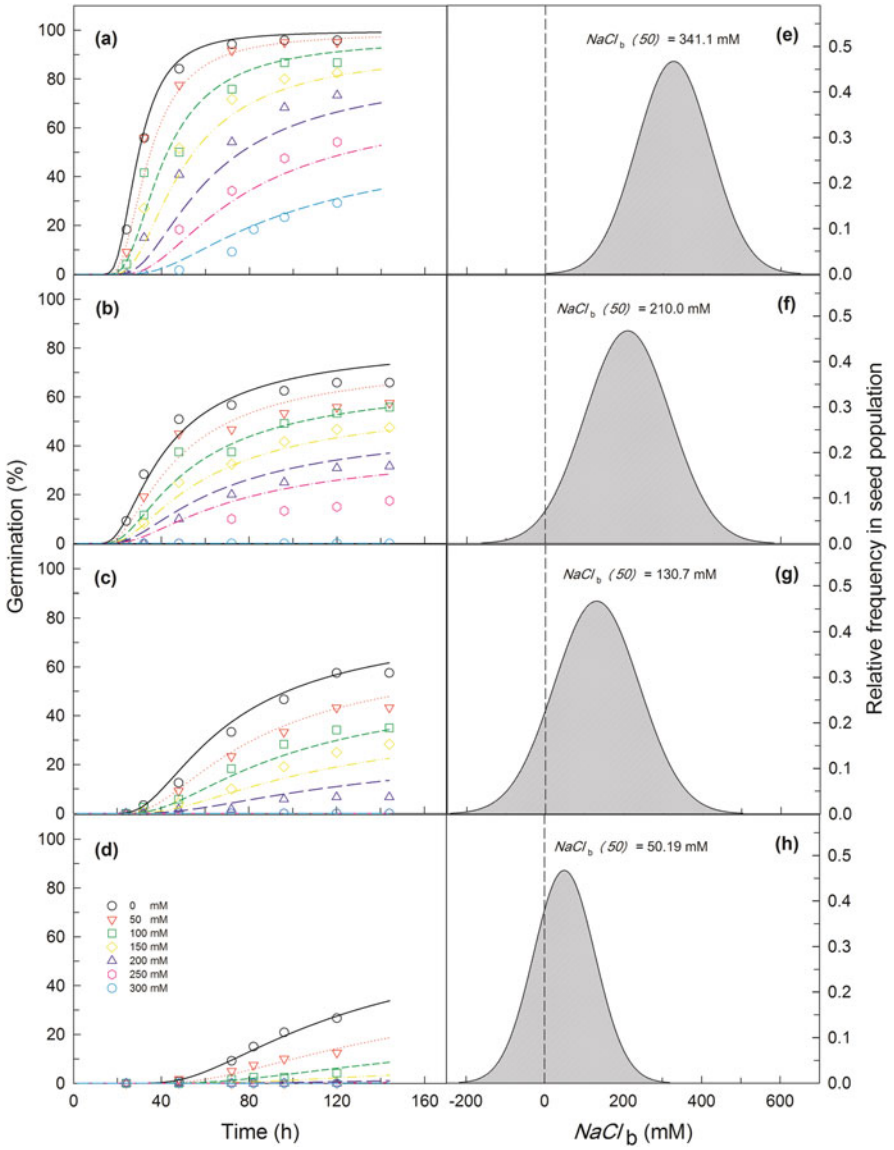

Fig. 22.1 Germination time courses of canola seeds (cv. "Dalgan") at various accelerated aging test periods (AATP) including 0 (**a**), 24 h (**b**), 48 h (**c**), and 72 h (**d**) and at each of the following different levels of NaCl concentration (mM). The symbols are the actual data, and the lines are the time courses predicted by the halotime model (Eq. (22.3)) based on the parameters presented in Table 22.2. (**e**–**h**) Normal distribution showing the relative frequencies of $NaCl_b(g)$ values in seed population at each AATP. The median or $NaCl_b(50)$ values are shown (see Table 22.2)

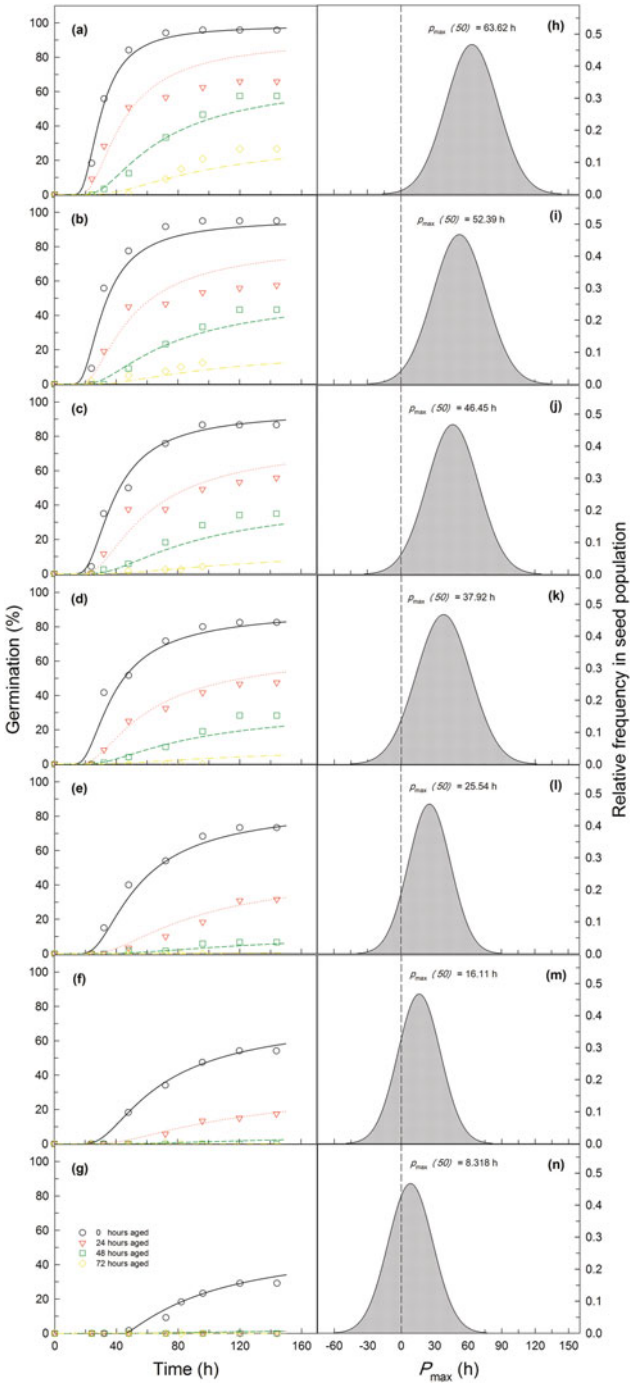

Fig. 22.2 (**a**–**g**) Germination time courses of canola seeds (cv. "Dalgan") at the different levels of NaCl concentration (mM) including 0 (**a**), 50 (**b**), 100 (**c**), 150 (**d**), 200 (**e**), 250 (**f**), and 300 (**g**) at each of the following various accelerated aging period. The symbols are the actual data, and the

22.3.3 Aging Model

The aging model (Eq. (22.4)) provided a good description of the germination response of canola seeds at a range of different NaCl concentrations with a R^2 ranging between 0.91 and 0.99 (Table 22.2 and Fig. 22.2). Our results showed that the θ_{age} values were not significantly affected by NaCl concentrations, ranged from 1797.7 h at 50 mM to 1972.2 h in the control condition (Table 22.2). The normal distribution showing the relative frequencies of $p_{max}(50)$ (that is, the maximum potential lifetimes under the storage conditions for 50th percentile) values in seed population at each NaCl is illustrated in Table 22.2 and Fig. 22.2h–n. Observing Table 22.2, it is obvious that $p_{max}(50)$ decreased significantly with the increasing NaCl concentrations (average all AATP). It ranged from a maximum value (63.62 h) at the control (0 mM) to a minimum value (8.318 h) at 300 mM NaCl (Table 22.2). The relationship between $p_{max}(50)$ and NaCl concentration is linear (with a slope of 0.185 h per mM NaCl), and the intercept is equal to 341.1 mM NaCl (equal to $NaCl_b(50)$ for the control) (Fig. 22.3b). The GR_{50}, based on the aging model, showed the same pattern as for the halotime model and its values significantly decreased with NaCl concentration (from 0.0322 h^{-1} at the control to 0.0047 h^{-1} at 300 mM NaCl, which was 85.4% lower than the control) (Table 22.2).

22.3.4 SMC and EC

The regression analysis to examine the effect of AATP on the SMC is illustrated in Fig. 22.3c. The SMC increased linearly from 14.1% (SMC before AATP) to 33.3% (at 24 h) with an increase of 2.28-fold compared to the control. Then, it increased linearly for AATP $>$ 24 h, reaching 41.8% after 72 h of AATP (2.85-fold more than the control) (Fig. 22.3c). The amount of rate were 0.79 and 0.178% per h increase in AATP in the first (AATP $<$ 24) and second (AATP $>$ 24) phases of the regression model, respectively (Fig. 22.3c). Based on our results, EC remained constant (89.8 μS cm^{-1} g^{-1}) for AATP$<$31.18 h and then increased linearly with the rate of 1.34 μS cm^{-1} g^{-1} per h increase in AATP, reaching its maximum (144.5 μS cm^{-1} g^{-1}) at 72 h of AATP (Fig. 22.3d).

Fig. 22.2 (continued) lines are the time courses predicted by the aging model (Eq. (22.4)) based on the parameters presented in Table 22.2. (**h**–**n**) Normal distribution showing the relative frequencies of $p_{max}(g)$ values in seed population at each NaCl. The median or $p_{max}(50)$ values are shown (see Table 22.2)

Table 22.2 Estimated parameters of the population-based threshold (PBT) models (i.e., halotime and aging models) for characterizing germination of canola seeds at seven levels of NaCl concentration at each accelerated aging test period (AATP)

Halotime model	AATP (h)	θ_{Halo} (mM h)	$NaCl_b(50)$ (mM)	σ_{NaClb} (mM)	R^2	GR50 (h^{-1})	$\psi_b(50)$ (MPa)[a]
Halotime	0	10238.9	341.1	115.7	0.95	0.0333	−1.52
	24	11452.3	210.0	179.4	0.87	0.0183	−0.94
	48	12734.8	130.7	142.8	0.93	0.0102	−0.58
	72	13585.0	50.19	105.5	0.95	0.0036	−0.22
Aging model	**NaCl (mM)**	**θ_{age} (h h)**	**p_{max}(50) (h)**	**σ_{pmax} (h)**	**R^2**	**GR50 (h^{-1})**	
	0	1972.2	63.62	26.86	0.94	0.0322	–
	50	1797.7	52.39	27.39	0.91	0.0291	–
	100	1965.3	46.45	26.57	0.95	0.0236	–
	150	1703.1	37.92	28.03	0.97	0.0222	–
	200	1690.9	25.54	21.71	0.98	0.0151	–
	250	1726.4	16.11	21.96	0.99	0.0093	–
	300	1764.6	8.318	23.06	0.92	0.0047	–

[a]The NaCl concentrations (mM) values were converted to water potentials (MPa) using the Van't Hoff equation

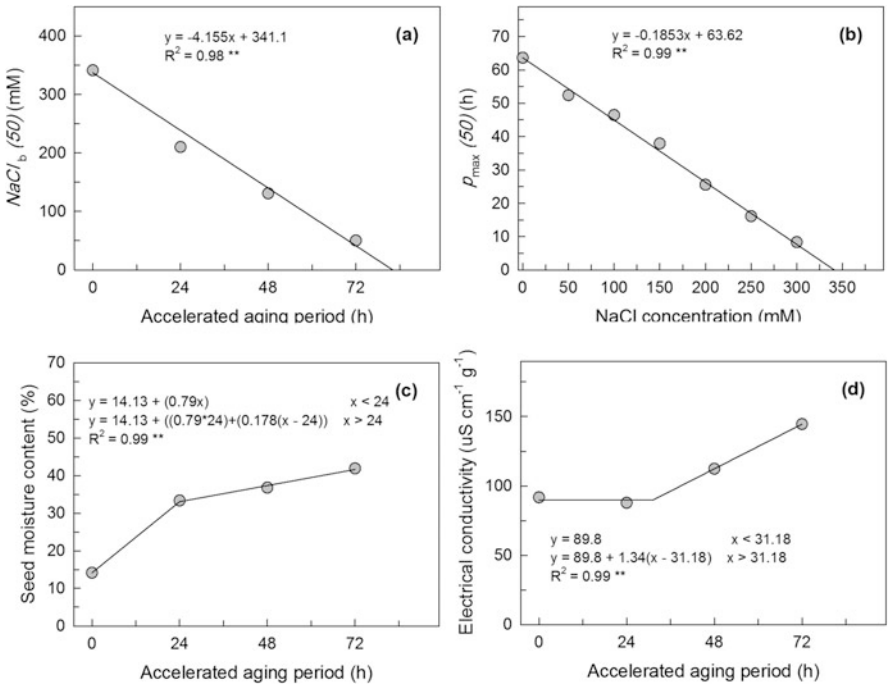

Fig. 22.3 Regression analysis to examine the effect of accelerated aging period on the $NaCl_b(50)$ (**a**), seed moisture content (**c**), and electrical conductivity (**d**) in canola seeds. (**d**) Relationship between $p_{max}(50)$ and NaCl concentration is shown. All regressions are statistically significant at 0.01 probability level. In panel a, $NaCl_b(50)$ = zero = 82.1 h, and in panel **b**, $p_{max}(50)$ = zero = 341.1 mM

22.4 Discussion

Seed germination characteristics (i.e., GP and GR_{50}) were at their maximum at control (0 mM NaCl and 0 h AATP) and were decreased with longer AATP and more concentrated medium growth with NaCl (Table 22.1). Furthermore, seeds germinated slower than the water when exposed to lower ψ caused by higher NaCl concentrations in the medium (Fig. 22.1a–d). Our results corroborate with many other researches showing that SG of several crops has been reported to decrease with increasing salt concentration (Egamberdieva 2009; Jalili et al. 2009; Shrivastava and Kumar 2015; Bakhshandeh et al. 2019). Based on the halotime model, we found that the $NaCl_b(50)$ was 341.1 mM. Contrarily, Silva and da Silveira (2014) reported that the final GP of canola seeds was reduced in all salinity levels and germination was totally inhibited at 250 mM. Al-Thabet et al. (2004) reported that the decrease in canola GP and GR was related to an increase in salinity concentrations superior to 100 mM NaCl and that this decrease was correlated with the osmotic effect due to the presence of NaCl in the medium. Decreased

osmotic potential can reduce water absorption and SG, as water plays an essential role in mobilizing seed reserves and during all germination phases (Bewley et al. 2012). Thus, the damaging effects of salinity on SG are linked to lower ψ, ion toxicity, and nutrition scarcity (Greenway and Munns 1980). In addition to osmotic stress, salinity is responsible for the initiation of primary effects such as ionic stress which in turn induce the oxidative stress in plants (Bybordi 2012). ROS, generated by the oxidative stress, are harmful for plants as they can destroy the structure and functions of biomolecules, such as proteins, nucleic acids, and membrane lipids. In higher concentrations, ROS cause death of the plant cells (Hernández et al. 2001; Ahmad and Sharma 2008; Ahmad et al. 2012; Bybordi et al. 2010). Also, salinity causes ion cytotoxicity (Zhu 2002; Wu et al. 2015). Under salinity stress, plants enhance Na^+ uptake, disturbing thereby the metabolic processes that necessitate high K^+, Ca_2^+, or both and low Na^+ to function normally (Shabala et al. 2013; Akhtar et al. 2015).

The estimated parameters of the halotime model for characterizing germination of canola seeds showed that there was a decrease in $NaCl_b(50)$ corresponding also to an increase of $\psi_b(50)$ values (became more positive) with longer AATP. This phenomenon could be related to the loss of cell membrane integrity and alteration of the enzyme activities. As was shown by other researches, the $\psi_b(50)$ is an important marker used to identify the radical emergence time at specific ψ and to recognize the initial seed vigor (Bakhshandeh and Gholamhossieni 2018; Derakhshan and Gharineh 2015; Soltani and Farzaneh 2014). Similarly, many crops have decreased both GP and GR at longer AATP and lower ψ (Bakhshandeh et al. 2015; Bakhshandeh et al. 2011; Kandil et al. 2013; Khaliliaqdam et al. 2013; Odoba et al. 2016). Our findings showed that at longer AATP, GP was reduced by 31.3, 40.0, and 72.2% at 24, 48, and 72 h of AATP compared to the control (0 mM NaCl). This diminution is greater than that obtained by Najafi et al. (2015), in canola seeds, showing a reduction of 15.5 and 65.0% at 41 °C for 48 and 96 h of seed deterioration, respectively. Besides, soybean seed deterioration at 41 °C for 48 h decreased GP by 22% in comparison with the control (Khaliliaqdam et al. 2013). Furthermore, the AATP of 48 h applied for soybean seeds decreased significantly GP, GR, and germination uniformity (GU) and led to an increase of electrolyte leakage (Mohammadi et al. 2012). However, for chickpea (*Cicer arietinum* L.), applied longer AATP (up to 144 h at 41 °C) declined GP by 66% (Bayat et al. 2016). It is also to be noticed that GR_{50} showed a decrease of 38.6, 69.7, and 86.3% at 24, 48, and 72 h of AATP compared to the control (0 mM NaCl), showing loss of seed vitality as GR is considered the most important marker of seed vigor (Matthews et al. 2011). As the AATP increases, seeds lost their vigor due to an increase in electrolyte leakage, an increase in lipid peroxidation, a decrease in enzyme activities. In our findings, the estimated parameters of the aging model showed a linear decrease in the $p_{max}(50)$ with increasing NaCl concentrations, reaching zero at 341.1 mM NaCl (equal to $NaCl_b(50)$ for the control). The results presented here show that loss of canola seed viability during AATP was largely influenced by salinity stress.

The SMC is a crucial factor affecting seed vigor during storage (Ellis et al. 1990; Ellis and Roberts 1980). We found that the SMC increased with AATP

corroborating with other results (Ghaderi-Far et al. 2010; Khaliliaqdam et al. 2013; Radha et al. 2014). Generally, an increase in SMC during seed aging can reduce seed vigor and vitality through seed respiration increase, decrease in enzyme activities, and cell membrane integrity (Bello and Bradford 2016; Kumar et al. 2014). As was shown in canola seed (Jayas and White 2003; McDonald 1999), the SMC increase is considered the most important factor influencing seed viability by accelerating seed deterioration.

The EC increased significantly and linearly with the AATP $>$ 31.18 h (Fig. 22.3d). This increase could be related to loss of membrane integrity (Elias and Copeland 1997). Similarly, it was shown that the increased leakage linked to aging is associated with membrane damage and loss of selective membrane permeability (Copeland and McDonald 1985). The EC or the electrolyte leakage was considered as an index of seed vigor (Abdul-Baki and Anderson 1972; Wilson Jr and McDonald Jr 1992). The electrolyte leakage increase due to membrane damage is associated with a decrease in transport, cellular integrity and metabolic energy, degradation of DNA (McDonald 1999; Priestley 1986; Ramos et al. 1988; Tatipata 2009). Thus, an increase in EC reflecting the loss of membrane integrity is directly related to a decrease in SG and vigor (Wilson Jr and McDonald Jr 1992).

22.5 Conclusions

In canola, GP and GR_{50} are significantly affected by AATP, NaCl, and their interactions. The halotime and aging models successfully described the response of GR and GP for this plant ($R^2 > 0.87$). Based on the model prediction, the SG of canola was totally inhibited at 82.1 h AATP and 341.1 mM NaCl. Both SMC and EC increased significantly with the AATP, suggesting a reduction in SG and vigor. The parameters estimated in this study can be used for simulating canola SG in similar conditions as well.

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Author Contribution Statement: EB, MJ, and RA designed the experiment. EB and MJ collected the data. EB conducted the modeling and interpreted the data. EB, MJ, RA, and FB co-wrote all drafts of this chapter and also approved the final draft for submission.

References

Abdul-Baki AA, Anderson JD (1972) Physiological and biochemical deterioration of seeds. Seed biology 2:283–316

Ahmad P, Kumar A, Ashraf M, Akram NA (2012) Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). Afr J Biotechnol 11(11):2694–2703

Ahmad P, Sharma S (2008) Salt stress and phyto-biochemical responses of plants. Plant Soil Environ 54(3):89–99

Akhtar SS, Andersen MN, Liu F (2015) Residual effects of biochar on improving growth, physiology and yield of wheat under salt stress. Agric Water Manag 158:61–68

Al-Thabet S, Leilah A, Al-Hawass I (2004) Effect of NaCl and incubation temperature on seed germination of three canola (Brassica napus L.) cultivars. Sci King Faisal Univ Basic Appl Sci 5 (1):81–92

Alvarado V, Bradford K (2002) A hydrothermal time model explains the cardinal temperatures for seed germination. Plant Cell Environ 25(8):1061–1069

Baalbaki R, Elias S, Marcos Filho J, McDonald MB (2009) Seed vigor testing handbook: contribuition n. 32 to The handbook on seed testing

Bailly C (2004) Active oxygen species and antioxidants in seed biology. Seed Sci Res 14(2):93–107

Bakhshandeh E, Atashi S, Hafeznia M, Pirdashti H, da Silva JAT (2015) Hydrothermal time analysis of watermelon (*Citrullus vulgaris* cv. 'Crimson sweet') seed germination. Acta Physiologiae Plantarum 37(1):1738

Bakhshandeh E, Ghadiryan R, Galeshi S, Soltani E (2011) Modelling the effects water stress and temperature on seed germination of soybean (*Glycine max* L.) and velvetleaf (Abutilon theophrasti med.). J Plant Prod 18:29–45

Bakhshandeh E, Gholamhossieni M (2018) Quantification of soybean seed germination response to seed deterioration under peg-induced water stress using hydrotime concept. Acta Physiol Plant 40(7):126

Bakhshandeh E, Pirdashti H, Vahabinia F, Gholamhossieni M (2019) Quantification of the effect of environmental factors on seed germination and seedling growth of eruca (*Eruca sativa*) using mathematical models. J Plant Growth Regul 39:190–204

Balešević-Tubić S, Tatić M, Miladinović J, Pucarević M (2007) Changes of faty acids content and vigor of sunflower seed during natural aging/cambios de contenido de ácidos grasos y germinación durante el envejecimiento natural de la semilla de girasol/changements du contenu d'acides gras et de la vigueur de la graine de tournesol au cours du vieillissement naturel. Helia 30(47):61–68

Bayat P, Ghobadi M, Ghobadi ME, Mohammadi G (2016) Calibration of accelerated aging test as a vigor test to predict the seedling emergence of chickpea (*Cicer arietinum* L.) in field conditions. Iran J Pulses Res 7:9–24

Bello P, Bradford KJ (2016) Single-seed oxygen consumption measurements and population-based threshold models link respiration and germination rates under diverse conditions. Seed Sci Res 26(3):199–221

Bewley JD, Bradford K, Hilhorst H (2012) Seeds: physiology of development, germination and dormancy. Springer Science & Business Media, New York

Botía P, Carvajal M, Cerdá A, Martínez V (1998) Response of eight Cucumis melo cultivars to salinity during germination and early vegetative growth. Agronomie 18(8-9):503–513

Bradford KJ (2002) Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. Weed Sci 50(2):248–260

Bradford KJ (2005) Threshold models applied to seed germination ecology. New Phytol 165 (2):338–341

Bradford KJ (2018) Interpreting biological variation: seeds, populations and sensitivity thresholds. Seed Sci Res 28(3):158–167

Bradford KJ, Benech-Arnold RL, Coˆme D, Corbineau F (2008) Quantifying the sensitivity of barley seed germination to oxygen, abscisic acid, and gibberellin using a population-based threshold model. J Exp Bot 59(2):335–347

Bradford KJ, Haigh AM (1994) Relationship between accumulated hydrothermal time during seed priming and subsequent seed germination rates. Seed Sci Res 4(2):63–69

Bradford KJ, Somasco OA (1994) Water relations of lettuce seed thermoinhibition. I. Priming and endosperm effects on base water potential. Seed Sci Res 4(1):1–10

Bradford KJ, Tarquis AM, Durán JM (1993) A population-based threshold model describing the relationship between germination rates and seed deterioration. J Exp Bot 44(7):1225–1234
Bybordi A (2012) Effect of ascorbic acid and silicium on photosynthesis, antioxidant enzyme activity, and fatty acid contents in canola exposure to salt stress. J Integr Agric 11 (10):1610–1620
Bybordi A, Tabatabaei SJ, Ahmadev A (2010) The influence of salinity stress on antioxidant activity in canola cultivars (*Brassica napus* L.). J Food Agric Environ 8(1):122–127
Carruthers JM, Cook SM, Wright GA, Osborne JL, Clark SJ, Swain JL, Haughton AJ (2017) Oilseed rape (*Brassica napus*) as a resource for farmland insect pollinators: quantifying floral traits in conventional varieties and breeding systems. GCB Bioenergy 9(8):1370–1379
Chang S, Sung J (1998) Deteriorative changes in primed sweet corn seeds during storage. Seed Sci Technol 26(3):613–625
Copeland L, McDonald M (1985) Principles of seed science and technology, 2nd edn. Edición Burguess Publising Company, 321 pp
Copeland LO, McDonald MB (2001) Seed vigor and vigor testing. In: Principles of seed science and technology. Springer, New York, pp 165–191
Corbineau F, Picard MA, Fougereux J-A, Ladonne F, Côme D (2000) Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. Seed Sci Res 10(3):329–339
Cuartero J, Fernández-Muñoz R (1998) Tomato and salinity. Sci Hortic 78(1-4):83–125
Delouche JC, Baskin CC (2016) Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci Technol J 1:427–452
Derakhshan A, Gharineh MH (2015) Application of hydrotime concept to predict seedling emergence of spring barley varieties in field. Iran J Seed Sci Res 2(2):1–14
Ebadie A, Parmoon G, Jahanbakhah S (2016) Effect of potassium nitrate on antioxidant enzymes activity of aged milk thistle (Silybum marianum) seeds. J Plant Process Funct 36(16):27–44
Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31(4):861–864
Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol Fertil Soils 45(6):563–571
El-Maarouf-Bouteau H, Mazuy C, Corbineau F, Bailly C (2011) DNA alteration and programmed cell death during ageing of sunflower seed. J Exp Bot 62(14):5003–5011
Elias S, Copeland L (1997) Evaluation of seed vigor tests for canola. Seed Technol 19:78–87
Ellis R, Hong T, Roberts E, Tao K-L (1990) Low moisture content limits to relations between seed longevity and moisture. Ann Bot 65(5):493–504
Ellis R, Roberts E (1980) The influence of temperature and moisture on seed viability period in barley (*Hordeum distichum* L.). Ann Bot 45(1):31–37
FAO (2016) FAOSTAT/Productionstat/Crops [Online]. Food and Agriculture Organization of the United Nations. Revised on 28 May 2018. http://www.fao.org/faostat/en/#data/QC
Garcia-Huidobro J, Monteith J, Squire G (1982) Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.) I. Constant temperature. J Exp Bot 33(2):288–296
Garza-Caligaris LE, Avendaño-Vázquez AO, Alvarado-López S, Zúñiga-Sánchez E, Orozco-Segovia A, Pérez-Ruíz RV, Gamboa-deBuen A (2012) At3g08030 transcript: a molecular marker of seed ageing. Ann Bot 110(6):1253–1260
Ghaderi-Far F, Bakhshandeh E, Ghadirian R (2010) Evaluating seed quality in sesame (*Sesamum indicum* L.) by the accelerated ageing test. Seed Technol 32:69–72
Gilbertson PK, Berti MT, Johnson BL (2014) Borage cardinal germination temperatures and seed development. Ind Crop Prod 59:202–209
Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol 31(1):149–190
Gul B, Ansari R, Flowers TJ, Khan MA (2013) Germination strategies of halophyte seeds under salinity. Environ Exp Bot 92:4–18

Gummerson R (1986) The effect of constant temperatures and osmotic potentials on the germination of sugar beet. J Exp Bot 37(6):729–741
Hameed A, Rasheed A, Gul B, Khan MA (2014) Salinity inhibits seed germination of perennial halophytes *Limonium stocksii* and *Suaeda fruticosa* by reducing water uptake and ascorbate dependent antioxidant system. Environ Exp Bot 107:32–38
Hampton J, Tekrony D (1995) Accelerated aging test. Handbook of vigour tests methods. pp 1–10
Hatzig SV, Frisch M, Breuer F, Nesi N, Ducournau S, Wagner M-H, Leckband G, Abbadi A, Snowdon RJ (2015) Genome-wide association mapping unravels the genetic control of seed germination and vigor in *Brassica napus*. Front Plant Sci 6:221
Hernández JA, Ferrer MA, Jiménez A, Barceló AR, Sevilla F (2001) Antioxidant systems and $O_2.-/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127(3):817–831
Holdsworth MJ, Bentsink L, Soppe WJ (2008) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol 179(1):33–54
International Seed Testing Association (ISTA) (2009) International rules for seed testing. International Seed Testing Association, Zurich
Jalili F, Khavazi K, Pazira E, Nejati A, Rahmani HA, Sadaghiani HR, Miransari M (2009) Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. J Plant Physiol 166(6):667–674
Jayas DS, White ND (2003) Storage and drying of grain in Canada: low cost approaches. Food Control 14(4):255–261
Kandil A, Sharief A, Sheteiwy M (2013) Effect of seed storage periods, conditions and materials on germination of some soybean seed cultivars. Am J Exp Agric 3(4):1020
Kayacetin F, Efeoglu B, Alizadeh B (2018) Effect of NaCl and PEG-induced osmotic stress on germination and seedling growth properties in wild mustard (*Sinapis arvensis* L.). Anadolu 28 (1):62–68
Khajeh-Hosseini M, Powell A, Bingham I (2003) The interaction between salinity stress and seed vigour during germination of soybean seeds. Seed Sci Technol 31(3):715–725
Khaliliaqdam N, Soltani A, Latifi N, Ghaderi Far F (2013) Soybean seed aging and environmental factors on seedling growth. Commun Soil Sci Plant Anal 44(12):1786–1799
Kumar TP, Asha A, Maruthi J, Vishwanath K (2014) Influence of seed treatment chemicals and containers on seed quality of marigold during storage. Bioscan 9(3):937–942
Luan Z, Xiao M, Zhou D, Zhang H, Tian Y, Wu Y, Guan B, Song Y (2014) Effects of salinity, temperature, and polyethylene glycol on the seed germination of sunflower (*Helianthus annuus* L.). Scientific World J 2014:170418
Matthews S, Wagner M, Ratzenboeck A, Khajeh-Hosseini M, Casarini E, El-Khadem R, El-Yakhlifi M, Powell A (2011) Early counts of radicle emergence during germination as a repeatable and reproducible vigour test for maize. Seed Testing Int 141:39–45
Mazza G (2007) Anthocyanins and heart health. Annali-Istituto Superiore Di Sanita 43(4):369
McDonald M (1999) Seed deterioration: physiology, repair and assessment. Seed Sci Technol 27:177–237
Merritt D, Senaratna T, Touchell D, Dixon K, Sivasithamparam K (2003) Seed ageing of four Western Australian species in relation to storage environment and seed antioxidant activity. Seed Sci Res 13(2):155–165
Mohammadi H, Soltani A, Sadeghipour H, Zeinali E (2012) Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. Int J Plant Product 5(1):65–70
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
Muscolo A, Panuccio M, Eshel A (2013) Ecophysiology of *Pennisetum clandestinum*: a valuable salt tolerant grass. Environ Exp Bot 92:55–63
Najafi G, Khamary S, Javadi A (2015) The response of rapeseed seed germination to seed vigor and hydro-priming changes. J Seed Res 5:54–70

Ni B-R, Bradford KJ (1993) Germination and dormancy of abscisic acid-and gibberellin-deficient mutant tomato (*Lycopersicon esculentum*) seeds (sensitivity of germination to abscisic acid, gibberellin, and water potential). Plant Physiol 101(2):607–617

Odoba A, Odiaka N, Gbanguba A, Bashiru M (2016) Germination characteristics of twenty varieties of soybean (*Glycine max* (L.) Merr) stored for seven months. Sci Agric 13(3):151–155

Pedras MSC, Zheng Q-A, Gadagi RS, Rimmer SR (2008) Phytoalexins and polar metabolites from the oilseeds canola and rapeseed: differential metabolic responses to the biotroph Albugo candida and to abiotic stress. Phytochemistry 69(4):894–910

Powell AA (1986) Cell membranes and seed leachate conductivity in relation to the quality of seed for sowing. J Seed Technol 10:81–100

Priestley DA (1986) Seed aging: implications for seed storage and persistence in the soil. Comstock Associates, Ithaca, NY

Radha B, Channakeshava B, Bhanuprakash K, Pandurange Gowda K, Ramachandrappa B, Munirajappa R (2014) DNA damage during seed ageing. IOSR J Agric Vet Sci 7:34–39

Rajjou L, Lovigny Y, Groot SP, Belghazi M, Job C, Job D (2008) Proteome-wide characterization of seed aging in Arabidopsis: a comparison between artificial and natural aging protocols. Plant Physiol 148(1):620–641

Ramos JMV, Lopez S, Vázquez E, Murillo E (1988) DNA integrity and DNA polymerase activity in deteriorated maize embryo axes. J Plant Physiol 133(5):600–604

Saravanakumar D, Samiyappan R (2007) ACC deaminase from Pseudomonas fluorescens mediated saline resistance in groundnut (*Arachis hypogea*) plants. J Appl Microbiol 102(5):1283–1292

SAS Institute Inc (2013) SAS/STAT user's guide. SAS Institute Inc., Cary

Seal CE, Barwell LJ, Flowers TJ, Wade EM, Pritchard HW (2018) Seed germination niche of the halophyte *Suaeda maritima* to combined salinity and temperature is characterised by a halothermal time model. Environ Exp Bot 155:177–184

Shabala S, Hariadi Y, Jacobsen S-E (2013) Genotypic difference in salinity tolerance in quinoa is determined by differential control of xylem Na+ loading and stomatal density. J Plant Physiol 170(10):906–914

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22(2):123–131

Sidari M, Santonoceto C, Anastasi U, Preiti G, Muscolo A (2008) Variations in four genotypes of lentil under NaCl-salinity stress. American Journal of Agriculture and Biological Science 3:410–416

Siger A, Nogala-Kalucka M, Lampart-Szczapa E (2008) The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. J Food Lipids 15(2):137–149

Silva VN, da Silveira TJ (2014) Canola seed germination and seedling initial growth under conditions of salinity. Científica 42(3):265–270

Soltani E, Farzaneh S (2014) Hydrotime analysis for determination of seed vigour in cotton. Seed Sci Technol 42(2):260–273

Still DW, Bradford KJ (1998) Using hydrotime and ABA-time models to quantify seed quality of *Brassicas* during development. J Am Soc Hortic Sci 123(4):692–699

Sung J, Jeng T (1994) Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated aging of peanut seed. Physiol Plant 91(1):51–55

Szydłowska-Czerniak A, Bartkowiak-Broda I, Karlović I, Karlovits G, Szłyk E (2011) Antioxidant capacity, total phenolics, glucosinolates and colour parameters of rapeseed cultivars. Food Chem 127(2):556–563

Szydłowska-Czerniak A, Trokowski K, Karlovits G, Szłyk E (2010) Determination of antioxidant capacity, phenolic acids, and fatty acid composition of rapeseed varieties. J Agric Food Chem 58(13):7502–7509

Taherkhani T, Rahmani N, Pazoki A (2013) The effect of hydro-priming on germination of mustard seeds under draught stress conditions. Life Sci J 10(3s)

Tatipata A (2009) Effect of seed moisture content packaging and storage period on mitochondria inner membrane of soybean seed. J Agric Technol 5(1):51–64

Vahabinia F, Pirdashti H, Bakhshandeh E (2019) Environmental factors' effect on seed germination and seedling growth of chicory (*Cichorium intybus* L.) as an important medicinal plant. Acta Physiologiae Plantarum 41(2):27

Van't Hoff JH (1887) The role of osmotic pressure in the analogy between solutions and gases. Z fur phys Chem 1:481–508

Wang P, Mo B, Long Z, Fan S, Wang H, Wang L (2016) Factors affecting seed germination and emergence of *Sophora davidii*. Ind Crop Prod 87:261–265

Wilson Jr D, McDonald Jr M (1992) Mechanical damage in bean (*Phaseolus vulgaris* L.) seed in mechanized and non-mechanized threshing systems. In: Proceedings of the International Seed Testing Association

Wu G-Q, Jiao Q, Shui Q-Z (2015) Effect of salinity on seed germination, seedling growth, and inorganic and organic solutes accumulation in sunflower (*Helianthus annuus* L.). Plant Soil Environ 61(5):220–226

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53 (1):247–273

Methods of Seed Enhancement

23

Vartika Sinha and Abhinav Kumar

Abstract

Seed is one of the most basic inputs for agriculture. It evolves over time to respond to a variety of environments including human behaviour. The adaptation of seed leads to sustainable crop production and its satisfactory performance over a period of time. Seed enhancement technique further improves seed performance. Agriculture productivity is directly proportional to viability of seeds. Normally only 20–25% of total seeds are able to germinate. In seed enhancement methods, seeds are pretreated physically, physiologically and biologically to overcome germination constraints. Various other techniques have been employed, which are followed by conceptual development of processes for germination rates and seedling vigour. This chapter considers post-harvest treatments that improve germination or seedling growth or facilitate the delivery of seeds and other materials required at the time of sowing. Other considerations are seed hydration, biological seed treatment and seed coatings.

Keywords

Seed priming · Seed enhancement · Seed colouring

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23.1 Introduction

Seed is the most fundamental source of food and crop and is also a key link in the food chain. No agriculture practice can improve a crop beyond the limit which is programmed by seed itself. Seed enhancement is a term which is popularly used to describe beneficial techniques applied on seeds post-harvest. There are some popular adages 'Care with the seed and joy with the harvest' or 'Good seed doesn't cost, it always pays', which throw light on the importance of quality seed. An acceptable definition for seed enhancement is post-harvest treatments that improve germination or seedling growth or facilitate the delivery of seeds and other materials required at the time of sowing. This definition examines three general methods: pre-sowing hydration treatments (priming), coating technologies and seed conditioning (Taylor et al. 1998). The current population of the world is approximately 7 billion and is expected to increase by 2.7 billion to be 9.7 billion by 2050 (The Food and Agriculture Organization of the United Nations, FAO 2009). Out of them an estimate of 840 million people may suffer from chronic malnutrition. Approximately 70% increase in food production is needed by 2050 to feed the 9.1 billion mouths (FAO 2009). Some countries are at high risk for food demand—India is one of them. So, there is immediate need to increase food production, which may go twofold sustainably on the same land area (1.5 billion ha) by 2050. Scientists and breeders are searching for these possibilities. Plant breeding and genetic engineering are the two most commonly used practices of enhancing food quality, but they have certain limitations like genetically modified (GM) crops are not easily acceptable by the people of several countries including India. So, agricultural scientists have evolved a new idea of enhancing food productivity by improving seed quality. The importance and potential of quality seed has not been realized by mankind recently, but long ago this necessity has been felt. The need for a viable seed for prosperity of human race is mentioned in Rigveda of ancient India. It is mentioned in the Primeval Manusmriti as 'Subeejam Sukshetre Jayate Sampadyate' which literally means 'A good seed in a good field will win and prosper'. The objective of this chapter is to provide an overview of methods involved in seed enhancement. The methods may not be exclusive, and a combination of techniques may be employed to achieve the objective. Future opportunity and possibilities are also highlighted in this chapter.

Seed priming, magnetic stimulation, seed pelleting and coating (Taylor et al.1988; Afzal et al. 2012; Farooq et al. 2006) are among some of the shotgun approaches, which have been in practice since the last two decades. Except all these approaches, physical methods of seed enhancement are also in practice without knowing the actual mechanism of seed invigoration. Despite the above methods, plasma seed technology, which applies radiation to seeds and was started in the early 1980s, is a proven technique of seed enhancement. Seed enhancement through magnetic field treatments is also being considered for many agronomic and horticultural crops with some limitations (Araújo et al. 2016).

23.2 Physiological Seed Enhancements

23.2.1 Seed Priming

The theory of seed priming was proposed by Heydecker in 1973. Generally, only 20–25% of seeds are able to germinate, so for a country to grow, seeds need to grow at the fullest, and this can be done by seed priming methods. Seed priming is an effective technology for enhanced growth, enhanced vigour and better yield of crops (Harris et al. 2007). Seed priming consists of several physical, physiological and biological treatments to overcome germination constraints. Alternatively, seed priming is a process in which seeds to be soaked are hydrated by keeping them in water or any solution for a specific time period. Seeds undergo their metabolic activities before actually sowing and then redry to their original weight (Bradford 1986). So, it is a pre-sowing approach to influence seedling development by stimulation of metabolic activities prior to seed germination (Taylor et al. 1998). This treatment to seeds is called as priming treatment which includes osmopriming by a salt solution or polyethylene glycol (PEG) or hydropriming and hormonal priming (Bakhtavar et al. 2015). Another one is solid matrix priming in which seeds are soaked in an inert medium also known as matrix potential (Bhosale and More 2013; Bradford 1986; Afzal et al. 2015; Khan 1992). Hydropriming, halopriming, osmopriming, solid priming and hormonal priming are some examples of seed priming methods. Hydropriming and halopriming are defined as soaking of seeds in water and in salt solutions, respectively (Ghassemi-Golezani et al. 2008). NaCl, KCl, KNO_3 and $CaCl_2$ are the commonly used salt solutions (Bajehbaj 2010). Osmopriming is the most widely accepted seed priming method in which seeds are soaked in aerated low-water-potential solution (e.g. mannitol or inorganic salts or polyethylene glycol), which makes it easier and economical under stressed environment (Guzman and Olave 2006). Pre-soaking of seeds in hormonal solution of GA_3, salicylic acid, ascorbic acid, cytokinins, etc. is known as hormonal seed priming. In solid priming seeds are mixed in an organic or inorganic carriers and water for a particular period of time. The moisture level of the matrix is further maintained to meet the requirement of radicle protrusion. The water potential of the seed is regulated by priming. There is one more priming type, matrix priming, in which seed is mixed with moist solid particulate materials (e.g. exfoliated vermiculite, diatomaceous earth) (Taylor et al. 1988). These priming treatments work well with extreme conditions like drought or very high or low temperatures or extreme salinity (Afzal et al. 2009, 2015) (Table 23.1).

The details of priming are explained below:

23.2.1.1 Hydropriming

Hydropriming is a controlled hydration process in which seeds are held at water potential that allows imbibition but prevents radical extension (Bradford 1986) and then redried to their initial moisture (McDonald 2000). It has been reported that 16 h of hydropriming resulted in the highest seedling emergence and also hydroprimed

Table 23.1 List of priming agents

Priming agents	Example
Water	
Salts	NaCl, Na_2SO_4, KNO_3, KCl, etc.
Growth regulators	GA_3, CCC, kinetin
Vitamins	Vitamin K3, nicotinic acid, pantothenic acid
Plant products	Garlic extract, coconut water, leaf extract of *Pongamia pinnata*, Albizia amara, etc.

seeds emerged earlier than those of unprimed seeds (Ghassemi-Golezani and Dalil 2014). If the drying process is non-uniform, it causes uneven germination (Pill and Necker 2001). Among other seed priming techniques, hydropriming is a promising treatment for drought and salinity stress, and also it is cost-effective, which are the reasons this method is highly acceptable among farmers (Janmohammadi et al. 2008). Hydropriming produces healthy seedlings which lead to uniform crop development and increased yield in crop production.

23.2.1.2 Osmopriming

Osmopriming is similar to hydropriming in which osmotic solution is used for seed priming. Osmotic solution can be polyethylene glycol or a salt solution, and it should be applied under controlled aerated conditions to permit imbibition but prevent extension. Polyethylene glycol regulates water uptake and controls radical extension (Pill et al. 1991). The nitrogen-providing salts commonly used for osmopriming are potassium chloride (KCl), potassium nitrate (KNO_3), sodium chloride (NaCl), magnesium sulphate ($MgSO_4$), potassium phosphate (K_3PO_4), calcium chloride ($CaCl_2$) and potassium hydrophosphate (KH_2PO_4). These provide nitrogen to the germinating seed, which is an important ingredient for protein synthesis. As compared to hydropriming, osmopriming induces more rapid and uniform germination and also shortens the mean germination time.

23.2.1.3 Hormonal Priming

Seed soaking in hormonal solution is known as hormonal priming. A single hormone can do many works together, and also many hormones can perform similar role, like Abscisic acid (ABA) has a negative role in germination but its effect can be neutralized using GA (gibberellic acid) and auxin (Chauhan et al. 2009). GA_3, salicylic acid, ascorbic acid and cytokinins are the widely used hormones for this. These hormones play a pivotal role in different phases of plant development, for example, cytokinins are important for all phases of plant development; gibberellic acid (GA_3) is known for breaking seed dormancy, increasing germination capacity and many more; while GA stimulates hydrolytic enzymes which enhance germination and accelerate seedling growth (Riefler et al. 2006; Karssen et al. 1989; Rood et al. 1990). This priming treatment acts well under dormant and abiotic stress

conditions. So, phytohormones, which are meant to alter plant growth, can naturally impact positively on seed germination.

23.2.1.4 Nutrient Priming

Nutrient priming can be referred as the most basic and cost-effective priming procedure in which micronutrients like Zn, B, Mo, Mn, Cu and Co are given for seed priming treatment for most of the field crops (Wilhelm et al. 1988; Peeran and Natanasabapathy 1980; Sherrell 1984). Seeds are simply soaked in nutrient solution overnight before planting (Harris et al. 2001). A different micronutrient has a different role in plant development like zinc salts increase growth and provide disease resistance to seedlings. Phosphorous is an important constituent of amino acids. Iron (Fe) is required for the formation of chlorophyll in plant cells. It serves as an activator for biochemical processes such as respiration, photosynthesis and symbiotic nitrogen fixation.

Seed priming has some molecular aspects also, and elucidation of mechanisms of these effects is essential for seed scientists and seed industry. So, factors associated with reduced vigour of seeds should be minimized.

In addition to above methods, plasma seed technology, which applies radiation to seeds and was started in the early 1980s, is a proven technique of seed enhancement. Seed enhancement through magnetic field treatments is also being considered for many agronomic and horticultural crops with some limitations.

23.2.1.5 Magnetic Fields for Seed Treatments

Seeds respond when exposed to magnetic field. So, it is essential to optimize the dose of field exposure, which affects seed germination which in turn leads to increased yield of crop production (Silva and Dobranszki 2016). Seeds, when pass through a magnetic funnel, are affected by the magnetic field on the passage and then are soaked. It was found that seeds germinate 1 day earlier after magnetic treatment and germination percentage increased by 33–45% in treated seeds related to the control (Ahmad et al. 2007). Magnetic exposure is defined as the product of flux density of magnetic field with timing of the exposure. The flux density is the number of magnetic lines of flux that pass through a certain point on a surface. So, it varies with static or alternating magnetic field exposure to seeds. It is experimentally proven that magnetic field not only enhances germination capacity of seeds but also increases crop yield and protects crop from pathogens (De Souza et al. 2006; Pietruszewski and Kania 2010).

23.2.1.6 Plasma Seed Treatments

Plasma application to seeds in agriculture and medicine is an example of recent advancement in seed enhancement techniques, which ensures every seed has treated in the best possible physical condition prior to germination (Sosnin et al. 2004; Akitsu et al. 2005). Recently this technique has come out as an alternative to traditional pre-seed sowing treatments. High-voltage plasma discharge, which is a resultant of bombardment from ions, oxygen radicals, nitrogen radicals and an assortment of charged particles, is applied to the seeds which cause disruption and

further oxidation of seed coats. Oxidized seed coats exhibit high permeability to water and nutrient uptake. This technique has come up with good results, which are increase in viability of seeds from 5% to 30% and decrease in seed germination time which is found to be ranging in between 20% and 50%. Plasma treatments were further upgraded into microwave plasma, magnetized plasma and atmospheric plasma treatments (Sera et al. 2010; Zhou et al. 2011). It has been reported that if plasmas are used with few gases such as aniline, cyclohexane and helium, germination and growth enhancement will be achieved early (Volin et al. 2000; Jiayun et al. 2014). Further, plasma contains reactive oxygen species which increases the quality of plant development by controlling thiol groups (Henselová et al. 2012). Plasma chemistry modifies seed germination by delaying or boosting with the application of plasma-treated deposits on seed surfaces (Volin et al. 2000). It has also been published that plasma helps to attain zero seed destruction, zero chemical use and environment-friendly treatments to seeds (Volin et al. 2000; Dhayal et al. 2006; Selcuk et al. 2008). It also improves seed quality and plant growth (Sera et al. 2010). Moreover, seeds exposed to plasma treatments result in alternations of enzymatic activity and sterilization of seed surface (Selcuk et al. 2008).

23.2.1.7 Radiation Seed Treatments

Ionizing radiation poses an impact on biological systems, and it has been proved that these radiations activate a number of physical and chemical steps inside the cell from absorption of radiation to injury. Among other ionizing radiations, gamma radiations are more effective and easily available and possess powerful penetration (Moussa 2006). Gamma radiations produce free radicals inside the cell of the organism by causing chemical interaction with biomolecules and water and damage cell components, which in turn effect some physiological and biochemical processes vital for cellular survival (Rogozhin et al. 2000). However, high dose of gamma radiation can alter protein synthesis, enzyme activity, hormone balance and leaf gas exchange (Al-Salhi et al. 2004; Hameed et al. 2008). Optimal radiation increases the root length and fresh weight of seedlings, and cellular mitotic divisions also remain normal (Mergen and Johansen 1963).

23.3 Biological Seed Enhancements

23.3.1 Bacterial Seed Agents

This treatment is also known as seed bacterization. It has been proven as a successful method for biological nitrogen fixation, solubilization of phosphorous and zinc and production of siderophores (molecules which can sequester Fe) in legume plants (Stacey et al. 1992). They have the capability to synthesize phytohormones to stimulate plant growth (Suslow and Schroth 1978; Graystone et al. 1991). Apart from this, it was found that this method also improves plant growth and provides biological control to plant diseases (Tahvonen 1982; Pierson and Weller 1994)). It is well known that rhizobacteria are soil-borne, free living bacteria, which induce plant

growth. These bacteria are alternatively called as plant-growth-promoting rhizobacteria (PGPR). Under stress conditions PGPR also synthesize ACC-deaminase enzyme by modulation of ethylene level (Glick et al. 1998; Nadeem et al. 2015). This biopriming technique is considered as a good example of seed enhancement, integrating biological and physiological aspects and plant disease control. It has been found that seed priming with beneficial microorganisms results in more rapid growth and increased crop yield under stress conditions. They can also be used as an alternative to chemical fertilizers (Bloemberg and Lugtenberg 2001; Vessey 2003). Rhizobacteria colonize roots and are symbiotic to their hosts. Reports of PGPR that have been successfully tested as co-inoculants with rhizobia include strains of the rhizobacteria such as *Azotobacter* [82], *Azospirillum* [83], *Bacillus* [84], *Pseudomonas* [85, 86], *Serratia* [86] and *Streptomyces* [87].

23.3.2 Fungal Seed Agents for Biopriming

Microorganisms including bacteria (discussed above) and fungi are used as biopriming agents to enhance seedling growth and vigour. Biopriming potential of *Trichoderma* and *Bacillus* spp. was compared with commercial products Agrotrich plus® and Rhizoliptus® for enhancing growth and yield of beans, and findings revealed better seedling growth with bioprimed seeds as compared to other techniques (Junges et al. 2016).

23.4 Seed Coating and Pelleting

Seed film coating comes under globally practiced seed treatments like pelleting, priming and inoculation (Thomas et al. 2003), which altogether aimed to improve seed germination, seed storage and enhanced plantability. Seeds vary greatly in size, shape and colour, whether small or irregular. Further, seeds are prone to attack from a range of pests, which include animals and pathogens. Seed coating refers to the application of chemicals to protect seeds from pathogens/pests and support seedling growth (Scott 1989). Initially coating is used to be done by just applying chemical slurry on the seeds and then dried, but disadvantages of this method are it was difficult to get uniform seed coating and, during transportation of seeds, most of the coatings were rubbed off. So, seed technologists have found out a new way termed as film coating to overcome these problems. In this technique, seeds are sprayed (chemicals applied in a polymer) along the path with a specialized machine, and later polymer is rapidly dried which results in dry polymer coating. These polymers can be growth regulators, inoculants, micronutrients, fungicides, insecticides and other seed protectants (Rehman et al. 2013).

Another operation is pelleting which is generally performed on drum or coating pan (Scott 1989). The speed varies with the diameter of apparatus used, and generally its range is from 10 to 35 rpm. This method, however, is a labour-intensive operation and required long working hours and skilled hands. The pelleting

process has become automated, and computer-controlled coating system is described in Scott (1989). In each case, seeds are coated with a combination of binder (adhesive) and filler (bulking agent). A number of materials can be used as binders and fillers (Scott 1989). The procedure involves broadly three stages: stamping, coating and rolling. To start with, seeds are uniformly coated with adhesive materials (gum arabic, gelatin, methylcellulose, polyvinyl alcohol, etc.) in correct quantity and concentration. Then, the filler materials (lime, gypsum, dolomite, rock phosphate, etc.) are sprinkled on the coated seeds and are rolled on the seeds for uniform coating. Finally, pelleted seeds are sown into the fields. A number of reports are available, which quote the success stories of this method. In sunflower, seed pelleting with a mixture of moringa leaf powder (250 g), DAP (100 g), *Azospirillum* (25 g) and *Trichoderma* (4–5 g) increases yield of 15% in irrigated and non-irrigated conditions (Anonymous 2001). Another report by Geetha and Bhaskaran (2013) indicated that seed hardening and pelleting treatment increases the yield of ragi (*Eleusine coracana*) varieties.

In conclusion, we can say that film coating provides an optimal method for the application of chemical and/or biological seed treatments (Taylor and Harman 1990; Taylor et al. 1994).

23.4.1 Seed Colouring

It is a method in which seeds are coloured using different dyes (naturally and artificial) so that it (seed) can acquire a distinct and attractive look. For natural dyes different parts of a plant (e.g. opuntia, jamun, basella, etc.) can be used. For example, the dried leaf powder of henna, when mixed with water, can be used as a colouring agent. Other examples are beetroot, the root portion of which is used as a colouring agent, and turmeric, which is also used in the same manner. In some cases, a flower part can be crushed and used (e.g. marigold and hibiscus).

Chemical dyes are also in use for the purpose of seed colouring. Different colours, which are generally used as colouring agents, are Congo red, bromocresol green, jade green, sky blue, direct chambagu, turquoise blue, etc.

23.4.2 Seed Hardening

It is the process of hydration and dehydration of seed to fix biochemical events. This method is done primarily to enhance the thermotolerance of the seed without loss of viability. Other benefits include rapid germination and growth rate of seedling and to impart resistance against various stress conditions.

23.4.3 Seed Conditioning

When seeds are brought in from the field, they are seldom pure and contain a number of unwanted materials such as pieces of stems, dust, weeds and poor-quality seed, which should be removed from that seed lot. Before the seed is sold for planting or other agricultural activities, the minimum quality standard should be met. Therefore, the series of measures, which involve mechanical operations, chemical treatment, packaging, distribution and marketing, are termed as seed conditioning. This process of upgrading can be considered as a seed enhancement as the germination and seedling growth characteristics of the seeds are further improved. The following are the steps in conditioning:

Drying: This is an important step in which various drying units are used. At the time of harvest, moisture content is high, so it should be minimized before storage.

Precleaning: A large amount of trash/green/dirt may be present in the seeds. Precleaning is often neglected, but this step is an important step. If this method is applied before drying, it enhances the capacity of the dryer.

Cleaning: Basic cleaning is accomplished by air-screen cleaner, which makes use of a series of screens and air separations to remove light, trash, dust and other unwanted materials. Sometimes this is a necessary step for direct marketing of seed.

Treatment: This step includes application of fungicide, insecticides, growth regulators, etc.

Packaging: This step consists of traditional bagging with the use of bulk bags. After conditioning storage and packaging are the final steps.

23.5 Molecular Aspects

Physiologically seed priming with nutrients increases the seed contents of primed nutrients and enhances nutrient uptake and also triggers the enzymes associated under deficient conditions to support the seedling development (Imran et al. 2013). Priming increased the production and activity of α-amylase within germinating seeds, which is directly proportional to the seed vigour (Afzal et al. 2012). At molecular level, priming beams favourable effects on RNA and protein synthesis and also initiates cell division and transportation of storage proteins (de Castro et al. 2000). *Arabidopsis* model plant was used for elucidation of several proteins and their precursors involved in seed germination or priming. It was found that actin isoform helps in imbibition and cytosolic glyceraldehyde-3-phosphate dehydrogenase plays a role in the seed dehydration process (Gallardo et al. 2001). In imbibition, a higher germination rate of seeds is the result of increased production of metabolites (Coolbear et al. 1980; Burgass and Powell 1984). At molecular level, peroxiredoxin-5, 1-Cys peroxiredoxin, embryonic protein DC-8, cupin and globulin-1 were found to be involved in improved seed germination, and their level in plants remained unaltered even after seed priming (Gong et al. 2013).

23.6 Conclusion

Germination of seed is an important event, and it depends upon two important factors: the state of the seed (dormant or not) and the environmental conditions. If a seed is dormant, it is necessary to treat it with chemicals or other things to break dormancy so as to initiate germination. There is a need of optimum condition of moisture, oxygen, temperature and light for a seed to germinate. Moisture and salinity are among the limiting factors which hinder seed germination and crop establishment. Seed enhancement technology provides a unique tool and idea to remove poor-quality seeds and also removal of unwanted materials from total seed lot. Priming is an important seed treatment practice, which can help the seeds to break dormancy and to minimize/overcome problems of abiotic stress such as moisture, salinity and extreme temperature. It is actually a pre-sowing seed treatment practice which provides sufficient moisture to start the pregermination metabolic process. This process rectifies the problems encountered during germination and emergence. Hence, it is useful for uniform emergence and growth. Priming can be done with water, different solutions and phytohormones. Cell membrane integrity can be indirectly measured through solute leakage measurement. However, seed enhancement technologies are not a new technique in agriculture as reported earlier by Evenari (1980). Seed coating is a much older technique, being practiced 2000 years back. The Chinese were well known to these techniques, so this 'modern seed enhancement' has certainly historical basis. This domain needs more research and detailed study. There are a number of challenges and opportunities exist, which depend upon several components of these technologies. This seed enhancement technique is one of the best solutions of germination-related problem when there is stress condition. It can increase the rate of germination and seedling even under different set of environmental conditions. Minimum exposure to toxicant, less time under stress condition, uniformity in the field and high turnover are some of the advantages of seed enhancement technology. This technique can prove the best technique if used wisely.

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References

Afzal I, Ashraf S, Qasim M, Basra SMA, Shahid M (2009) Does halopriming improve germination and seedling vigor in marigold (Tagetus sp.). Seed Sci Technol 37:436–445

Afzal I, Hussain B, Basra SMA, Rehman H (2012) Priming with MLE reduces imbibitional chilling injury in spring maize. Seed Sci Technol 40:271–276

Afzal I, Noor MA, Bakhtavar MA, Ahmad A, Haq Z (2015) Improvement of spring maize (Zea mays) performance through physical and physiological seed enhancements. Seed Sci Technol 43(2):1–12

Ahmad M, Galland P, Ritz T, Wiltschko R, Wiltschko W (2007) Magnetic intensity affects cryptochrome-dependent responses in *Arabidopsis thaliana*. Planta 225:615–624
Akitsu T, Ohkawa H, Tsuji M, Kimura H, Kogoma M (2005) Plasma sterilization using glow discharge at atmospheric pressure. Surface Coatings Technol 193:29–34
Al-Salhi M, Ghannam MM, Al-Ayed MS, El-Kameesy SU, Roshdy S (2004) Effect of gamma irradiation on the biophysical and morphological properties of corn. Nahrung 48:95–98
Anonymous (2001) Processing of the oil seed meet. Tamil Nadu Agriculture University, Coimbatore
Araújo SS, Paparella S, Dondi D, Bentivoglio A, Carbonera D, Balestrazzi A (2016) Physical methods for seed invigoration: advantages and challenges in seed technology. Front Plant Sci 7:646
Bajehbaj AF (2010) The effects of NaCl priming on salt tolerance in sunflower germination and seedling grown under salinity conditions. African J Biotechnol (12):1764–1770
Bakhtavar MA, Afzal I, Basra SMA, Ahmad A, Noor M (2015) Physiological strategies to improve the performance of spring maize (Zea mays L.) planted under early and optimum sowing conditions. PLoS One 10(4):e0124441
Bhosale RS, More AD (2013) Effect of EMS (ethyl methane sulfonate) on seed germination, seedling height and seedling injury in Withania somnifera, (L.) Dunal. Int J Life Sci 1 (2):158–160
Bloemberg GV, Lugtenberg BJ (2001) Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Opin Plant Biol 4(4):343–350
Bradford KJ (1986) Manipulation of seed water relations via osmotic priming to improve germination under different field conditions. Res J Agric Biol Sci 22:33–37
Burgass RW, Powell AA (1984) Evidence for repair processes in the invigoration of seeds by hydration. Ann Bot 53:753–757
Chauhan JS, Tomar YK, Singh IN, Ali S, Debarati A (2009) Effect of growth hormones on seed germination and seedling growth of black gram and horse gram. J Am Sci 5:79–84
Coolbear P et al (1980) "Osmotic pre-sowing treatments and nucleic acid accumulation in tomato seeds", (LycopersiconCycopersicum). Seed Sci Technol 8:289–303
de Castro RD, van Lammeren AAM, Groot SPC, Bino RJ, Hilhorst HWM (2000) Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. Plant Physiol 122:327–335
De Souza A, Garcia D, Sueiro L, Gilart F, Porras E, Licea L (2006) Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. Bioelectromagnetics 27:247–257
Dhayal M, Lee SY, Park SU (2006) Using low-pressure plasma for Carthamus tinctorium L. seed surface modification. Vacuum 80:499–506
Evenari M (1980) The history of germination research and the lesson it contains for today. Israel J Bot 29:4–21
Farooq M, Basra SMA, Tabassum R, Afzal I (2006) Enhancing the performance of direct seeded fine rice by seed priming. Plant Prod Sci 9(4):446–456
Gallardo K, Job C, Groot SPC, Puype M, Demol H, Vandekerckhove J, Job D (2001) Proteomic analysis of Arabidopsis seed germination and priming. Plant Physiol 126:835–848
Geetha VV, Bhaskaran M (2013) Ragi seed quality enhancement techniques under rainfed conditions of tribal habitations of Hosur forest division. J Acad Ind Res 2(1):2278–5213
Ghassemi-Golezani K, Aliloo AA, Valizadeh M, Moghaddam M (2008) Effects of hydro and Osmo-priming on seed germination and field emergence of lentil (Lens culinaris Medik). Not Bot Hort Agrobot Cluj 36:29–33
Ghassemi-Golezani K, Dalil B (2014) Effects of seed vigor on growth and grain yield of maize. Plant Breed Seed Sci 70:81–90
Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J Theor Biol 190:63–68

Gong F, Wu X, Wang W (2013) Comparative proteomic identification of embryo proteins associated with hydropriming induced rapid-germination of maize seeds. Plant Omics 6 (5):333–339

Graystone SJ, Stephens JH and Nelson LM (1991) Field and greenhouse studies on growth promotion of spring wheat inoculated with co-existent rhizobacteria. In: Keel C, Koller B De'fago G (eds) Plant growth promoting rhizobacteria-progress and prospects. IOBC/WPRS Bulletin XIV, pp 11–16

Guzman M, Olave J (2006) Response of growth and biomass production of primed melon seed (Cucumis melo L. cv. Primal) germination to salinity level and N-forms in nursery. J Food Agri Enviro Ethics 4:163–165

Hameed A, Mahmud TS, Atta BM, Haq MA, Sayed H (2008) Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. Pak J Bot 40:1033–1041

Harris D, Raghuwanshi BS, Gangwar JS, Singh SC, Joshi KD, Rashid A, Hollington PA (2001) Participatory evaluation by farmers of 'on-farm' seed priming in wheat in India, Nepal and Pakistan. Exp Agric 37(3):403–415

Harris D, Rashid A, Hollington A, Jasi L, Riches C (2007) Prospects of improving maize yield with on farm seed priming. In: Rajbhandari NP, Ransom JK (eds) Sustainable maize production systems for Nepal. NARC and CIMMYT, Kathmandu, pp 180–185

Henselová M, Slováková Ľ, Martinka M, Zahoranová A (2012) Growth, anatomy and enzyme activity changes in maize roots induced by treatment of seeds with low temperature plasma. Biologia 67:490–497

Heydecker W, Higgins J, Gulliver RL (1973) Accelerated germination by osmotic seed treatment. Nature 246:42–46

Imran M, Mahmood A, Römheld V, Neumann G (2013) Nutrient seed priming improves seedling development of maize exposed to low root zone temperatures during early growth. Eur J Agron 49:141–148

Janmohammadi M, Moradi DP, Sharifzadeh F (2008) Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. Gen Appl Plant Physiol 34(3–4):215–226

Jiayun T, Rui H, Xiaoli Z, Ruoting Z, Weiwen C, Size Y (2014) Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of Andrographis paniculata. Plasma Sci Technol 16:260

Junges E, Muniz MFB, Bastos BDO, Oruoski P (2016) Biopriming in bean seeds. Acta Agric Scand Sect B Soil Plant Sci 66:207–214

Karssen CM, Zagorski S, Kepczynski J, Groot SPC (1989) Key role for endogenous gibberellins in the control of seed germination. Ann Bot 63:71–80

Khan AA (1992) Preplant physiological seed conditioning. Horticult Rev 13:131–181

McDonald M (2000) Seed priming. In: Black M, Bewley JD (eds) Seed technology and its biological basis. Sheffield Academic Press, Sheffield, pp 287–325

Mergen F, Johansen TS (1963) Effect of ionizing radiation on microsporogenesis in *Pinus rigida* mill. Rad Bot 3:321–331

Moussa JP (2006) Role of gamma irradiation in regulation of NO3 level in rocket (Eruca vesicaria subsp. sativa) plants. Russ J Plant Physiol 53:193–197

Nadeem SM, Naveed M, Ahmad M, Zahir ZA (2015) Rhizosphere bacteria for biomass production and improvement of stress tolerance: mechanisms of action, applications and future prospects. In: Arora NK (ed) Plant microbe symbiosis—applied facets. Springer, The Netherlands, pp 1–36

Peeran SN, Natanasabapathy S (1980) Potassium chloride pretreatment on rice seeds. Int Rice Res Newslett 5:19

Pierson EA, Weller DM (1994) Use of mixtures of fluorescent pseudomonads to suppress take-all and improve the growth of wheat. Phytopathology 84:940–947

Pietruszewski S, Kania K (2010) Effect of magnetic field on germination and yield of wheat. Int Agrophys 24:275–302

Pill WG, Necker AD (2001) The effect of seed treatment on germination and establishment of Kentucky blue grass (Poa pretenses L.). Seed Sci Technol 29:65–72
Pill WG, Frett JJ, Morneau DC (1991) Germination and seedling emergence of primed tomato and asparagus seeds under adverse conditions. Hort Sci 26:1160–1162
Rehman A, Farooq M, Ata Z, Wahid A (2013) Role of boron in leaf elongation and tillering dynamics in fine-grain aromatic rice. J Plant Nutr 36(1):42–54
Riefler M, Novak O, Strnad M, Schmülling T (2006) Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. Plant Cell 18:40–54
Rogozhin VV, Kuriliuk TT, Filippova NP (2000) Change in the reaction of the antioxidant system of wheat sprouts after UV-irradiation of seeds. Biofizika 45:730–736
Rood SB, Williams PH, Pearce D, Murofushi N, Mander LN, Pharis R (1990) A mutant gene that increases gibberellin production in Brassica. Plant Physiol 93:1168–1174
Scott JM (1989) Seed coatings and treatments and their effects on plant establishment. Adv Agron 42:43–83
Selcuk M, Oksuz L, Basaran P (2008) Decontamination of grains and legumes infected with Aspergillus spp. and Penicillium spp. by cold plasma treatment. Bioresour Technol 99:5104–5109
Sera B, Spatenka P, Sery M, Vrchotova N, Hruskova I (2010) Influence of plasma treatment on wheat and oat germination and early growth. IEEE Trans Plasma Sci 38:2963–2968
Sherrell CG (1984) Effect of molybdenum concentration in the seed on the response of pasture legumes to molybdenum. New Zealand J Agric Res 27:417–423
Silva JAT, Dobranszki J (2016) Magnetic fields: how is plant growth and development impacted. Protoplasma 253:231–248
Sosnin EA, Stoffels E, Erofeev MV, Kieft IE, Kunts SE (2004) The effects of UV irradiation and gas plasma treatment on living mammalian cells and bacteria: a comparative approach. IEEE Trans Plasma Sci 32:1544–1550
Stacey G, Burris RH, Evans HJ (1992) Biological nitrogen fixation. Chapman & Hall, New York
Suslow TV, Schroth MN (1978) Bacterial culture preservation in methylcellulose (abstr.). Phytopathol News 12:136
Tahvonen R (1982) Preliminary experiments into the use of *Streptomyces* spp. isolated from peat in the biological control of soil and seed-borne diseases in peat cultures. J Sci Agric Soc Finl 54:29–36
Taylor AG, Harman GE (1990) Concepts and technologies of selected seed treatments. Annu Rev Phytopathol 28:321–339
Taylor AG, Klein DE, Whitlow TH (1988) SMP: solid matrix priming of seeds. Scientia Hort 37:1–11
Taylor AG, Harman GE, Nielsen PA (1994) Biological seed treatments using *Trichoderma harzianum* for horticultural crops. HortTech 4:105–109
Taylor AG, Allen PS, Bennett MA, Bradford KJ, Burris JS, Misra MK (1998) Seed enhancements. Seed Sci Res 8:245–256
Thomas B, Murphy DJ, Murray BG (2003) Encyclopedia of applied plant sciences (3 volume set). Elsevier Science BV
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2):571–586
Volin JC, Denes FS, Young RA, Park SM (2000) Modification of seed germination performance through cold plasma chemistry technology. Crop Sci 40:1706–1718
Wilhelm NS, Graham RD, Rovira AD (1988) Application of different sources of manganese sulphate decreases take-all (Gaeumannomyces graminis var. tritici) of wheat grown in a manganese deficient soil. Austr J Agric Res 39:1–10
Zhou Z, Huang Y, Yang S, Chen W (2011) Introduction of a new atmospheric pressure plasma device and application on tomato seeds. Agric Sci 2:23

Seed Biotechnology for Improvement of Staple Crops

24

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Abstract

Cereals are the major sources of energy for both human and other animals as they possess carbohydrate, protein, fibres, nutrients and many other important components. There are several factors which affect the quality of cereals along with crop productivity and yield directly or indirectly. Maintaining the seed quality reflects in the production of improved yield, longer storage period as well as high nutritional valued cereals. Biotechnological approaches take the centre stage in significant contribution towards maintaining the seed quality. The preference of these approaches over the traditional ones is due to the less time consumption, cost-effectiveness and robust outputs. Major biotechnological approaches, such as biofortification and transgenics, for insect resistance are of prime importance to many cereal crops. Important cereals, such as rice, maize, wheat, etc., have been biofortified to produce high nutrients for animal consumption. It has been able to combat nutrient starvation by increasing micronutrients such as iron, zinc, vitamins and other biotic compounds. Insect infestation is a major issue for both developing and underdeveloped countries as it causes up to 40% damage to the crops. Transgenic approaches have been successfully employed to get resistance against various insects and storage pests in important cereals. Presently, the scientific community is more focused towards the enrichment of cereals, which ultimately can solve the basic issues such as malnutrition, increasing demand for food supply and starvation. Seed biotechnology has a great potential to offer the high nutrient value cereals for upcoming generations. The present chapter aims at providing a brief scenario of the basic issues related to the

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seed quality and important biotechnological approaches to overcome those constrains.

Keywords

Cereals · Seed biotechnology · Biofortification · Insect resistance

24.1 Introduction

Staple crops are primarily grown for harvesting of mature grains or seeds and consumed as the main sources of carbohydrate, protein, fibre and other nutrients. Most of the cereals fall under the category of staple crops as they are consumed in different forms by a large population across the globe. The major cereals cultivated worldwide are wheat, rice, maize, barley, sorghum, pearl millet, etc. In underdeveloped countries, the important part of diet contains cereals along with plant or animal supplements, whereas in the developed countries, it is also the major source of dietary components or processed food. Although the most dominating cereals around the world are wheat, rice and maize, more than ten other minor cereals are also grown worldwide. Cereals and their products are important sources of carbohydrate, protein, fibre as well as micronutrients such as zinc, magnesium and vitamins. Other than these nutrients, cereals also contain a variety of bioactive compounds. Thus, cereals are either directly or indirectly related with the potential health status of a large population worldwide.

As cereals hold an important position among horticultural and agronomic crops, it is very essential to produce high-quality seeds in terms of purity, high nutrition value, disease-free material and good physiological characteristics. Although there are many issues which affect the production of quality seeds, most of the cereals are nutritionally rich and play a major role in the health aspects of a large population globally. The problems related with seed quality of cereals can be summarized as the following:

1. Non-availability of important nutrients in cereals for human consumption:
   As previously described, cereals provide important nutrients like carbohydrate, protein, fibres and micronutrients such as iron, zinc, vitamin precursors, etc. But the availability of these nutrients for human consumption is constrained by several factors. The cooking and food processing practices play an important role in the consumption of these nutrients available in cereals. For example, the use of polished rice became popular in the 1970s as a staple food widespread. The polishing of rice involves removing of husk, barn and germ from rice seeds. Although polishing provides bright, white and shiny-appearing seeds, there are several reports available on nutrient loss caused by polishing and milling of rice (Thomas 2019). The polished rice has less moisture, biotin, niacin, protein and fatty acid content than the unpolished or brown rice (Thomas 2019). Similarly, in

other cereals also the loss of nutrients and micronutrients was reported due to various food processing practices.

2. Storage pest of cereal seeds:
   Another important problem related with the seed quality is the loss caused by various pests and insects during storage of seeds. There are various reports available on the crop loss due to storage pests (Dhaliwal et al. 2013). The annual loss of grain crop is estimated about one quarter or one third during storage around the world, and most of these pests are insects. The most preferred part of cereal seeds damaged by insect pest is grain embryos, which reduces the protein content of seeds and affects the germination rate (Dept. of Primary Industries and Regional Development's Agriculture and Food Division, Govt. of Western Australia 2019). Although the storage pest problems occur in several grain-producing crops, the cereals get affected the most. The commonly known storage pests include the lesser grain borer, rice weevil and rust red flour etc.
3. Lower yield and production of cereals:
   The crop production is greatly affected by several elements of biotic and abiotic factors which was noticed long ago by the scientific community. Various biotic factors, such as insect pests, bacteria, virus, fungi, nematode, etc., and abiotic factors, such as temperature, drought, wind, flood, salinity, etc., greatly impact the yield of cereals in different climatic zones.

   Maintaining the quality of cereals for human consumption or animal fodder as well as livestock seeds for the next cropping season is very essential. Therefore, the worldwide scientific community has been putting efforts to resolve these issues since ages. Various conventional methods such as adopting alternative cropping system, breeding approaches, hybrid selection, etc. are being employed to increase the nutrition, yield and resistance development against pathogens in cereals. Although these diverse attempts are effective and being used for a very long time, these conventional methods are time-consuming, and the desired results are very difficult to obtain. Since the last few decades, an extensive research has been done to deal with the challenges related with seed quality of cereals.

   The seed enhancement uses various modern technologies, such as biofortification, transgenic approaches, genome editing approaches based on molecular marker, etc., to enhance the nutritional quality of cereals. The biofortification is one of the emerging areas of research which has been able to produce several modified cereal crops with enhanced vitamins, iron, zinc and other micronutrients. Similarly, the development of transgenic crops as well as molecular marker-based genome editing approaches also has a major contribution to biotechnological research. So far, the resistance against insect pest and storage pest has been achieved in crops, such as cotton, maize, brinjal, canola and soybean, using transgenic approaches (Johnson and O'Connor 2015). The maintenance of cereals seed quality is very essential to feed the vast growing world population as well as to deal with the nutritional value of existing cereal crops, which are being cultivated globally. In this chapter, we summarize the problems related with seeds quality and storage of cereal crops, the importance of

biotechnological approaches in seed biotechnology and a brief review of major biotechnological approaches being used for the improvement of seed quality.

24.2 Importance of Seed Biotechnology

The global population is growing very rapidly which is at present about more than 7 billion, and according to the current projected growth rate, it is estimated that it would reach 10 billion in 2050 (Population Division, USA 2019). Therefore, the staple crops need a serious upsurge in production and yield as well as seed quality maintenance in terms of nutritional value and pest resistance.

The prevalence of malnutrition, vitamin deficiency and lack of other important micronutrient in staple food were reported in underdeveloped countries (Muller and Krawinkel 2005). According to the report of the WHO (2001) in South and South-east Asia, iron deficiency (ID) and iron deficiency anaemia (IDA) are highly prevalent among young women and children. It was also reported that about 60–80% (6 billion people) were iron deficient and about 30% were found to be zinc deficient (Kennedy et al. 2003). On one hand, the 20% death of children under the age of five can be attributed by vitamin A, Zn, Fe and/or I deficiency (Prentice et al. 2008). On the other hand, cereals provide about more than 60% of the energy and protein requirement of human consumption. In developing countries, it is about 75% of the daily calorie intake, which comes from cereals only, and most of these consumed cereals are very low in micronutrients (Cakmak and Hoffland 2012). The highly populated countries of Asia, Africa and Latin America face a serious health problem of vitamin A deficiency. The basic cause of this problem is consumption of polished rice which is prepared after removing oil-rich aleurone layer. The edible part of polished rice lacks several essential nutrients including provitamin A (Ye et al. 2000). The insect pests have found to cause a huge loss up to 40% of the crops in developing countries despite the use of various insect control methods (Oerke 2006). Various economically important cereals like corn, sorghum, wheat and pulses get affected by these insect pests, leading to the yield and quality loss. Moths and butterflies are considered the most damaging insect species, which comes under Lepidoptera order (Srinivasan et al. 2006).

Thus, considering the fact of quality and quantity loss in cereal crops, it is very essential to develop more reliable and robust approaches to deal with these issues. The use of biotechnological methods, such as biofortified crops, transgenic crops expressing insect resistance genes, high-yield varieties, etc., is the most effective solution to overcome these challenges.

24.3 Application of Biotechnological Approaches

24.3.1 Biofortification

Biofortification is the process of increasing the nutritional quality of food crops through conventional breeding, agronomic practices and/or genetic engineering approaches without compromising on agriculturally important traits (WHO 2019). It is a food-based approach to combat nutrient starvation by providing highly nutritious food even to the lower income groups (Vinoth and Ravindhran 2017). Micronutrient deficiency such as iron deficiency anaemia (IDA) poses a serious threat to the society affecting around 2 billion people worldwide (WHO 2016). Women and children are the most vulnerable to iron malnutrition. Micronutrient deficiency is also known as hidden hunger since their effect can only be detected after irreparable damage has been caused. In order to control micronutrient deficiency, the WHO and UNICEF have recommended several approaches including dietary diversification, supplementation and fortification (Boonyaves et al. 2017). However, these strategies are not always beneficial. For instance, iron supplementation may increase the severity of other infectious diseases during malaria outbreak, while iron fortification may make the food distasteful (Boonyaves et al. 2017). In addition, these approaches are expensive and not practicable in the Third World countries. Biofortification is advantageous than conventional approaches, such as supplementation and fortification in controlling micronutrient deficiency, since these strategies are expensive and difficult to implement especially in the developing countries, where majority of the population is poor (Bashir et al. 2013). Biofortification differs from conventional fortification in that it focuses to increase the nutritional value in crops during growth and development rather than adding nutrients when they are processed into food (Manwaring et al. 2016). Biofortification through conventional breeding is able to produce biofortified food crops aiming improved human nutrition. However, in cases where the target nutrient is unavailable in the required amounts in the natural germ plasm, transgenic approach is the only choice to produce the desired biofortified crop with elevated nutritional quality as well as farmer preferred traits (Bouis and Saltzman 2017). Biofortification Challenge Program under the HarvestPlus initiative of the Consultative Group for International Agricultural Research (CGIAR) targeted seven staple crops, viz. rice, wheat, maize, pearl millet, beans, cassava and sweet potato for increasing the levels of iron, zinc and vitamin D (Welch and Graham 2004; Vinoth and Ravindhran 2017).

24.3.1.1 Iron Biofortification

Genetic engineering approaches for biofortification mainly focus on producing iron-rich crops. Iron biofortification heavily relies on transgenic approaches since genetic variability of endosperm iron content especially in rice germ plasm is extremely narrow (Boonyaves et al. 2017). These approaches depend on the genetic arrangement that controls the uptake and remobilization of minerals in the roots, its translocation to the shoots and its storage in the edible parts (Shahzad et al. 2014).

Transgenic approaches for iron biofortification have fundamentally concentrated on increasing the production of chelating compounds; increasing the concentrations of metal-binding proteins such as ferritin and lactoferrin; increasing promoter compounds such as ascorbic acid, β-carotene and cysteine-containing peptides; and reducing antinutrients, such as phytate and tannins in the edible parts (White and Broadley 2005; Boonyaves et al. 2017). Although iron is available in high amounts in the soil, its bioavailability is limited especially in aerobic and neutral pH conditions. Under aerobic condition, iron is present in ferric form (Fe^{3+}), which cannot be used by plants. In order to overcome this problem of iron non-bioavailability plants have developed sophisticated mechanisms. Most dicots follow strategy I or reduction strategy, where ferric chelate reductase is produced to convert ferric form to ferrous form on the root surface before its subsequent absorption. Monocots, on the other hand, follow the chelation strategy or strategy II, where phytosiderophores (PS) are released that form complex with Fe^{+3} (Boonyaves et al. 2017).

Iron in rice seeds is mostly found in the aleurone layers, scutellum and integuments, which are lost during milling. Hence, in order to increase the iron concentration in rice seeds, it is necessary to target the endosperm (Goto et al. 1999; Lee et al. 2009; Grillet et al. 2014). Therefore, iron biofortification programs in rice primarily concentrate on targeting the endosperm. It has been demonstrated that constitutive expression of *nicotianamine synthase* (*NAS*), the most widely used gene in the synthesis of PS, can increase iron levels by two to four fold in polished rice grains (Masuda et al. 2009; Johnson et al. 2011; Lee et al. 2012).

Another approach of iron biofortification is overexpression of iron storage protein *ferritin*. Rice globulin (*OsGlb1*) or glutelin (*OsGluB1*) promoter-driven expression of *ferritin* could elevate iron concentration. When *SoyferH-1* gene under the control of two endosperm-specific promoters, *OsGlb1*and *OsGluB*, was overexpressed in rice, it led to increase in the concentration of Fe in grains (Qu et al. 2005). However, they observed no significant increase in Fe concentration in rice seeds when compared to expression driven by a single endosperm-specific promoter. Transgenic rice plants generated by overexpression of *ferritin* gene under the control of *OsGluB1* promoter showed 2- to 3.7-fold increase in Fe concentration polished grains (Paul et al. 2012; Oliva et al. 2014). Even though there was a significant increase in Fe concentration in transgenic rice with *ferritin* driven by endosperm-specific promoter, the levels were low as compared to non-transgenic lines (Masuda et al. 2013). Therefore, in addition to increased Fe storage in seeds, enhanced uptake of Fe from the soil and its subsequent translocation within the plant body are required to further improve the Fe biofortification of rice seeds (Masuda et al. 2013). For example, *soybean ferritin* (*SferH-2*) under the control of an endosperm-specific promoter, *Hordeum vulgare nicotianamine synthas*e gene (*HvNas1*), two *nicotianamine aminotransferase* genes (*HvNAAT-A and HvNAAT-B*) and a *mugineic acid synthase* gene (*IDS3*) were introduced in rice plants with an objective to enhance mugineic acid production using a marker free vector for public acceptance. The iron content in this pyramided transgenic rice increased by 2.5- to 4-fold depending on the soil type (Masuda et al. 2013). Transgenic lines harbouring rice

nicotianamine synthase (*OsNAS2*) and *soybean ferritin* (*SferH-1*) genes showed no yield loss or altered grain quality (Trijatmiko et al. 2016). Furthermore, the concerted expression of *Arabidopsis thaliana iron regulator transporter 1* (*AtIRT1*), *Arabidopsis thaliana nicotianamine synthase 1* (*AtNas1*) and *Phaseolus vulgaris ferritin* (*PvFER*) in rice resulted in an increase in iron content up to 10.46 $\mu g g^{-1}$ dry weight.

Like rice, in wheat also iron is accumulated in the aleurone layers, which are lost during the processing. Furthermore, iron in these tissues mostly concentrates in the plant storage vacuoles (Regvar et al. 2011). In these vacuoles, iron is usually bound to phytate, making it unavailable to humans (Borg et al. 2009). Ferritin, an iron storage protein that can attract around 4500 iron ions within its vicinity (Theil 1987; Andrews et al. 1992), is found to be present in the endosperm amyloplast (Balmer et al. 2006). So biofortification in wheat is also concerned with the endosperm. Wheat transformation efficiency, however, lags behind rice transformation efficiency. Nevertheless, it is in the path of improvement since a wide range of promoters are now available that can target specific tissues or developmental stages (Harwood 2012). Moreover, *Agrobacterium*-mediated transformation in wheat is now possible with the help of a patented technology (PureIntro; WO 95/06722) from Japan tobacco, which has been licensed to various institutions, and its transformation efficiency is as high as 30% (Borrill et al. 2014). But the problem is not over yet. Wheat transformation protocols are not cost-effective, and there are no reported genome-independent transformation procedures in wheat since most of the protocols exploit Bobwhite or Fielder, which is not feasible for commercialization (Li et al. 2012). The capacity to transform any cultivar of wheat at low costs can improve the present status of wheat transformation (Borrill et al. 2014). Presently, wheat transformation is mainly concentrated on endosperm-specific expression of wheat or soybean *ferritin* genes, which resulted in 1.5- to 1.9-fold and 1.1- to 1.6-fold increase of grain iron content, respectively (Borg et al. 2012; Sui et al. 2012). Likewise, in maize, co-expression of soybean *ferritin* and *Aspergillus niger phytase* genes can improve iron content in the grains (Drakakaki et al. 2005).

24.3.1.2 Biofortification by Lowering Antinutrient Quantity

Iron biofortification can be improved by reducing antinutrients such as phytic acid. Antinutrients are commonly removed by decortication, malting, fermentation, roasting, flaking and grinding (Vinoth and Ravindhran 2017). For significant improvement of iron bioavailability, complete degradation of phytate is necessary (Hurrell et al. 2003; Sandberg and Andlid 2002). Complete degradation of phytate increases iron absorption by fivefold (Hurrell et al. 2003).

Genetically modified crops either with low-phytate content or high phytase activity have been considered (Bohn et al. 2008). For example, overexpression of *A. niger phytase* gene (*phy A2*) in maize seeds driven by endosperm-specific globulin-1 promoter led to 30% decrease in seed phytate concentration (Chen et al. 2008). In order to increase the bioavailability of iron in wheat, elevated phytase activity by expression of phytochrome gene *phyA* has been reported (Brinch-

Pederson et al. 2000). Furthermore, silencing of wheat transporter ABCC13 can reduce phytic acid concentration (Bhati et al. 2016).

Endosperm-specific expression of *ferritin* along with *phytase* and constitutive expression of *NAS* (NFP rice) resulted in a sixfold increase in endosperm iron content in polished grains. Genes controlling phytic acid biosynthetic pathway are well understood in major cereal crops (Raboy and Bowen 2006). Three enzymes, namely, myo-inositol-3-phosphate synthase (MIPS), myo-inositol-3-phosphate 5/6-kinase (MIK) and inositol 1,3,4,5,6-pentakisphosphate 2-kinase (IPK1), expressed in different levels of the biosynthetic pathway are the molecular targets for producing low-phytate crops (Kuwano et al. 2009). Silencing IPK1 gene by RNAi technology produced low-phytate rice and the yield was comparable with wild type (Ali et al. 2009). Insertional mutagenesis of IPK1 gene by site-specific nucleases, such as zinc finger nucleases (ZFNs), resulted in low-phytate maize (Shukla et al. 2009).

24.3.1.3 Zinc Biofortification

There is some kind of crosstalk between iron and zinc transport network since transgenic plants with overexpressed Fe transporters also showed increased Zn accumulation. For example, enhanced expression of a rice *nicotianamine synthase* gene (*OsNAS3*) resulted in the increase of Fe and Zn in both vegetative tissues and mature seeds (Lee et al. 2009). Another example can be drawn from the work done by Trijatmiko et al. (2016), where they reported 15 μg g^{-1} Fe and 45.7 μg g^{-1} Zn in polished grain using rice *nicotianamine synthase* (*OsNAS2*) and *soybean ferritin* (*SoyferH-1*) genes.

24.3.1.4 Vitamin Biofortification

Rice is targeted for biofortification programs aiming to reduce malnutrition since it is a staple crop to half of the world's population. Vitamin deficiency is one such challenge affecting people especially the poorer section of the society who cannot meet the expense of vitamin nutrition in their diets. The most famous example of vitamin biofortified rice is the Golden rice, which is a rich source of β- carotene, a precursor of vitamin A (Ye et al. 2000). Golden rice was generated by introducing two genes involved in β-carotene biosynthesis, viz. *phytoene synthase* (*psy*) from daffodil (*Narcissus pseudonarcissus*) and *carotene desaturase* (*crtI*) from soil bacterium *Erwinia uredovora* (Burkhardt et al. 1997; Ye et al. 2000; Beyer et al. 2002; Datta et al. 2003; Paine et al. 2005). A team from Syngenta in 2005 developed Golden rice II by bringing together *phytoene synthase* gene from maize and the original *carotene desaturase* gene from Golden rice. This produced 23 times more carotenoids than the original Golden rice (up to 37 μg/g) and accumulates more β-carotene (up to 31 μg/g of 37 μg/g) (Paine et al. 2005). By overexpressing genes encoding *Arabidopsis* GTP-cyclohydrolase I (GTPCHI) and aminodeoxychorismate synthase (ADCS), folate concentration in rice has been found to be increased by 150-fold (Storozhenko et al. 2007; Blancquaert et al. 2015). Similarly, in wheat provitamin A content has been observed to be increased by the introduction of bacterial *psy* and *carotene desaturase* genes (*Crt B* and *Crt I*) (Cong et al. 2009; Wang et al. 2014). In maize also provitamin A content has been demonstrated to

have increased in the endosperm by expressing bacterial *crtB* (Aluru et al. 2008) and multiple carotenogenic genes (Decourcelle et al. 2015; Zhu et al. 2008). Vitamin C (L-ascorbic acid) concentration in maize has been found to be increased by 100-fold through recycling oxidized ascorbic acid to reduced form by the expression of dehydroascorbate reductase (DHAR) (Chen et al. 2003). A multiple vitamin containing maize was developed with 169-fold of the normal amount of β-carotene, double the normal amount of folate and sixfold of the normal amount of ascorbate by engineering three metabolic pathways (Naqvi et al. 2009).

24.3.2 Transgenics for Insect Resistance

The development of insect resistance in cereal crops is very essential as the insect causes up to 40% damage to the crops, especially in the developing countries. As described previously, most of the crop damages are caused by Lepidoptera, which is the second largest order of insects and comprises of moths and butterflies. The genetic modification has the potential to provide the much larger range of resistance against insect pest and herbicides, which is not possible using the conventional breeding methods (Stevens et al. 2012). To overcome the emerging problem, the use of biotechnological approaches, such as transgenic crops expressing endotoxins, vegetative insecticidal protein, biotin binding protein, chitinase, cholesterol oxidase, amylase inhibitors, protease inhibitors, trypsin modulating factor, etc., has become very popular and successful to some extent. Similarly, the modified crops expressing herbicide resistance genes can also provide protection to many important crops. There are various reports available describing the use of these biotechnological methods, and some modified crops are being commercially cultivated also (Table 24.1).

The insecticidal Bt toxin (*Bacillus thuringiensis*) is the most popular and widely used toxin to develop the insect-resistant transgenic crops. The Bt toxin comprises of a large range of insecticidal genes and exhibits different insecticidal activity against pests. The mode of action includes accumulation of crystalline inclusion bodies produced by bacterium on sporulation (Cry proteins, Cyt proteins) or expression of vegetative insecticidal proteins (Vip) during bacterial growth. The extensively studied cry proteins have three domains, and the mechanism involves proteolytic activation and oligomerization followed by osmotic lysis of midgut cells. In cereals, the maize (*Zea Mays*) crop providing resistance to both lepidopteran and coleoptern has become extensively used in agricultural around the globe and reported to lead a reduction in the production cost and pesticide uses (Toenniessen et al. 2003; Brookes and Barfoot 2005). Likewise, the fusion protein expression in transgenic maize and rice provides resistance against the larvae of stemborers (*Chilo suppressalis*) and leaf armyworm (*Spodoptera littoralis*), but unlikely the plants expressing the unmodified Cry1Ac were reported to be susceptible for both the insects (Xu et al. 2018). Additionally, the resistance against hemipteran pest, the leafhopper (*Cicadulina mbila*), was also reported from the plants expressing fusion protein, as the Bt toxins are not effective against hemipterans, so the lectin domain may cause this effect.

Table 24.1 Biotechnological tools for improvement of seed quality

Crop	Technology used	Outcome	References
Nutritional quality improvement			
Rice (*Oryza sativa*)	Introducing two genes involved in β-carotene biosynthesis, viz. *phytoene synthase* (*psy*) from daffodil (*Narcissus pseudonarcissus*) and *carotene desaturase* (*crtI*) from soil bacterium *Erwinia uredovora*	Vitamin A-enriched rice (Golden rice)	Ye et al. (2000)
	Phytoene synthase gene from maize and the original *carotene desaturase* gene from Golden rice	23 times more carotenoids than the original Golden rice (Golden rice II)	Lee et al. (2009)
	Expression of a rice *nicotianamine synthase* gene (*OsNAS3*)	Increase of Fe and Zn	Trijatmiko et al. (2016)
	Rice *nicotianamine synthase* (*OsNAS2*) and *soybean ferritin* (*SoyferH-1*) genes	Increase of Fe and Zn in polished rice	Drakakaki et al. (2005)
	Endosperm-specific expression of *ferritin* along with *phytase* and constitutive expression of *NAS* (NFP rice)	Increase of endosperm iron content in polished grains	Masuda et al. (2009), Johnson et al. (2011)
	Constitutive expression of *nicotianamine synthase* (*NAS*)	Increase iron levels by two- to fourfold in polished rice grains	Lee et al. (2012)
	SoyferH-1 gene under the control of two endosperm-specific promoters, *OsGlb1*and *OsGluB*, was overexpressed	Increase in the concentration of Fe in grains	Qu et al. (2005)
	Overexpression of *ferritin* gene under the control of *OsGluB1* promoter	2- to 3.7-fold increase in Fe concentration polished grains	Paul et al. (2012), Oliva et al. (2014)
Maize (*Zea mays*)	Barley HGGT gene was overexpressed	An eightfold increase in total tocols (tocopherols and tocotrienols) vitamin E-enriched maize	Cahoon et al. (2003)
	Expressing bacterial *crtB* and multiple carotenogenic genes	Increase in provitamin A content	Aluru et al. (2008), Decourcelle et al. (2015), Zhu et al. (2008)
	Recycling oxidized ascorbic acid to reduced form by the expression of dehydroascorbate reductase	Vitamin C-enriched maize	Chen et al. (2003)

(continued)

Table 24.1 (continued)

Crop	Technology used	Outcome	References
	Multiple vitamin containing maize by engineering three metabolic pathways	169-fold the normal amount of β-carotene, double the normal amount of folate and 6-fold of the normal amount of ascorbate	Naqvi et al. (2009)
	Overexpression of *A. niger phytase* gene (*phy A2*) in maize seeds driven by endosperm-specific globulin-1 promoter	30% decrease in seed phytate concentration	Chen et al. (2008)
Wheat (*Triticum sp.*)	Endosperm-specific expression of wheat or soybean *ferritin* genes	Increase in grain iron content up to1.5- to 1.9-fold	Borg et al. (2012), Sui et al. (2012)
	Elevated phytase activity by expression of phytochrome gene *phyA*	Increase in the bioavailability of iron	Brinch-Pederson et al. (2000)
	Silencing of wheat transporter ABCC13	Reduce phytic acid concentration	Bhati et al. (2016)
	Introduction of bacterial *psy* and *carotene desaturase* genes (*Crt B* and *Crt I*)	Increase in wheat provitamin A content	Cong et al. (2009), Wang et al. (2014)
Insect pest resistance			
Maize (*Zea mays*)	Transgenic maize producing dsRNA directed against *V-type ATPase* of corn rootworm	Suppression of mRNA in the insect and reduction in feeding damage compared to controls	Baum et al. (2007)
	Expression of avidin in transgenic maize	Initially aimed to produce a high-value product but resistance occurred in seeds containing more than 0.1% avidin (of total protein) to larvae of three different coleopteran storage pests	Kramer et al. (2000)
	Fusion protein expression in transgenic maize	Resistance against the larvae of stemborers (*Chilo suppressalis*) and leaf armyworm (*Spodoptera littoralis*)	Xu et al. (2018)
	Gene stacking methodology	Containing both insect resistance genes and herbicide resistance genes	Grainnet (2007)
Rice (*Oryza sativa*)	Proteinase inhibitor II, (trypsin, chymotrypsin, oryzin, subtilisin, elastase)	Decrease weight of *Sesamia inferens*	Duan et al. (1996)
	Fusion protein expression in transgenic rice	Resistance against the larvae of stemborers (*Chilo suppressalis*) and leaf armyworm (*Spodoptera littoralis*)	Xu et al. (2018)

(continued)

Table 24.1 (continued)

Crop	Technology used	Outcome	References
Lowering the antinutrients			
Maize (*Zea mays*)	Overexpression of *A. niger phytase* gene (*phy A2*) in maize seeds driven by endosperm-specific globulin-1 promoter	30% decrease in seed phytate concentration	Chen et al. (2008)
Wheat (*Triticum sp.*)	Expression of phytochrome gene *phyA*	Increase the bioavailability of iron in wheat, elevated phytase activity	Brinch-Pederson et al. (2000)
	Silencing of wheat transporter ABCC13	Reduce phytic acid concentration	Bhati et al. (2016)
Rice (*Oryza sativa*)	Silencing IPK1 gene by RNAi technology	Low-phytate rice but	Ali et al. (2009)
	Endosperm-specific expression of *ferritin* along with *phytase* and constitutive expression of *NAS* (NFP rice)	Sixfold increase in endosperm iron content in polished grains	
Maize (*Zea mays*)	Insertional mutagenesis of IPK1 gene by site-specific nucleases like zinc finger nucleases (ZFNs)	Resulted in low-phytate maize	Shukla et al. (2009)

Further, the strong insecticidal activity of avidin was also reported, although the susceptibility was different between insect species. The transgenic maize expressing avidin, which was initially aimed to produce a high-value product, showed a full resistance to the larvae of three different coleopteran storage pests (Kramer et al. 2000). Another well-known method, i.e. RNAi, is also being used to disrupt the gene function and develop resistance against insects in various crops. The mRNA level in modified crops confirms the gene expression, and these transgenic plants producing the double-stranded RNAs were reported to exhibit the partial resistance against insect pests (Turner et al. 2006). In the report of Baum et al. 2007, the dsRNA producing maize directed against V-type ATPase of corn rootworm provided suppression of mRNA in the insect and reduction in the insect damage. Moreover, improvement in the transformation methods, such as the range extension of *Agrobacterium*-mediated gene transfer to monocots, also provided insect resistance. The introduction of plasmid vectors containing multiple transgene in a single locus confers as a one-stop solution to pest and weed problems. This gene stacking methodology was reported in transgenic maize containing both insect resistance genes and herbicide resistance genes. The transgenic lines provide resistance against corn rootworm and lepidopteran pests (rootworm; Cry34Ab1, 1 Cry35Ab1, modified Cry3Bb1: Lepidoptera; Cry1F, Cry1A.105, Cry2Ab2) and tolerance to herbicides (glyphosate and glufosinate-ammonium) (Grainnet 2007).

Thus, a major scientific success has been achieved by production of transgenic insect-resistant plants. Although the practical success of these genetically modified crops is limited to the some countries, more established regulatory system for release of genetically modified crops is needed worldwide.

24.3.3 Quality Improvement of Cereals Using Biotechnological Approaches

Major cereal crops, such as rice, maize and wheat, improved for quality characteristics through various biotechnological approaches in seed biotechnology are summarized in Table 24.1.

24.4 Conclusion

Cereals form an important part of the staple diet as it is consumed by the large population in the world. As cereals are rich in carbohydrates, proteins, vitamins, micronutrients, etc., they have always formed the centre-point of interest since civilization. Though conventional strategies are being continuously used to improve the seed quality and increase the nutrient level in cereals, these methods are cumbersome and generally do not deliver the desired results. Hence, biotechnological interventions are necessary to address the issues of non-availability of important nutrients, loss of yield due to insects and pests as well as other environmental factors. Biofortification has the potential to make cereal crops loaded with important micronutrients and vitamins. This approach of food enrichment proves to be convenient especially in the developing and underdeveloped countries that cannot afford costlier alternatives, such as supplementation or fortification. Biotechnological methods are also being used to prevent insect damage in important cereals. An example can be drawn from the very popular "Bt", where crystalline proteins (Cry) proteins from *Bacillus thuringiensis* are being used to confer resistance against Lepidopteran insects. In addition, vegetative insecticidal protein, chitinase, amylase, protease inhibitors, etc. were also used to provide resistance against various insect pests. On the other hand, the yield and productivity of cereals can also be increased through biotechnological approaches especially by increasing the seed size and quantity.

With the advent of genome editing tools such as CRISPR, new avenues have opened up in the improvement of cereals. This technique has prospective to find new niches in the production of climate-ready cereals, cereals with better resistance to insects and improved seed quality. CRISPR and other genome editing techniques may take seed biotechnology to new frontiers that will produce improved cereals, which in turn will definitely improvise human health.

References

Ali N, Paul S, Gayen D, Sarkar SN, Datta K, Datta SK (2009) Development of low phytate rice by RNA mediated seed-specific silencing of *inositol 1,3,4,5,6-pentakisphosphate 2- kinase* gene (*IPK1*). PLoS One 8:68161

Aluru M, Xu Y, Guo R, Wang Z, Li S, White W, Wang K, Rodermel S (2008) Generation of transgenic maize with enhanced provitamin A content. J Exp Bot 59(13):3551–3562

Andrews SC, Arosio P, Bottke W, Briat JF, von Darl M, Harrison PM, Laulhere JP, Levi S, Lobreaux S, Yewdall SJ (1992) Structure, function, and evolution of ferritins. J Inorg Biochem 47(3–4):161–174

Balmer Y, Vensel WH, Dupont FM, Buchanan BB, Hurkman WJ (2006) Proteome of amyloplasts isolated from developing wheat endosperm presents evidence of broad metabolic capability. J Exp Bot 57:1591–1602

Bashir K, Takahashi R, Nakanishi H, Nishizawa NK (2013) The road to micronutrient biofortification of rice: progress and prospects. Front Plant Sci 4(15):1–7

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J (2007) Control of coleopteran insect pests through RNA interference. Nat Biotechnol 25:1322–1326

Beyer P, Al-Babili S, Ye X, Lucca P, Schaub P, Welsch R, Potrykus I (2002) Golden rice: introducing the β-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132(3):506–510

Bhati KK, Alok A, Kumar A, Kaur J, Tiwari S, Pandey AK (2016) Silencing of ABCC13 transporter in wheat reveals its involvement in grain development, phytic acid accumulation and lateral root formation. J Exp Bot 67(14):4379–4389

Blancquaert D, Van daele J, Strobbe S, Kiekens F, Storozhenko S, De Steur H, Glleynck X, Lambert W, Stove C, Van Der Straeten D (2015) Improving folate (vitamin B9) stability in biofortified rice through metabolic engineering. Nat Biotechnol 33:1076–1078

Bohn L, Meyer AS, Rasmussen SK (2008) Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J Zhejiang Univ Sci B 9:165–191

Boonyaves K, Ting-Ying W, Gruissem W, Bhullar NK (2017) Enhanced grain iron levels in rice an iron regulated metal transporter, nicotianamaine synthase, and ferritin gene cassette. Front Plant Sci 8:130

Borg S, Brinch-Pedersen H, Tauris B, Holm P (2009) Iron transport, deposition and bioavailability in the wheat and barley grain. Plant Soil 325:15–24

Borg S, Brinch-Pedersen H, Tauris B, Madsen LH, Darbani B, Noeparvar S, Holm PB (2012) Wheat ferritins: improving their on content of the wheat grain. J Cereal Sci 56:204–213

Borrill P, Connorton JM, Balk J, Miller AJ, Sanders D, Uauy C (2014) Biofortification of wheat grain with iron and zinc: integrating novel genomic resources and knowledge from model crops. Front Plant Sci 5(53):1–8

Bouis HE, Saltzman A (2017) Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. Glob Food Sec 12:49–58

Brinch-Pederson H, Olesen A, Rasmussen SK, Holm PB (2000) Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. Mol Breed 6:195–206

Brookes G, Barfoot P (2005) GM crops: the global economic and environmental impact: the first nine years 1996-2004. AgBio Forum 8:15

Burkhardt PK, Beyer P, Wuenn J, Kloeti A, Armstrong GA, Schledz M, von Lintig J, Potrykus I (1997) Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. Plant J 11(5):1071–1078

Cahoon EB, Hall SE, Ripp KG, Ganzke TS, Hitz WD, Coughlan SJ (2003) Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. Nat Biotechnol 21(9):1082–87

Cakmak I, Hoffland E (2012) Zinc for the improvement of crop production and human health. Plant Soil 361:1

Chen Z, Young TE, Ling J, Chang SC, Gallie DR (2003) Increasing vitamin C content of plants through enhanced ascorbate recycling. Proc Natl Acad Sci U S A 100:3525–3530

Chen R, Xue G, Chen P, Yao B, Yang W, Ma Q, Fan Y, Zhao Z, Tarczynski MC, Shi J (2008) Transgenic maize plants expressing a fungal phytase gene. Transgenic Res 17:633–643

Cong L, Wang C, Chen L, Liu H, Yang G, He G (2009) Expression of *phytoene synthase1* and *carotene desaturase crtI* genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). J Agric Food Chem 57(18):8652–8660

Datta K, Baisakh N, Oliva N, Torrizo L, Abrigo E, Tan J, Rai M, Rehana S, Al-Babili S, Beyer P, Potrykus I, Datta SK (2003) Bioengineered 'golden' Indica rice cultivars with beta-carotene metabolism in the endosperm with hygromycin and mannose selection systems. Plant Biotechnol J 1:81–90

Decourcelle M, Perez-Fons L, Baulande S, Steiger S, Couvelard L, Hem S, Zhu C, Capell T, Christou P, Fraser P, Sandmann G (2015) Combined transcript, proteome, and metabolite analysis of transgenic maize seeds engineered for enhanced carotenoid synthesis reveals pleotropic effects in core metabolism. J Exp Bot 66(11):3141–3150

Department of Primary and Regional Development's Agriculture and Food Division, Govt. of Western Australia (2019). https://www.agric.wa.gov.au/. Retrieved 27 July 2019

Dhaliwal GS, Singh R, Jindal V (2013) A textbook of integrated pest management. Kalyani Publishers, New Delhi

Drakakaki G, Marcel S, Glahn RP, Lund EK, Pariagh S, Fischer R, Christou P, Stoger E (2005) Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus* phytase in maize results in significant increases in the levels of bioavailable iron. Plant Mol Biol 59 (6):869–880

Duan X, Li X, Xue Q, Abo-Ei-Saad M, Xu D, Wu R (1996) Transgenic rice plants harbouring an introduced potato proteinase inhibitor II gene are insect resistant. Nat Biotech 14:494–498

Goto F, Yoshihara T, Shigemoto N, Toki S, Takaiwa F (1999) Iron fortification of rice seed by the soybean ferritin gene. Nat Biotech 17:282–286

Grainnet (2007) Monsanto and Dow Agrosciences launch "SmartStax", industry's first-ever eight-gene stacked combination in corn. Grainnet. http://www.grainnet.com

Grillet L, Mari S, Schmidt W (2014) Iron in seeds–loading pathways and subcellular localization. Front Plant Sci 4(536):1–8

Harwood WA (2012) Advances and remaining challenges in the transformation of barley and wheat. J Exp Bot 63:1791–1798

Hurrell RF, Redd MB, Juillerat MA, Cook JD (2003) Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am J Clin Nutr 77:1213–1219

Johnson D, O'Connor S (2015) These charts shows every genetically modified food people already eat in the U.S. All the GMOs approved in the US. Time. Com. Retrieved 7 July 2019

Johnson AA, Kyriacou B, Callahan DL, Carruthers L, Stangoulis J, Lombi E, Tester M (2011) Constitutive overexpression of the *OsNAS* gene family reveals single-gene strategies for effective iron-and zinc-biofortification of rice endosperm. PLoS One 6:e24476

Kennedy G, Nantel G, Shetty P (2003) The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. Food Nutr Agric 32:8e16

Kramer KJ, Morgan TD, Throne JE, Dowell FE, Bailey M, Howard JA (2000) Transgenic avidin maize is resistant to storage insect pests. Nat Biotechnol 18:670–674

Kuwano M, Mimura T, Takaiwa F, Yoshida KT (2009) Generation of stable 'low phytic acid' transgenic rice through antisense repression of them 1D-myo–inositol 3-phosphate synthase gene (*RINO1*) using the 18-kDa oleosin promoter. Plant Biotechnol J 7:96–105

Lee S, Jeon US, Lee SJ, Kim YK, Persson DP, Husted S, Schjorring JK, Kakei Y, Masuda H, Nishizawa NK, An G (2009) Iron fortification of rice seeds through activation of the nicotianamine synthase gene. Proc Natl Acad Sci U S A 106:22014–22019

Lee SC, Kim Y, Jeon U, Kim Y, Schjoerring JK, An G (2012) Activation of rice *nicotianamine synthase 2* (*OsNAS2*) enhances iron availability for biofortification. Mol Cells 33:269–275

Li JR, Ye XG, An BY, Du LP, Xu HJ (2012) Genetic transformation of wheat: current status and future prospects. Plant Biotechnol Rep 6:183–193

Manwaring HR, Bligh HFJ, Yadav R (2016) The challenges and opportunities associated with biofortification of pearl millet (*Pennisetum glaucum*) with elevated levels of grain iron and zinc. Front Plant Sci 7:1944

Masuda H, Usuda K, Kobayashi T, Ishimaru Y, Kakei Y, Takahashi M, Higuchi K, Nakanishi H, Mori S, Nishizawa NK (2009) Overexpression of the barley *nicotianamine synthase* gene *HvNAS1* increases iron and zinc concentrations in rice grains. Rice 2:155–166

Masuda H, Kobayashi T, Ishimaru Y, Takahashi M, Aung MS, Nakanishi H, Mori S, Nishizawa NK (2013) Iron-biofortification in rice by the introduction of three barley genes participated in mugineic acid biosynthesis with soybean ferritin gene. Front Plant Sci 4:132

Muller O, Krawinkel M (2005) Malnutrition and health in developing countries. CMAJ 173 (3):279–286

Naqvi S, Zhu C, Farre G, Ramessar K, Bassie L, Breitenbach J, Perez Conesa D, Ros G, Sandmann G, Capell T, Christou P (2009) Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proc Natl Acad Sci U S A 106(19):7762–7767

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43

Oliva N, Chadha-Mohanty P, Poletti S, Abrigo E, Atienza G, Torrizo L, Garcia R, Duenas C Jr, Poncio MA, Balingdon J, Manzanilla M, Montecillo F, Zaidem M, Barry G, Herve P, Shou H, Slamet-Loedin IH (2014) Large-scale production and evaluation of marker-free *indica* rice IR64 expressing phytoferritin genes. Mol Breed 33:23–37

Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Silverstone AL, Drake R (2005) Improving the nutritional value of golden rice through increased pro-vitamin a content. Nat Biotechnol 23:482–487

Paul S, Ali N, Gayen D, Datta SK, Datta K (2012) Molecular breeding of *OsFer 2* gene to increase iron nutrition in rice grain. GM Crops Food 3:310–316

Population Division-United Nations (2019) World population prospects. Population.un.org. Retrieved 26 Mar 2019

Prentice A, Goldberg GR, Schoenmakers I (2008) Vitamin D across the lifecycle: physiology and biomarkers. Am J Clin Nutr 88(2):500S–506S

Qu LQ, Yoshihara T, Ooyama A, Goto F, Takaiwa F (2005) Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. Planta 222:225–233

Raboy V, Bowen D (2006) Genetics of inositol polyphosphates. In: Majumder AL, Biswas BB (eds) Biology of Inositols and Phosphoinositides. US Govt, Netherlands, pp 71–102

Regvar M, Eichert D, Kaulich B, Gianoncelli A, Pongrac P, Vogel-Mikus K, Ivan K (2011) New insights into globoids of protein storage vacuoles in wheat aleurone using synchrotron soft X-ray microscopy. J Exp Bot 62:3929–3939

Sandberg AS, Andlid T (2002) Phytogenic and microbial phytases in human nutrition. Int J Food Sci Technol 37(7):823–833

Shahzad Z, Rouached H, Rakha A (2014) Combating mineral malnutrition through iron and zinc biofortification of cereals. Compr Rev Food Sci Food Saf 13:329–346

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill P, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, Rebar EJ, Gregory PD, Urnov FD (2009) Precise genome modification in the crop species *Zea mays* using zinc-finger nucleases. Nat Lett 459:437–443

Srinivasan A, Giri A, Gupta V (2006) Structural and functional diversities in lepidopteran serine proteases. Cell Mol Biol Lett 11:132–154

Stevens J, Dunse K, Fox J, Evans S, Anderson M (2012) In: Soundararajan RP (ed) Biotechnological approaches for the control of insect pests in crop plants, pesticides - Advances in chemical and botanical pesticides. Intech Open. https://doi.org/10.5772/46233

Storozhenko S, De Brouwer V, Volckaert M, Navarrete O, Blancquaert D, Zhang GF, Lambert W, Van Der Straeten D (2007) Folate fortification of rice by metabolic engineering. Nat Biotechnol 25(11):1277–1279

Sui X, Zhao Y, Wang S, Duan X, Xu L, Liang R (2012) Improvement Fe content of wheat (*Triticum aestivum*) grain by soybean ferritin expression cassette without vector backbone sequence. J Agric Biotechnol 20:766–773

Theil EC (1987) Ferritin: structure, gene regulation, and cellular function in animals, plants, and microorganisms. Annu Rev Biochem 56(1):289–315

Thomas L (2019) Should we eat polished rice? News-Medical. https://www.news-medical.net/. Retrieved 27 July 2019

Toenniessen GH, O'Toole JC, DeVries J (2003) Advances in plant biotechnology and its adoption in developing countries. Curr Opin Plant Biol 6:191–198

Trijatmiko KR, Duenas C, Tsakirpaloglou N, Torrizo L, Arines FM, Adeva C, Balindong J, Oliva N, Sapasap MV, Borrero J, Rey J, Francisco P, Nelson A, Nakanishi H, Lombi E, Tako E, Glahn RP, Stangoulis J, Chadha-Mohanty P, Johnson AA, Tohme J, Barry G, Slamet-Loedin IH (2016) Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. Sci Rep 6:19792

Turner CT, Davy MW, MacDiarmid RM, Plummer KM, Birch NP, Newcomb RD (2006) RNA interference in the light brown apple moth, Epiphyas postvittana (Walker) induced by double-stranded RNA feeding. Insect Mol Biol 15:383–391

Vinoth A, Ravindhran R (2017) Biofortification in millets: a sustainable approach for nutritional security. Front Plant Sci 8:29

Wang C, Zeng J, Li Y, Hu W, Chen L, Miao Y, Deng P, Yuan C, Ma C, Chen X, Zang M, Wang Q, Li K, Chang J, Wang Y, Yang G, He G (2014) Enrichment of provitamin a content in wheat (*Triticum aestivum* L.) by introduction of the bacterial carotenoid biosynthetic genes *CrtB* and *CrtI*. J Exp Bot 65(9):2545–2556

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

White PJ, Broadley MR (2005) Bio-fortifying crops with essential mineral elements. Trends Plant Sci 10:586–593

World Health Organization (2001) The World health report: 2001: Mental health: new understanding, new hope. World Health Organization

World Health Organization (2016) Micronutrient deficiencies. http://www.who.int/nutrition/topics/ida/en/. Accessed 15 Dec 2016

World Health Organization (2019) Biofortification of staple crops. https://www.who.int/elena/titles/biofortification/en/. Accessed June 2019

Xu C, Cheng J, Lin H, Lin C, Gao J, Shen Z (2018) Characterization of transgenic rice expressing fusion protein Cry1Ab/Vip3A for insect resistance. Sci Rep 8:15788

Ye X, Al-Babili S, Kloti A, Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287(5451):303–305

Zhu C, Naqvi S, Breitenbach J, Sandmann G, Christou P, Capell T (2008) Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. Proc Natl Acad Sci U S A 105(47):18232–18237

Production of Healthy Cane Seedlings in Northeast Brazil

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Abstract

In Northeast Brazil, the urge for rapid expansion of sugarcane fields after continuous periods of water stress and economic crisis has brought about the creation of new mechanisms that use less infrastructure, machinery, workforce, and, consequently, lower costs to develop sugarcane nursery and fields. The premise of all the techniques is heat treatment, which helps to improve seed health conditions as well as the sturdiness and longevity of sugarcane fields. Therefore, the aim of this research is to present the main techniques used in seedling nurseries in Northeast Brazil, such as production of cane seedlings with heat treatment, pre-sprouted seedling system, Interrotation Method Occurring Simultaneously, Cantosi, and the association of those methods under field conditions.

Keywords

Sugarcane · Seedling nursery · Thermotherapy · Plant health

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25.1 Introduction

Brazil is the world's greatest producer of sugarcane (*Saccharum* spp.), followed by India, China, and Thailand; for the 2018/2019 harvest, sugarcane production exceeded 620,000 million tons per 8589.2 ha. Even though the production is significant in the sugarcane agribusiness, yields revolve around 72.0 t/ha.

The Brazilian sugar-alcohol sector is considered one of the most modern in the world. Brazil leads the global ranking of sugarcane ethanol production, which is significant due to its market value and for being one of the main sources of clean energy known globally.

Since it is a country with continental proportions, sugarcane croplands are distributed over the most diverse regions, with different edaphoclimatic conditions, which may favor or hinder agricultural yield.

Sugarcane fields stretch over a coastal strip of approximately 200 km. The presence of uneven relief in coastal zones contributes even more for this ecosystem variability, which includes coastal forests and scrubs on sandy soils. The climate is tropical humid, with usually high temperatures and rainfall during autumn and winter. The native vegetation was Atlantic Forest, but it is almost extinct currently and has been replaced by sugarcane plantations since the beginning of the colonization in the fourteenth century.

In this regard, the Sugarcane Genetic Improvement Program of the Interuniversity Network for the Development of the Sugarcane Industry (RIDESA) engages with the distribution of nearly 70% of the national stands, using RB varieties, which are more adapted to the different local cultivation regions.

Additionally, crop protection issues also aggregate to decreases in sugarcane production in Brazil, such as ratoon stunting disease (RSD) deserved attention because it occurs in every Brazilian producer state.

The bacterium *Leifsonia xyli* subsp. *xyli*, a causal agent of the ratoon stunting disease, is Gram-positive, coryneform, nonmobile, obligatory aerobic, and xylem limited. The bacterial cells are pleomorphic rods, ranging from 0.25 to 0.5 μm in width and 1–4 μm in length. They can be straight or slightly curved and occasionally have swollen extremities or central regions.

The pathogen shows slow growth on nutrient-rich culture media, taking from 10 to 15 days for the appearance of colonies and more than 3 weeks for reasonable growth in liquid culture medium. Colonies are nonpigmented and circular when in semisolid media.

This bacterium is limited to xylem vessels of the plant, being restricted to the vessel elements, parenchyma, and lacunas; when under water stress condition, the bacterium causes the obstruction of water and nutrient translocation in the plant.

The ratoon stunt is considered the most important sugarcane disease and may cause 30% average yield losses, depending on the genotype and water conditions; it can even infect up to 100% of a given sugarcane field.

The biggest problem related to this disease is the nonspecificity of the symptoms in the field, which regularly misleads farmers into assuming the field has abiotic problems, making it difficult to give a proper diagnosis.

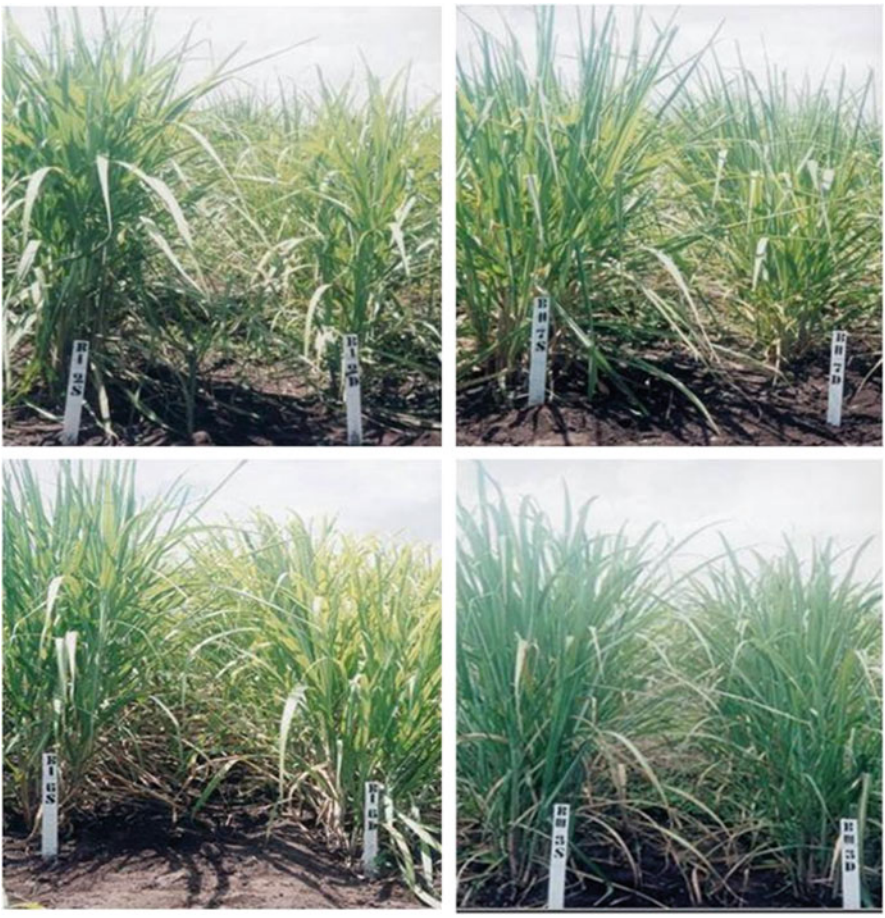

Fig. 25.1 Comparison of the development of two sugarcane plants in the field, a healthy plant (left side, shows bigger size and received heat treatment) and an infected plant by Lxx (right side, less developed plant). The genotypes used for this study were as follows: A. RB763710; B. SP79–1011; C. B8008; D. SP78–4764. (Photos: Chaves et al. 2002)

Commonly, diseased plants are stunted, with reduced size, impaired growth, and shortened internodes. These symptoms may be masked by water availability, plant age and genotype, and soil type.

In drier years, it is common to observe this behavior in plants; however, when there is no water deficit, this behavior is not visualized.

The studies carried out in Northeast Brazil show expressive differences in the development of 10 commercial varieties of sugarcane inoculated with Lxx and healthy plants (Fig. 25.1), resulting in up to 25% of yield loss for diseased plants.

In addition to reducing agricultural yield of sugarcane, another important factor of this disease is reducing the number of cuts in highly infected plots by impairing plant growth.

Genetic resistance of the genotypes to RSD would be the most desirable technique by farmers and researchers of the sugar-alcohol sector; however, there is a great difficulty in selecting resistant varieties due to the complexity of rapid and efficient diagnosis of the disease.

The use of healthy seedlings for planting is the most applied technique to control RSD in Brazil.

25.2 Technologies Used in the Production of Healthy Sugarcane Seedlings in Northeast Brazil

There are some technologies used to produce healthy seedlings, and that integrates high yield in the field with longer life span of sugarcane fields. The most important one, which should be readily used by sugarcane technicians and producers, would be the development of nurseries with heat treatment. However, a considerable portion of production units seems to have forgotten this practice, formerly common in sugarcane fields, putting at risk the success of the nurseries themselves.

Thus, firstly, it will be discussed how to plan seedling nurseries with heat treatment, and, secondly, a few other practices will be addressed that are common when using seedlings in the Northeast region.

25.2.1 Seedling Nurseries with Heat Treatment

25.2.1.1 Nursery Planning

In order to implement seedling nurseries with heat treatment, it is necessary to have an execution plan at least 2 years prior to planting.

The Genetic Improvement Program of the Sugarcane Experimental Station of Carpina (EECAC-UFRPE) in Pernambuco points out that this is the first aspect that must be taken into consideration to set up nurseries to improve sugarcane yield while also considering some of the following steps:

- Site selection
  The site where the nursery will be built must have good fertility, drainage and irrigation conditions, and easy access and, ideally, must be close to the production unit. The producer must use fertilizers from the production unit and amend organic matter to the plots when possible.
- Treatment period
  In Northeast Brazil, the heat treatment is carried out during hot and humid seasons, preferably from late August to late November.
- Seedling quality

The seedling should be healthy, free of diseases and pathogens, physiologically mature, and aged from 12 to 14 months for the cane plant and 10–12 months for the ratoon cane, in order to obtain a better growth result. Another important point is to use the middle third of the stalk. If the material has been previously heat treated or has received roguing inspection to eliminate diseased plants and varietal mixture, the result is even better.

Nursery planning should not surpass the commercial planting of a given variety. It should be less than 20% of the total cropland of the production unit.

25.2.1.2 Special Care

Planting area must be moistened through irrigation, 1 day prior to sowing.

A sprouting test must be programmed by selecting 10 stalks in the plots that will be cut, separated in 100 healthy buds, which will be planted in a well-prepared bed, with results around 20 days, defining its planting in the field.

Nitrogen fertilization increases the vegetative vigor and decreases sugar contents under field conditions; therefore, it is advised to add a dose of nitrogen of 20 kg/ha at 50 days after sowing.

Nurseries must be placed in areas far from disease hot spots, and proper farming practices must always be performed, in addition to weed control, regularly.

25.2.1.3 Preparing Material for Treatment

The hygiene of the selected stalks is performed manually to avoid mechanical damages to the buds, and the knives or guillotines used to cut and prepare the sugarcane billets must be sterilized (according to the model recommend by EECAC-UFRPE).

The size of sugarcane billets will depend on the most convenient for producers and the capacity of the heat treatment unit used by the company.

25.2.1.4 Production of Seedlings with Heat Treatment

The most common heat treatments are the short-term heat treatment (SHT), which lasts for 30 min at 52 ºC, and the long-term heat treatment (LHT), which lasts for 2 h at 50 ºC. After immersing the cane into the tank with previously heated water, there will be a cooling process (temperature shock), making the temperature to drop between 3 and 4 ºC. Therefore, the treatments can have a 0.5-ºC increase in temperature. It is recommended to begin the treatment when the thermostat recovers or reaches the adequate temperature inside the water tank.

In addition, it is advised to use a mercury thermometer with a 0.1-ºC precision, to help in measuring the temperature.

Taking precautions with the balance between the water tank temperature and the time the billets will be immersed is important because errors resulting from this process incur in health impairment of the material or in an increase in failures of bud growth.

It is important to highlight that the SHT and LHT do not remove bacteria completely; leaks commonly occur, which result in retreatment of billets from the

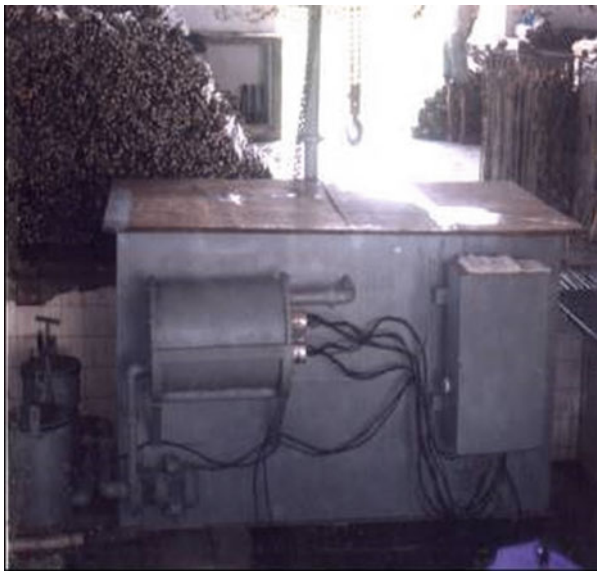

Fig. 25.2 Implanor heat tank

nurseries, so that the farmer has more safety in relation to the material's health. Since it is faster, the SHT is used more frequently at sugarcane production units.

In order to produce seedlings on a large scale, it is recommended to use an Implanor heat treatment unit (Fig. 25.2), water tank with 2500-L capacity, six 1000-W resistances, a motor for forced circulation of water, and a set of automated relay-thermostat to control water temperature. Usually, this tank is used in conjunction with the long-term heat treatment (LHT), which enables the pairing temperature x time in 50.5 °C during 120 min.

The working capacity of the heat treatment unit is 500 kg for each 120 min.

At some production units, the use of Copersucar heat tank (Fig. 25.3) is more adequate. This tank has a capacity for 250 L with 0.1-°C variation; generally, this tank is used for the short-term heat treatment (52 °C/30 min), faster for the production unit logistics. Apart from that, the advantage of the mentioned temperature x time pairing in relation to the traditional one is greater performance in addition to demonstrably stimulating bud germination under such conditions.

After the heat treatment, the billets are taken out of the cages and cooled under room temperature for 15–20 min.

As a protection measure, it is important to change the water from the tank every 48 h. This procedure is associated with cleaning the tank with a sanitizing solution. Cleaning the tank avoids microorganism proliferation in the water during the treatments and that may impair the bud germination in the field.

The disinfection of manual or mechanized cutting blades must occur for both nurseries and commercial planting areas. This technique should be repeated at least for each plot cut or for each variety harvested within the plots.

Fig. 25.3 Copersucar heat tank

25.2.1.5 Fungicidal Treatment

After the cooling time of the heat treatment, the billets are placed into an immersion bath with fungicide for approximately 5 min. Commonly it is used with azoxystrobin +cyproconazole (Priori Xtra)- or pyraclostrobin (Comet)-based fungicides, which are the registered products for sugarcane in Brazil.

It is important to renew the suspensions with fungicide every day at the beginning of each series.

25.2.1.6 Field Planting

- *Precautions with the Cut and Material Transportation*
  The stalk from nurseries must be cut with flamed machetes (fire-based sterilization), and it is advised not to remove sugarcane straw to avoid mechanical damages to the buds during the transportation for the treatment site. Another important aspect consists of avoiding throwing or beating the treated bushels due to posttreatment bud fragility.
- *Planting Density*
  Billet planting must obey the sowing system used by the production unit. Fertilizer application during planting must follow the soil analysis results and specific recommendations. Nitrogen application in installments, together use fertigation starting at 90 days after planting, acts as an auxiliary fertilization and provides better seedling growth.
- *Irrigation and Farming Practices*
  Before sowing the billets, it should be performed with a light irrigation in order to provide adequate soil moisture within planting furrows, thus facilitating billet settlement and germination. After planting, irrigation frequency will be maintained according to the daily need until the establishment of seedlings in the field.

It is recommended the use of herbicides with direct jet dispensing system to control weeds. Under manual or mechanic cultivation, it is recommended to thoroughly wash the agricultural implements before their use. In addition to washing, it is highly advisable to disinfect the implements with quaternary ammonia at 2%.

- *Roguing*
  Roguing operation frequency is recommended to occur monthly. In order to facilitate and speed up the farmer's work, he/she should use glyphosate herbicide at 6% diluted in water and applied with a 5-L sprayer, with jet directed to the leaf whorl of stalks from the diseased clump.
- *Multiplication by Clump Division or Stalk Breaking in the Clump*
  The nursery area may be increased by the clump division process of seedlings with at least 3 months of age or when a clump presents most of its tillers with own roots or when 6- to 7-month-old cane plant stalks are manually broken on its basis and replanted.
- *Disinfection of Agricultural Tools*
  Ratoon stunt and leaf scald are transmitted by using contaminated cutting tools that cause wounds in the sugarcane stalks. Therefore, the cutting in nurseries should be avoided when using any kind of tools or implements previously used in other areas, without prior disinfection with quaternary ammonia.

25.2.1.7 Nursery Categories

- *Basic or Preprimary Nurseries*
  They are the initial nurseries, built at a production unit, where heat-treated billets are used.
- *Primary Nurseries*
  They follow up the preprimary or basic nurseries. Their areas are 10- to 15-fold greater than the basic nurseries.
- *Secondary Nurseries*
  They are the nurseries following up the previously mentioned nurseries and are located at strategic points for commercial planting. There is a very close ratio, where in order to make 10.0 commercial ha, it is necessary to make one tenth of this treated area for nurseries, that is, 1.0 ha.

  The lifetime of a seedling nursery, or the number of multiplication one nursery can have, depends exclusively on the crop protection precautions performed during the field stage.

25.2.2 Pre-Sprouted Seedling System (PSS)

One technology that has been gaining attention in sugarcane planting is the pre-sprouted seedling system (PSS). It presents rapid multiplication associated to a high crop protection standard, vigor, and uniformity.

The PSS main advantage is to increase the efficiency and economic profits in implementing nurseries, replanting, and commercial areas. It is based on the planting

Fig. 25.4 Pre-sprouted seedling system (PSS)

Fig. 25.5 PSS planting, sugarcane field without failure

of a seedling derived from a single bud already sprouted (Fig. 25.4), creating a new planting logistics in nurseries and replanting and, more recently, in commercial areas.

Thus, seedlings rather than stalks are used for planting, minimizing sprouting failures and increasing the uniformity of the planting rows (Fig. 25.5).

In the Northeast, the single bud is known as mini billets, or single-bud billets (Fig. 25.6), which originates the PSS. The mini billets are used to initiate the system,

Fig. 25.6 Single-bud mini billet

and they are extracted from stalks derived from basic nurseries with 7–10 months of age.

All management practices and protocols to obtain healthy pest-free seedlings are used inside the nurseries. From the harvested stalks, the mini billets that receive fungicide-based treatments are chosen, and they can be associated with bio-stimulants and rooting promoters.

The mini billets are packaged in sprouting boxes and kept inside greenhouses for approximately 10–15 days (Fig. 25.7), with irrigation and temperature around 8 mm/day and 35 °C, respectively. Sprouted buds are then transferred individually to tubes (Fig. 25.9), and the ones that did not sprout are discarded from the process.

Seedling acclimatization occurs in the yard under direct sunlight (Fig. 25.8) with irrigation, according to the vegetative development and leaf pruning, to stimulate development. This step lasts from 21 to 30 days, totaling a 60-day cycle approximately.

Planting can be either manual or mechanized. For manual planting, the following procedures must be performed: furrowing, fertilization, and agricultural pesticide application; while for mechanized planting, it is necessary an adequate soil preparation. Spacing between seedlings must be around 0.35–0.50 m, and the spacing between furrows should follow the farming practice adopted by the farmer.

In the manual planting, the boxes are distributed along the furrows, based on the cultivar. Subsequently, seedlings are placed in the furrow, and then they are covered with soil using a hoe (Fig. 25.9). Planting may also be performed through jab planters (Fig. 25.10) with two people: one fills the jab planter and the other plants. The production is estimated in approximately 10,000 seedlings per day.

In the mechanized planting, a drawn planter is used in the threefold operation, furrowing, fertilization, and seedling covering. The boxes are placed on the

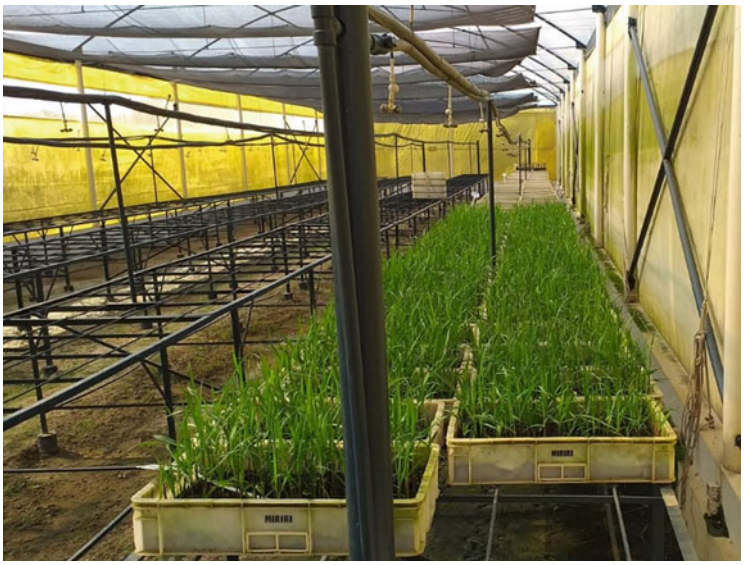

Fig. 25.7 Mini billets in tubes inside a greenhouse

Fig. 25.8 Acclimatization of PSS in the yard under direct sunlight

platforms of the machine, and two people distribute the seedlings using a rotating table, making up two planting rows. The yield is estimated to be around 3.0 ha/day with the described equipment.

Fig. 25.9 Manual planting with hoes (Photo: Flavio Costa/RIDESA)

Fig. 25.10 Planting with jab planters (Photo: Agnaldo Stenico/RIDESA)

25.2.3 Interrotation Method Occurring Simultaneously (MEIOSI)

The MEIOSI is a planting system developed by the end of the 1980s. It arose as an alternative to sugarcane foundation, considering the importance taken between the partnership of sugarcane and grains in São Paulo State (Fig. 25.13).

In the South-Central region of Brazil, the system consists of planting two sugarcane furrows in the spring in the plot to be renovated and allowing some space between the furrows to enable commercial crop cultivation (soybean and peanut) and legume for green manure. After harvesting or management of crops in the summer, plants planted in the spring act as seedlings for the new sugarcane field.

Currently, it has gained expressive attention in the sugar-alcohol sector mainly due to the GPS technology within the tractors, which is a tool that enables to precisely keep the parallelism of furrows. In addition, the possibility of using pre-sprouted seedling favors the increase in multiplication rates, thus improving the efficiency of the system and reducing planting costs.

In Northeast Brazil, this system had to be modified since a second or interspersed cultivation is not traditional in the region. Difficulties such as smaller plots, uneven relief and slopes, as well as rainfall distribution and irrigation needs during planting have made this system poorly used. Some production units make use of MEIOSI in summer planting, claiming less planting implementation costs, but still there is a need for irrigation, so the seedlings can develop. This cost reduction is mainly related to the cutting, loading, and transportation (CLT) of seeds. Once the seed is already in the planting plot, thus avoiding loading and transportation. In between furrows, legume is planted which will be used for green manure.

25.2.4 MEIOSI and PSS

MEIOSI system has been improved with PSS; now, the rows that consist of MEIOSI are planted with PSS. This method has enabled multiplication rates of up to 1:14, which is important for rapid use of varietal census of sugarcane.

A common strategy is to combine the methods to apply at nursery areas, multiplication and renovating areas, and new sugarcane field implementation. The great benefit of this association is the high multiplication rate, leading to a yield increase.

MEIOSI with PSS arose from the need to speed up the expansion of sugarcane cultivation, allowing seedling preselection and aiming to eliminate pathogens and pests; thus, seedlings that are going to the field present greater vigor and inspection during the acclimatization for pests, diseases, and even weeds.

25.2.5 Cantosi System

The benefits from the PSS principle also allow farmers to adjust to methods to implement nursery and commercial areas, as the case of MEIOSI system (Interrotation Method Occurring Simultaneously). The latter consists of planting a

percentage of the area in a way that its own production is used as seedling for the remainder of the area, exploiting a "free" region to produce other crops or, in the case of the Northeast region, the planting of legumes for green manure.

The principle of Cantosi method consists of planting a percentage of the seedlings the same way as in MEIOSI, but under a preestablished percentage and place in the plot, around 20%, using the remaining 80% to plant grains and legumes. In the Northeast, it happens in the beginning of winter, when the precipitation and temperature indices are favorable for the seedling development, and the irrigation is not needed. The seedling cutting occurs by the end of the rainy season, August, when there is enough moisture for the sugarcane to sprout.

Also in the Northeast, some production units use Cantosi in summer planting with irrigation in January. As the area is clustered in a place and percentage of the plot, the irrigation is feasible. The seedling cutting will occur in the beginning of winter with the precipitation favorable to sprouting and development of the sugarcane field.

25.2.6 Pre-Sprouted Seedling in the Field (PSSF)

In Northeast Brazil, the need of making a rapid expansion of the sugarcane field after consecutive periods of water stress and economic crisis has impacted the creation of new mechanisms that use less infrastructure, machinery and work force, and, consequently, less costs to implement sugarcane nurseries and fields.

The implementation of PSSF is based on thermotherapy (TT), which will produce healthy seedlings. The system begins with the single-bud billet planting after TT directly in the field to develop primary nurseries. This planting occurs by the end of the rainy season in August, implementing the Cantosi system. Subsequently, 8 months after (April) the planting in the field, when the stalks are developed, occurs the cutting of the apical portion below the meristem (Fig. 25.11) to develop the PSSF. Thus, from May to June, 40–50 days after the cutting, the PSSF are already formed in the sugarcane stalks under natural field conditions.

Fig. 25.11 Apical portion cutting in the field

Fig. 25.12 Development of PSSF in the field

Fig. 25.13 (**a**, **b**) Cutting of PSSF in field

The apical portion cutting below the meristem helps in breaking the bud dormancy, making the lateral sprouting to occur (Fig. 25.12). One can cut the PSSF with the aid of a cutting plier attached to a wooden or iron structure in the field (Fig. 25.13a,b). Leaf pruning and a crop protection treatment are recommended as well as the use of rooting promoters.

Fig. 25.14 Manual planting of PSSF in the field

Fig. 25.15 Aspect dry leaves after planting PSSF in the field

The planting of PSSF can be manual with the seedlings placed in the furrows and covered with soil using hoes (Fig. 25.14), with jab planters or even mechanized with drawn planter in the threefold operation, furrowing, fertilization, and covering of seedlings.

Days after the planting, the PSSF will have an aspect of dry leaves since PSSF do not present rooting (Fig. 25.15). However, as they are in a stalk supply, vigor, and a good development, this is sufficient enough to occur rooting and new leaves appear with good tillers (Fig. 25.16).

Fig. 25.16 Settlement of PSSF in the field after 45 days

25.3 Conclusions

The use of more practical and sustainable practices on sugarcane fields in the Northeast Brazil region has been noted as an evolution for sugarcane field improvement, both in using less infrastructure and work force and less costs for the development of sugarcane nurseries and fields.

The increase in vigor, health, and consequently longevity of sugarcane fields, in addition to rapid multiplication of seedlings under field conditions, envisions periods with greater agricultural yield and growth of the sugar-alcohol sector in the region.

References

Barcelos JET (1984) Meiosi - Cana e Alimentos - Método inter-rotacional ocorrendo simultaneamente. Revista Saccharum 7(31):10–18

Barcelos JET (1989) Comparação entre dois sistemas de rotação da cana-de-açúcar (*Saccharum* spp.). 135f.Dissertação (Mestrado em Agronomia) Universidade Estadual Paulista, Jaboticabal

Benda GTA (1994) Serial hot-water treatment for sugarcane disease control. In: Rao GP, Gillaspie AG Jr, Upadhyaya PP, Bergamin Filho A, Agnihotri VP, Chen CT (eds) Current trends in sugarcane pathology. International Books and Periodicals Supply Service, New Delhi, pp 297–310

Cardoso COM (1986) Isolamento da bactéria do raquitismo (Clavibacter xyli subsp. xyli) no Brasil. Copersucar, Piracicaba; 55p (Boletim Técnico, 34)

Chaves A, Pedrosa EMR, Cavalcante JFD, Araújo CFS, Ferreira GE (2002) Comportamento de variedades comerciais de cana-de-açucar em relação ao raquitismo da soqueira (Leifsonia xyli subsp. xyli) na região Nordeste do Brasil – avaliações em cana-planta. In: Congresso Nacional

Da STAB, 8. 2002. Recife, Resumos...Recife: Sociedade dos Técnicos Açucareiros e Alcooleiros do Brasil, 2002, pp 27–34

Chaves A, Almeida RR, Calsa Junior T (2016) Raquitismo da soqueira: a realidade da ação de um discreto agente nocivo em canaviais brasileiros. In: Gama MAS, Nicoli A, Guimarães LMP, Lopes UP, Michereff SJ (eds) Estudo da Arte em Fitobacterioses Tropicais, 1st edn. EDUFRPE, Recife, pp 275–290

Companhia Nascional De Abastecimento – CONAB (2019) Acompanhamento da Safra Agrícola: Cana-de-açúcar. v. 4, n.1. - Safra 2018/19. Disponível em: file:///C:/Users/Deborah/Downloads/BoletimZCanaZ4ZLevantamentoZ18–19.pdf. Acesso em: 23 Jul 2019

Comstock JC, Lentini RS (2002) Sugarcane mosaic virus disease. Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. SS-AGR-209

Copersucar (1989) Binômio tempo x temperatura no controle do raquitismo da soqueira (RSD) da cana-de-açúcar, pelo processo de termoterapia em gemas isoladas. Cadernos COPERSUCAR 25:1–5

Damann KE Jr, Benda GTA (1983) Evaluation of commercial heat-treatment methods for control of ratoon stunting disease of sugarcane. Plant Dis 67:966–967

Davis MJ, Bailey RA (2000) Ratoon stunting. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (eds) A guide to sugarcane diseases. Centre de Cooperation International en RechercheAgronomique Pour Le Development (CIRAD) and International Society of Sugar Cane Technologists (ISSCT), Montpellier, pp 49–54

Davis MJ, Purcel AH, Thomson SV (1980) Isolation media for the Pierce's disease bacterium. Phytopathology 70:425–429

Grisham MP (2004) Ratoon stunting disease. In: Rao GP, Saumtally AS, Rott P (eds) Sugarcane pathology. Bacterial and nematodes diseases. Science Publishers, Enfield, NH, pp 77–96

Haapalainen M, Mattinen J, Metzler MC (2000) The growth of a plant-parasitic bacterium, *Clavibacter xyli* subsp. *cynodontis*, is enhanced by xylem fluid components. Physiol Mol Plant Pathol 56:147–155

Landell MGA, Campana MP, Figueiredo P (1990) Sistema de multiplicação de cana-de-açúcar com uso de mudas pré-brotadas (MPB), oriundas de gemas individualizadas. Documento IAC, n. 109. Campinas: Instituto Agronômico, 2012.16p

Porto ACF, Pedrosa EMR, Guimaraes LMP, Maranhao SRVL, Cardoso MSO (2018) Management of plant-parasitic nematodes on sugarcane under tropical conditions. In: Priyanka S, Tiwari AK (Org) Sustainable sugarcane production, vol 1, 1st ed. Apple Academic Press, Oakville, pp 271–286

Rossetto R, Santiago AD (2007) Cana-de-açúcar: doenças. Disponível em: http://www.agencia.cnptia.embrapa.br/gestor/cana-de-acucar/arvore/CONTAG01_55_711200516718.html. Acesso em: 25 abr 2016

Sanguino A (2008) Tecnologias na Agroindustria Canavieira. In: II Simpósio do Setor Sucroalcooleiro de Jaboticabal, 2008, Jaboticabal-SP. As Principais doenças da cana-de-açúcar, vol 1. FCAV-Unesp, Jaboticabal-SP, pp 75–86

Teakle DS (1983) The sugarcane ratoon stunting disease bacterium. In: Fahy, P. C.; Persley, G.J. (Eds.). Plant bacterial diseases: a diagnostic guide. Academic Press, North Ryde, cap 11, pp 247–257

Teakle DS, Smith PM, Steindl DRL (1973) Association of a small coryneform bacterium with the ratoon stunting disease of sugarcane. Aust J Agric Res 24:869–874

Tokeshi H (1997) Doenças da cana-de-açúcar. In: Kimati H et al (eds) Manual de fitopatologia: doenças das plantas cultivadas, vol 2. Agronômica Ceres, São Paulo, pp 207–225

Tokeshi H, Rago A (2005) Doenças da cana-de-açúcar. In: Kimati H, Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA (eds) Manual de Fitopatologia- Doenças das plantas cultivadas, 4th edn. Agronômica Ceres, São Paulo, pp 185–196

Improvement of Seed Quality: A Biotechnological Approach

26

Indrani Baruah and Geetanjali Baruah

Abstract

Agriculture is the key to a stable civilization, and it applies to the whole world. The most important factor for stable agricultural productivity is the delivery of healthy and shockproof seeds. Seeds are encapsulated form of gene pools as well as an integral part of the basis for plant life on earth. As there is a vivid change in the environmental conditions and change in farming procedures, the seeds also need to be competent enough to be able to withstand the harsh alterations in the environment and perform well with better production level. Therefore, there is an urgent need for reviewing the measures taken to uplift the production level by enhancing the seed quality and productivity. In this chapter, we have taken into consideration the role of application of different tools of agricultural biotechnology in augmentation of seed quality and prospects in the agricultural scenario.

Keywords

Seed quality · Agricultural biotechnology · Priming · Artificial seeds · High vigour

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26.1 Introduction and Background

India holds the second position after China in population count in the world map with 1.37 billion in 2019 as per United Nations Population Fund report (https://www.unfpa.org/). India's population rose at an average annual rate of 1.2% between the years 2010 and 2019, which is why it is expected that India would beat China in population by 2028 (http://worldpopulationreview.com/countries/india/). As a result, India faces the utmost burden of better production rate in agricultural sectors with limited resources due to its higher food demand and limited area of cultivation. Therefore, it is very essential to increase the production rate with available land areas. It is very challenging to achieve higher and constant production rate every year due to soil fertility loss, pathogen attack as well as insect infestation and side effects caused by the application of chemical fertilizers.

The development of biotechnology has brought a new golden era to the progress of scientific and agricultural productivity. There are several genetic, physiological or biochemical defence pathways in plants which are vital for them to survive under various stress conditions. Each pathway is linked to various important genes, mRNAs, proteins and transcription factors. These factors act in different ways such as inducer, suppressor, enhancer or signalling molecule to confer defence to the plant. To date several biotechnological tools have been employed to improve seed quality, which is pioneer to crop productivity. Seed quality improvement through genetic manipulation is quite challenging because of the host complexity and the diversity of the nature. Certain tissue culture approaches such as micropropagation, cybridization, anther culture, induced male sterility and molecular markers have helped in the initialization of quality improvement. To enhance the seed vigour, such approaches have played a very crucial role. After improvement of seed quality, there is a big challenge to store these seeds for prolonged period without interfering its nutrition level, quality and viability. Breakthrough genetic engineering approaches such as transcription activator-like effector nucleases (TALEN), zinc-finger nucleases (ZFN), clustered regularly interspaced short palindromic repeat/CRISPR-associated protein (CRISPR/Cas9) and CRISPR from *Prevotella* and *Francisella* 1 (CRISPR/Cpf1) are very much essential for viability and stability of the seed quality over the generations. Due to genomic complexity of several crops and other environmental factors, only a few successes have been achieved in the agricultural scenario. Therefore, research studies need to be carried out in model plant systems such as *Arabidopsis thaliana*, *Nicotiana benthamiana* and *Solanum lycopersicum* for basic understanding of seed improvement through biotechnological tools. Along with further improvisation of these genome-editing technologies, superior-quality seeds can be achieved in great quantity, and the conservation of improved variety of germ plasm would also be possible. All the defensive and metabolic pathways in plants include cascade of gene signalling. To date several important genes have been revealed, which play critical role in disease resistance, seed size, oil content, high yield, early fruiting and biotic and abiotic stress tolerance. The recent report says seeds of various plant species such as *Raphanus sativus*, *Vigna unguiculata* (cowpea), *Phytolacca americana and*

Mirabilis jalapa contain several molecules that secrete various proteins such as β-1,3-glucanases and casein-rich antifungal proteins (AFPs) to confer pathogen resistance (Terras et al. 1995; De Bolle et al. 1995; Rose et al. 2006). Certain proteins such as hybrid proline-rich proteins, dehydrins, methylglyoxalases, ethylene response factors and transcription factor genes; DEAD-box RNA helicases as positive and negative regulators of stress signalling pathway; stress proteins including aquaporins, molecular chaperons, heat shock proteins, proline and ABA; and proteins that are responsible for antifungal secretion, seed viability, enhancement of oil content and antioxidant content are the current research targets for the development of high yield containing stress and disease resistance crop seeds (Baruah et al. 2017; Chikkaputtaiah et al. 2017; Marwein et al. 2019; Raviv et al. 2017). Persistent progress in biotechnological approaches would certainly develop new generations of sustainable seeds with high yield productivity, prolonged storage viability and stress and disease resistance.

Agricultural biotechnology is in its nascency especially in India, yet it holds the power to hold remarkable growth in less time. Therefore, it has the potential to deliver new prospects for accomplishing boosted crop production to overcome insufficiency, recover from food security issues and better nutrition and uphold sustainable usage of available natural resources (Srinivas et al. 2017).

26.2 Tissue Culture Approaches in the Development of Seed Quality

26.2.1 Micropropagation

Micropropagation is the preliminary and widely accepted tissue culture approach in seed modification. Micropropagation basically involves four steps including selection of mother plant, multiplication, acclimatization and final transplantation into soil. Tissue culture has contributed numerous ways to preserve the germ plasm and storage of seeds for long period of time (Sugandh 2017). In vitro conservation through tissue culture approach is an alternative way to seed storage. Slow growth and cryopreservation are the two major categories for in vitro seed storage. Slow growth involves reduction of chemicals or lowering of temperature with subculture at regular intervals, whereas in cryopreservation growth of the plant is suspended by preserving it in ultralow temperature generally in liquid nitrogen (−196 °C). For long-term storage of fruits, the shelf life should be maintained. Seedless fruits are better and have long shelf life for storage. Parthenocarpy is the process in which the fruits are developed from the ovary without any pollination or fertilization of the ovule. Such fruits are seedless and of better quality. Modern biotechnology has introduced many technical strategies to develop seedless fruits.

Tissue culture approaches used for the improvement of seed quality are discussed below.

26.2.2 Hybridization and Cybridization

For crop improvement hybridization is the most opportune way. Through hybridization technology the vigorous crop variety can be hybridized with the poor-quality variety to improve the seed quality of the desired crop plant. Sexual hybridization involves nuclear fusion of both parental cells. In this case, genetic fusion of two parent cells gives rise to a vigorous healthy hybrid variety. However, in somatic hybridization, the cytoplasm of two parent cells is fused together. Generally, protoplast fusion tissue culture method is used for this purpose. One kind of somatic hybridization is known as cybridization. Cybridization involves fusion of nucleus of one cell with the extranuclear genome of the other parent cell. Such approaches are time-consuming and complex, but cybridization has helped in successfully developing some high-quality seed products.

26.2.3 Somatic Embryogenesis

Somatic embryogenesis has been considered as a very important tissue culture approach in the development of improved quality plant without sexual fertilization. For large-scale multiplication of seed production, the seedlings are regenerated from the explants or callus without sexual fertilization of the zygotic embryo (Suman and Kumar 2016). However, studies have shown that regeneration of seeds from the primary somatic embryo is more efficient and uniform than that of callus tissues (Maheswaran and Williams 1984). This approach can be initiated through embryogenesis of zygotic culture or explants of shoots and leaf. Once the embryo is established, multiplication of the mature somatic embryo is performed to generate high-quality seedling and seed production. Somatic embryogenesis has been reported to play essential role in the development of various major crops and ornamental plants. The recent report says that somatic embryogenesis technology has been successfully applied in buckwheat, such as *Fagopyrum esculentum* and *F. cymosum*, and grapevines (Woo and Kim 2016). Rapid vegetative propagation can be achieved by raising secondary somatic embryogenesis. Secondary embryogenesis occurs when the primary somatic embryo undergoes successive embryo cycles (Kumar and Loh 2012). In case of genetic transformation, clonal propagation and mutant induction, secondary embryos take the lead for seedling regeneration.

26.2.4 Artificial Seed Development

Somatic embryogenesis alone is not enough for the development of some elite agronomical and horticultural crops. Seed heterozygosity, endosperm deficiency, tiny seed size and lack of mycorrhizal fungi association with seed for germination such as orchid hamper successful seed propagation. Artificial seed concept has arisen as a powerful tool to recover all such difficulties. Murashige et al. (1978)

for the first time have given the idea of artificial seed formation. By taking this concept, the artificial seed development started initially by surrounding the somatic embryo with protective jelly. For a large-scale production in the commercial field, the somatic embryo is encased in a sterile coating of agarose, alginate-chitosan, alginate-gelatin or calcium alginate. Somatic embryo has been considered as a prerequisite of artificial seed. Considering not only the somatic embryo but also the encapsulation of non-endospermic seed and protocorms of essential plant species has brought progress in artificial seed development (Kumar and Loh 2012). Artificial seed development extends to simulate endosperm and seed coat surrounding the somatic embryo and protocorms of rooted shoot tip of desired plant species (Kumar and Loh 2012). The rapid delivery of in vitro-derived propagules into the germination field has reduced the labour cost and has taken a lead in clonal propagation approach.

26.2.5 Embryo Rescue

Some of the plant species are unable to germinate from seeds in spite of the fact that they produce seeds abundantly. Some of such examples are grapes, tomato, citrus and orchids. The cause could be many, such as absence of food reserve in the seed, low survival under natural conditions, etc. To overcome such hurdles in mass propagation of these plants, a tissue culture technique, i.e. embryo rescue, has come into the play. Embryos excised from the developing seed at or near maturity stage are fully autotrophic. These embryos could be germinated and grown on a simple inorganic medium by adding an energy source. Defining an optimum culture medium supporting its normal growth and development is the most important part in embryo rescue (Hu and Zanettini 1995).

26.3 Genome-Editing Approaches in the Development of Seed Quality

Genetic engineering has taken a great leap forward to the advanced technology in seed improvement and crop yield. It has opened numerous possibilities to counteract the critical situations of seed quality deprivation. Advanced gene-editing approaches can target the specific sequences of such essential genes to improve the quality of seeds. The sequence-specific gene-editing approaches include zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 or CRISPR from *Prevotella* and *Francisella* 1. This preferred nuclease gene-editing technology is engineered with two domains, i.e. binding domain and nuclease domain (Khan et al. 2017). The binding domain binds specifically to the target sequence of the double-strand DNA, and nuclease domain creates double-strand breaks (DSBs) by acting in pair. The purpose of creating DSBs in this technology is to do further desired editing such as deletion, addition or other modification of the

Table 26.1 Biotechnology tools targeting crops for developing shockproof varieties

Plant	Biotechnology tool	Target	Reference
Arabidopsis	TALEN	*ADH1, TT4, MAPKKK1, DSK2B, NATA2, GLL22a, GLL22b*	Cermak et al. (2011), Christian et al. (2013)
	ZFN	*ADH1, TT4*	Zhang et al. (2010)
	TALEN	*CLV3*	Forner et al. (2015)
Tomato	TALEN	*ANT1*	Cermak et al. (2015)
	CRISPR/Cas9	*SHR, SCR, lncRNA1459, SP5G*	Brooks et al. (2014), Li et al. (2018), Ron et al. (2014), Soyk et al. (2017)
Rice	CRISPR/Cas9	*OsERF922, GW2, GW5 and TGW6. OsPDS, OsBADH2 and OsMPK2*	Shan et al. (2013), Wang et al. (2016), Xu et al. (2016)
	TALEN	*OsMST8, OsMST7, OsEPSPS*	Zhang et al. (2015), Wang et al. (2015)
	TALEN	*DEP1, BADH2, CKX2, SD1*	Shan et al. (2013), Shan et al. (2015)
Barley	TALEN	*HvPAPhy_a*	Wendt et al. (2013)
Wheat	CRISPR/Cas9	*TaMLO, GASR7*	Wang et al. (2014), Zhang et al. (2016a, b)
	TALEN	*MLO*	Wang et al. (2014)
Zea mays	ZFN	*IPK1*	Shukla et al. (2010)
	TALEN	*Glossy2* locus	Char et al. (2015)
	CRISPR/Cas9	*ALS*	Svitashev et al. (2015)
Soybean	ZFN	*DCL4a* and *DCL4b*	Townsend et al. (2009)
	TALEN	*FAD2-1A, FAD2-1B*	Haun et al. (2014)
	CRISPR/Cas9	*ALS*	Li et al. (2015)
Potato	TALEN	*Vlnv*	Clasen et al. (2015)
Nicotiana benthamiana	TALEN	*FucT, XylT*	Li et al. (2016)
	CRISPR/Cas9	Regions in the viral genome	Baltes et al. (2015), Ji et al. (2015)
Citrus	CRISPR/Cas9	*CsLOB1*	Jia et al. (2017), Peng et al. (2017)
Camelina sativa	CRISPR/Cas9	*FAD2*	Morineau et al. (2016), Jiang et al. (2017)

target site. These breaks are repaired through either non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Malzahn et al. 2017). NHEJ leads to a frameshift mutation, usually creating knockouts, and HDR is useful in creating gene stacking, gene replacement and fusion proteins (Zhang et al. 2017). Seed storage and germ plasm conservation is also a very essential part of seed preservation and modification. Breakthrough genetic engineering technology has increased the shelf life and modified the seed quality of diverse species of crop seeds (Table 26.1). Bt (*Bacillus thuringiensis*) chickpea, Bt cotton, soybean, castor bean, *Jatropha curcas*

and peanut are few examples of targeting various genes for pest resistance, storage viability and increasing its shelf life and oil content (Meitei et al. 2018; Long et al. 2018; Villanueva-Mejia and Alvarez 2017).

26.4 Zinc-Finger Nucleases

Zinc-finger nucleases (ZFNs) are the combination of zinc-finger domain having DNA-binding specificity and nuclease with DNA-cleavage domain (FoK1) (Zhang et al. 2018). Each finger of 4–6 zinc-finger protein domains recognizes and hence binds to 3 bp of DNA (Petolino 2015). Designing of ZFNs is complex and quite challenging as it is time-consuming. Moreover, the success rate in using ZFNs is relatively low because of its poor target specificity, large number of off-target cleavages and labour-intensive procedure (Jaganathan et al. 2018). ZFNs have been widely used in various plant species including *Arabidopsis*, rice, apple, tobacco, maize and soybean to develop high yield generating seeds (Zhang et al. 2010, 2018). ZFNs induce site-specific mutagenesis or base substitution, resulting in alteration of the gene expression, and preferably generate knockout (Urnov et al. 2010). Insertional disruption of IPK1 locus in maize plant has shown herbicide tolerance and alteration of inositol phosphate profile in developing seeds (Shukla et al. 2010). ZFN targeting of two paralogous *DICER-LIKE (DCL4b)* genes, *DCL4a* and *DCL4b*, has shown heritable mutagenesis in soybean seeds (Petolino 2015).

26.5 Transcription Activator-Like Effector Nucleases

Transcription activator-like effector nuclease (TALEN) genetic tool is derived from a phytopathogenic bacterium *Xanthomonas oryzae*, which produces transcription activator-like effectors (TALEs) (Doyle et al. 2013). TALE proteins have a unique feature of the central DNA-binding domain composed of 6 to 33 repeats, which are made of 34 amino acids (Boch and Bonas 2010). TALEs bind to the promoter region of the target DNA and activate gene expression (Sedeek et al. 2019). The fusion of TALE protein with nuclease produces an enzyme named TALEN, which can bind to the target-specific DNA sequences, and further, manipulation can be achieved (Mahfouz et al. 2011). Alteration in number and type of repeats in TALEs gives the flexibility to bind to any DNA strand (Li et al. 2012). TALEN has been used in a variety of major crops to improve its trait quality. Shan et al. (2015) have produced improved rice seeds with fragrance by knocking out OsBADH2 gene through TALEN. Similarly, storage tolerance trait enhancement has been done in rice seeds by targeting *lipoxygenase LOX3 gene* (Ma et al. 2015). TALEN has been successfully applied in rice, soybean, *Arabidopsis*, tobacco, wheat, barley and tomato, targeting various genes to improve seed quality (Forner et al. 2015; Du et al. 2016; Li et al. 2016; Lor et al. 2014; Wang et al. 2014; Wendt et al. 2013; Zhang et al. 2016a, b). TALEN mediates site-directed mutagenesis in the target site (Khan et al. 2017). The essential genes in seed development could be targeted

through mutagenesis. Improvement of cold storage and processing traits has also been targeted in potato (Clasen et al. 2016). TALEN-directed mutagenesis of fatty acid desaturase 2 (FAD2) promotes the oleic acid content in peanut (Wen et al. 2018).

26.6 Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-Associated Protein

Clustered regularly interspaced short palindromic repeat/CRISPR-associated protein (CRISPR/Cas9) is a breakthrough technology which can create precise site-specific DSBs and can target multiple sites simultaneously. In this era, CRISPR/Cas9 has taken over other genome-editing tools because of its efficiency, simplicity, rapid methodology and cost-effective characteristics. CRISPR/Cas9 was first developed in 2012 from the concept of adaptive immune response of bacterial cells against invading phages (Jinek et al. 2012). CRISPR was first observed in *Escherichia coli* as tandem repeats flanked by non-repetitive DNA stretches in the downstream of *iap* genes (Ishino et al. 1987). CRISPR system is mainly classified into two major categories, namely Type I and Type II. CRISPR/Cas9, a type II genome-editing technology, cleaves the target site with Cas9 endonuclease from *Streptococcus pyogenes*, which is guided by a single-guide RNA (SgRNA) (Sedeek et al. 2019). CRISPR/Cas9 has been modified to make it compatible to be used in diverse species of plants and animals as prime genome-editing tool. The modified CRISPR/Cas9 is composed of a 20-bp CRISPR RNA (CrRNA), which binds to the complementary sequence of the target DNA, and Cas9 guided by transactivating crRNA (tracrRNA) forms the complex for cleavage (Puchta 2017). Cas9 endonuclease, guided by CrRNA, cleaves the DNA region of 3–4 nucleotides upstream of a three-nucleotide protospacer-adjacent motif (PAM) in downstream of the target DNA sequence (Bortesi and Fischer 2015). The development of CRISPR/Cas9 has made it easier to target the genes responsible for seed vigour. The modified ARGOS8 variant I maize has been developed by using CRISPR/Cas9 that has shown increased grain yield under drought condition and no yield loss under well-watered condition (Shi et al. 2017). Improvised CRISPR/Cas9 technology has been used extensively to target multiple genes in a major crop plant to develop sustainable productivity (Lowder et al. 2015). In rice eight agronomically important multiple genes have been edited using a single binary vector (Shen et al. 2018). The promoter region of rice OsRAV2 has also been targeted by CRISPR/cas9 for its functional role in salt stress tolerance (Duan et al. 2016).

26.7 CRISPR from *Prevotella* and *Francisella* 1

Apart from CRISPR/Cas9, another genome-editing tool has been discovered called CRISPR from *Prevotella* and *Francisella* 1 (CRISPR/Cpf1). CRISPR/Cpf1 is a third-generation genome-editing tool, which is categorized under type II, subtype

V CRISPR technology. This technology is composed of Cas12a enzyme also known as Cpf1, which creates DSBs with staggered ends and requires a T-rich (5′-TTTN-3′ or 5′-TTN-3′) PAM sequence located at the 5′ end of the target sequence (Zetsche et al. 2015). Because of this unique characteristic, CRISPR/Cpf1 has been accepted for gene editing in thymine-rich genome sequences of plants (Endo et al. 2016). CRISPR/Cpf1 has opened the path to target the sequences of thymine-rich crops such as rice and soybean and even in model plants such as *Nicotiana benthamiana* for seed quality improvement (Zhang et al. 2018). FnCpf1 from *Francisella novicida*, AsCpf1 from *Acidaminococcus* sp. and LbCpf1 from *Lachnospiraceae* bacterium are the three modified CRISPR/cpf1 systems developed recently (Zhang et al. 2017). These three systems have been widely utilized in diverse plant species including *Arabidopsis*, rice, soybean and tobacco (Endo et al. 2016; Kim et al. 2017; Tang et al. 2017; Wang et al. 2017; Xu et al. 2017).

26.8 Enhancement of Seed Vigour

The importance of seeds could be conferred by the fact that three crop species (wheat, rice and maize) are responsible for more than 50% of total calories consumed by the worldwide community (Macovei et al. 2012). A crucial factor on which the performance of crop seeds depends is seed vigour. An approach to deal with the loss of vigour due to factors such as pathogen attack and harsh environment and for better survival in the field is bio-priming of the seeds. Seed bio-priming is a process of coating the seeds with beneficial microbes. But, as seed vigour is an intertwined physiological trait integrating genetic makeup, metabolic pathways, and hormonal signalling pathways (Rajjou et al. 2012). A promising way to enhance seed vigour involves improving the accumulation of protein L-isoaspartyl methyltransferase (PIMT) in seeds, which is an enzyme known for recognizing and catalysing the repair of damaged L-isoaspartyl and D-aspartyl groups in proteins (Ryttersgaard et al. 2002). Yet, no precise proteins have been allotted to the known seed vigour-associated QTLs (quantitative trait loci). Efforts in increasing vigour is expected to be successful with rigorous study revealing genetic base contributing to higher vigour and its applications in agriculture (Wu et al. 2017).

26.9 Conservation of Germ Plasm Through Seeds

Seeds play a key role in germ plasm conservation. Seed banking could preserve genetic material and empower agricultural modernization (Peres 2016). This account of crop genetic conservation therefore shows how breeders and geneticists sought to create their own seed archives from whence the evolutionary history of crops could be made accessible in ways that are useful for the future. The importance of germ plasm conservation is well known. Earth has a diverse biodiversity all around, and we are responsible for its conservation. Climate change and habitat destruction are two of the greatest threats to global biodiversity (Travis 2003). The powerful

combination of elite genetic makeup and cutting edge biotechnological tools is well known. To date many such gene pools have been lost due to various reasons such as anthropogenic activities including mining operations, deforestation, industrialization, pollution and climate change causing an irreversible loss (Bharali and Khan 2011; Sundström et al. 2014). Therefore, conservation of the existing biodiversity in the form of seeds is a key to conserve the unique gene pools for future generations.

26.10 Conclusion and Future Perspectives

A seed is a primary and salient contribution in promoting sustainable agriculture. There is a tremendous pressure under the seed production sector as it has multidimensional applicability such as food grain production, starting material for multiple crops and suitability for germ plasm conservation. Also, there is a gradual increase of new threats in the form of new diseases, stronger pests and drastic climatic events, which are affecting the demand for healthy seeds. Agricultural biotechnology is therefore seen as an emerging field in creating solutions for the production of healthy seeds with enhanced vigour and high reproducibility. However, there is a sense of scepticism among common people towards the involvement of biotechnology in the food business due to safety concerns and lack of scientific explanations. Still, a major section of the society has started to believe in solutions driven by biotechnological input for combating the complex problems of today's world (Table 26.2 and Fig. 26.1). This support is evident from the request from Nobel laureates led by Richard Roberts, who won the Nobel in Physiology or Medicine in 1993, stating that by implementing genetic modification of plants, increase in yield, tolerance to pests and nutrients could be achieved. He also said that traditionally bred plants are likely to be more dangerous.

In June, 2019, farmers from Maharashtra have come up for the support of the banned GM crops herbicide-tolerant Bt cotton and Bt Brinjal seeds. Although GM cotton is allowed to cultivate in India, this variant is not. This protest has been termed by the national media as the first farmer stir for the support of the GM crops. It is the prime duty of the scientific community as well as the government sectors to work hand in hand to deliver the high-quality seeds to the peasants of the county with the ability to fight adversities thrown by climatic changes, population growth, new plant diseases and/or insect pests to establish a stable economic status. Government bodies should be formed for regular monitoring of the seeds and seed products resulted from biotechnological intervention and the harm to nature and its components if any.

As per the report of the National Academies of Sciences, Engineering and Medicine (2017), the emerging trends and new products in the field of biotechnology would involve DNA sequencing, editing and synthesis. It is predicted due to the exponential price drop in sequencing and synthesis techniques throughout the last decade. Also, lucrative invention such as nanopore sequencing is making the sequencing of genomes and synthesis of artificial constructs very cheap. Likewise, advances in the RNA interference (RNAi) and CRISPR/Cas9 technologies are to dominate the field of agricultural biotechnology for the enhancement of nutritional

Table 26.2 Gradual increase in land area implementing agricultural biotechnology tools

Sl No.	Year	Hectares (million)
1	1996	1.7
2	1997	11
3	1998	27.8
4	1999	39.9
5	2000	4.2
6	2001	56.6
7	2002	58.7
8	2003	67.7
9	2004	81
10	2005	90
11	2006	102
12	2007	114.3
13	2008	125
14	2009	134
15	2010	148
16	2011	160
17	2012	170.3
18	2013	175.2
19	2014	181.5
20	2015	179.7
21	2016	185.1
22	2017	189.8

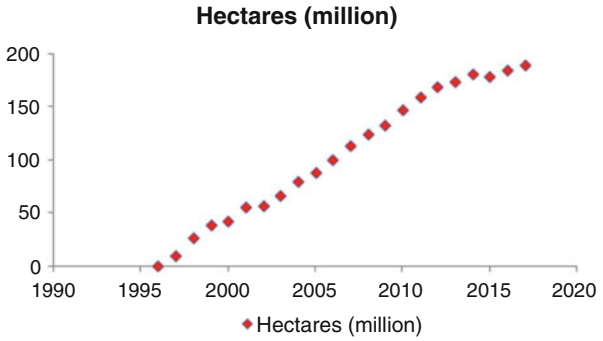

Fig. 26.1 Plot showing land area (hectares) in *x*-axis vs year in *y*-axis from Table 26.2 data

qualities of seeds as well as insect-pest control in agricultural crops. Combination of genome-editing tools such as ZFN, TALEN, CRISPR/Cas9 and CRISPR/Cpf1 with tissue culture approaches would pave the path of detailed molecular understanding of the seed development mechanism under adverse condition. It would translate the fundamental concept into major crops such as rice, wheat, tomato, barley and maize to generate vigorous, viable and sustainable high-quality seeds. These groundbreaking biotechnological approaches help in forward and reverse functional genetic

analysis in research to improve crop yield by developing sustainable vigorous seeds even under adverse condition.

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References

Baltes NJ, Hummel AW, Konecna E, Cegan R, Bruns AN, Bisaro DM, Voytas DF (2015) Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system. Nature 1:15145

Baruah I, Debbarma J, DekaBoruah HP, Keshavaiah C (2017) The DEAD-box RNA helicases and multiple abiotic stresses in plants: a systematic review of recent advances and challenges. Plant Omics 10:252–262

Bharali S, Khan ML (2011) Climate change and its impact on biodiversity; some management options for mitigation in Arunachal Pradesh. General Article Curr Sci 101:7

Boch J, Bonas U (2010) Xanthomonas AvrBs3 family-type III effectors: discovery and function. Annu Rev Phytopathol 48:419–436

Bortesi L, Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. Biotech Adv 33:41–52

Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014) Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiol 166:1292–1297

Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res 39(12):e82

Cermak T, Baltes NJ, Cegan R, Zhang Y, Voytas DF (2015) High-frequency, precise modification of the tomato genome. Genome Biol 16(1):232

Char SN, Unger-Wallace E, Frame B et al (2015) Heritable site-specific mutagenesis using TALENs in maize. Plant Biotechnol J 13:1002–1010

Chikkaputtaiah C, Debbarma J, Baruah I, Havlickova L, DekaBoruah HP, Curn V (2017) Molecular genetics and functional genomics of abiotic stress-responsive genes in oilseed rape (Brassica napus L.): a review of recent advances and future. Plant Biotechnol Rep 11:365–384

Christian M, Qi Y, Zhang Y, Voytas DF (2013) Targeted mutagenesis of *Arabidopsis thaliana*using engineered TAL effector nucleases. G3 (Bethesda) 3(10):1697–1705

Clasen BM, Stoddard TJ, Luo S et al (2015) Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnol J 14(1):169–176. https://doi.org/10.1111/12370

Clasen BM, Stoddard TJ, Luo S, Demorest ZL, Li J, Cedrone F, Tibebu R, Davison S, Ray EE, Daulhac A et al (2016) Improving cold storage and processing traits in potato through targeted gene knockout. Plant Biotechnol J 14(1):169–176

De Bolle MF, Eggermont K, Duncan RE, Osborn RW, Terras FR, Broekaert WF (1995) Cloning and characterization of two cDNA clones encoding seed-specific antimicrobial peptides from *Mirabilis jalapa* L. Plant MolBiol 28:713–721

Doyle EL, Stoddard BL, Voytas DF, Bogdanove AJ (2013) TAL effectors: highly adaptable phytobacterial virulence factors and readily engineered DNA-targeting proteins. Trends Cell Biol 23:390–398

Du H, Zeng X, Zhao M, Cui X, Wang Q, Yang H, Cheng H, Yu D (2016) Efficient targeted mutagenesis in soybean by TALENs and CRISPR/Cas9. J Biotechnol 217:90–97

Duan YB, Li J, Qin RY et al (2016) Identification of a regulatory element responsible for salt induction of rice OsRAV2 through ex situ and in situ promoter analysis. Plant Mol Biol 90(1–2):49–62

Endo A, Masafumi M, Kaya H, Toki S (2016) Efficient targeted mutagenesis of rice and tobacco genomes using Cpf1 from Francisellanovicida. Sci Rep 6:38169

Forner J, Pfeiffer A, Langenecker T, Manavella P, Lohmann JU (2015) Germline-transmitted genome editing in Arabidopsis thaliana using TAL-effector-nucleases. PLoS One 10:e0121056

Haun W, Coffman A, Clasen BM et al (2014) Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. Plant Biotechnol J 2:934–940

Hu CY, Zanettini MHB (1995) Embryo culture and embryo rescue for wide cross hybrids. In: Gamborg OL, Phillips GC (eds) Plant cell, tissue and organ culture, Springer lab manual. Springer, Berlin, Heidelberg

Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A (1987) Nucleotide sequence of the IAP gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. J Bacteriol 169:5429–5433

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan H, Venkataraman G (2018) CRISPR for Crop Improvement: An Update Review. Front. Plant Sci. https://doi.org/10.3389/fpls.2018.00985

Ji X, Zhang H, Zhang Y, Wang Y, Gao C (2015) Establishing a CRISPR-Cas-like immune system conferring DNA virus resistance in plants. Nat Plants 1:15144

Jia H, Zhang Y, Orbovic V, Xu J, White F, Jones J, Wang N (2017) Genome editing of the disease susceptibility gene CsLOB1 in citrus confers resistance to citrus canker. Plant Biotechnol J 15:817–823

Jiang WZ, Henry IM, Lynagh PG, Comai L, Cahoon EB, Weeks DP (2017) Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing. Plant Biotechnol J 15:648–657

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012) A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science 337:816–821

Khan Z, Khan SH, Mubarik MS, Sadia B, Ahmad A (2017) Use of TALEs and TALEN technology for genetic improvement of plants. Plant Mol Biol Rep 35(1):1–19

Kim H, Kim SZ, Ryu J, Kang BC, Kim JS, Kim SG (2017) CRISPR/Cpf1-mediated DNA-free plant genome editing. Nat Commun 8:14406

Kumar PP, Loh CS (2012) Plant tissue culture for biotechnology. Plant Biotechnology and Agriculture. pp 131–138. ScholarBank@NUS Repository. https://doi.org/10.1016/B978-0-12-381466-1.00009-2

Li L, Piatek MJ, Atef A, Piatek A, Wibowo A, Fang X et al (2012) Rapid and highly efficient construction of TALE-based transcriptional regulators and nucleases for genome modification. Plant Mol Biol 78:407–416

Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, Ward RT, Clifton E, Falco SC, Cigan M (2015) Cas9-guide RNA directed genome editing in soybean. Plant Physiol 169:960–970

Li J, Stoddard TJ, Demorest ZL, Lavoie PO, Luo S, Clasen BM, Cedrone F, Ray EE, Coffman AP, Daulhac A et al (2016) Multiplexed, targeted gene editing in *Nicotianabenthamiana*for glyco-engineering and monoclonal antibody production. Plant Biotechnol J 14(2):533–542

Li R, Fu D, Zhu B, Luo Y, Zhu H (2018) CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. Plant J 94:513–524

Long L, Guo DD, Gao W, Yang WW, Hou LP, Ma XN, Miao YC, Botella JR, Song CP (2018) Optimization of CRISPR/Cas9 genome editing in cotton by improved sgRNA expression. Plant Meth 14:85

Lor VS, Starker CG, Voytas DF, Weiss D, Olszewski NE (2014) Targeted mutagenesis of the tomato PROCERA gene using TALENs. Plant Physiol 166:1288–1291

Lowder LG, Zhang D, Baltes NJ, Paul JW, Tang X, Zheng X et al (2015) A CRISPR/Cas9 toolbox for multiplexed plant genome editing and transcriptional regulation. Plant Physiol 169:971–985

Ma L, Zhu F, Li Z, Zhang J, Li X, Dong J, Wang T, Yang D (2015) TALEN-based mutagenesis of lipoxygenase LOX3 enhances the storage tolerance of rice (Oryza sativa) seeds. PLoS One 10 (12):e0143877

Macovei A, Gill SS, Tuteja N (2012) microRNAs as promising tools for improving stress tolerance in rice. Plant Signal Behav 7:1296–1301

Maheswaran G, Williams EG (1984) Direct somatic embryoid formation in immature embryos of *Trifoliumrepens*, *T. pretense* and *Medicago sativa*, and rapid clonal propagation of *T. repens*. Ann Bot 54:201–211

Mahfouz MM, Li L, Shamimuzzaman M, Wibowo A, Fang X, Zhu JK (2011) De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks. Proc Natl Acad Sci U S A 108:2623–2628

Malzahn A, Lowder L, Qi Y (2017) Plant genome editing with TALEN and CRISPR. Cell Bio Sci 7:21

Marwein R, Debbarma J, Sarki YN, Baruah I, Saikia B, DekaBoruah HP, Velmurugan N, Chikkaputtaiah C (2019) Genetic engineering/Genome editing approaches to modulate signaling processes in abiotic stress tolerance. In: Plant signalling molecules, 1st edn. Elsevier Publishers, Germany, pp 63–82

Meitei AL, Bhattacharjee M, Dhar S, Chowdhury N, Sharma R, Acharjee S, Sarmah BK (2018) Activity of defense related enzymes and gene expression in pigeon pea (Cajanuscajan) due to feeding of Helicoverpaarmigera larvae. J Plant Interact 13(1):231–238

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogue F, Faure JD (2016) Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelinasativa. Plant Biotechnol J 15:729–739

Murashige T (1978) The impact of plant tissue culture on agriculture. In: Thorpe TA (ed) Frontiers of plant tissue culture. International Association for plant Tissue Cult, Calgary, pp 15–56

National Academies of Sciences, Engineering, and Medicine (2017) Preparing for future products of biotechnology. The National Academies Press, Washington, DC. https://doi.org/10.17226/24605

Peng A, Chen S, Lei T, Xu L, He Y, Wu L, Yao L, Zou X (2017) Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. Plant Biotechnol J 15(12):1509–1519. https://doi.org/10.1111/pbi.12733

Peres S (2016) Saving the gene pool for the future: Seed banks as archives. Stud Hist Philos Biol Biomed Sci 55:96–104

Petolino JF (2015) Genome editing in plants via designed zinc finger nucleases. In Vitro Cell Dev Biol Plant 51(1):1–8

Puchta H (2017) Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. Curr Opin Plant Biol 36:1–8

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C et al (2012) Seed germination and vigor. Annu Rev Plant Biol 63:507–533

Raviv B, Aghajanyan L, Granot G, Makover V, Frenkel O, Gutterman Y, Grafi G (2017) The dead seed coat functions as a long-term storage for active hydrolytic enzymes. PLos One 12(7): e0181102. https://doi.org/10.1371/journal.pone.0181102

Ron M, Kajala K, Pauluzzi G, Wang D, Reynoso MA, Zumstein K et al (2014) Hairy root transformation using *Agrobacterium rhizogenes* as a tool for exploring cell type-specific gene expression and function using tomato as a model. Plant Physiol 166:455–469

Rose TL, Conceicao ADS, Jose XF, Okorokov LA, Fernandes KVS, Marty F et al (2006) Defense proteins from Vignaungaiculata seed exudates: characterization and inhibitory activity against *Fusariumoxysporum*. Plant Soil 286:181–191

Ryttersgaard C, Griffith SC, Sawaya MR, MacLaren DC, Clarke S, Yeates TO (2002) Crystal structure of human L-isoaspartylmethyltransferase. J Biol Chem **277**(12):10642–10646

Sedeek KEM, Mahas A, Mahfouz M (2019) Plant Genome Engineering for Targeted Improvement of Crop Traits. Front Plant Sci 10:114

Shan Q, Wang Y, Chen K, Liang Z, Li J, Zhang Y, Zhang K, Liu J, Voytas DF, Zheng X, Zhang Y, Gao C (2013) Rapid and efficient gene modification in rice and Brachypodium using TALENs. Mol Plant 6(4):1365–1368

Shan Q, Zhang Y, Chen K, Zhang K, Gao C (2015) Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology. Plant Biotechnol J 13(6):791–800

Shen L, Wang C, Fu Y, Wang J, Liu Q, Zhang X et al (2018) QTL editing confers opposing yield performance in different rice varieties. J Integr Plant Biol 60:89–93

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207–216

Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM, Rock JM, Wu YY, Katibah GE, Zhifang G, McCaskill D, Simpson MA, Blakeslee B, Greenwalt SA, Butler HJ, Hinkley SJ, Zhang L, Rebar EJ, Gregory PD, Urnov FD (2010) Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Proc Natl Acad Sci U S A 459(7245):437–441

Soyk S, Lemmon ZH, Oved M, Fisher J, Liberatore KL, Park SJ et al (2017) Bypassing negative epistasis on yield in tomato imposed by a domestication gene. Cell 169:1142–1155

Srinivas J, Ravinder Reddy K, Saidaiah P, Umarajashekar A (2017) Biotechnological Tools to Improve Quality Parameters in Temperate Vegetables. Int J Curr Microbiol App Sci 6 (6):2549–2556

Sugandh S (2017) Plant tissue culture: a promising tool of quality material production with special reference to micropropagation of banana. Biochem Cell Arch 17:1–26

Suman S, Kumar H (2016) Plant regeneration via somatic embryogenesis from the cultured immature male floral buds of banana genotype 'Dwarf Cavendish'. Natl J Life Sci 13(2)

Sundström JF, Albihn A, Boqvist S et al (2014) Future threats to agricultural food production posed by environmental degradation, climate change, and animal and plant diseases – a risk analysis in three economic and climate settings. Food Sec 6:201

Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM (2015) Targeted mutagenesis, precise gene editing and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol 169:931–945

Tang X, Lowder LG, Zhang T, Malzahn AA, Zheng X, Voytas DF, Zhong Z, Chen Y, Ren Q, Li Q (2017) A CRISPR-Cpf1 system for efficient genome editing and transcriptional repression in plants. Nat Plants 3:17018

Terras FR, Eggermont K, Kovaleva V, Raikhel NV, Osborn RW, Kester A et al (1995) Small cysteine-rich antifungal proteins from radish: their role in host defense. Plant Cell 7:573–588

Townsend JA, Wright DA, Winfrey RJ, Fu F, Maeder ML, Joung JK, Voytas DF (2009) High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 459:442–445

Travis JMJ (2003) Climate change and habitat destruction: a deadly anthropogenic cocktail. Proc R Soc London Ser B 270:467–473

Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD (2010) Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11:636–646

Villanueva-Mejia D, Alvarez JC (2017) Genetic improvement of oilseed crops using modern biotechnology. Adv Seed Biol. https://doi.org/10.5772/intechopen.70743

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C et al (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32(9):947–951

Wang X, Wang Y, Wu X et al (2015) Unbiased detection of off-target cleavage by CRISPR-Cas9 and TALENs using integrase-defective lentiviral vectors. Nat Biotechnol 33:175–178

Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Liu YG, Zhao K (2016) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PloS One 11:e0154027

Wang M, Mao Y, Lu Y, Tao X, Zhu JK (2017) Multiplex gene editing in rice using the CRISPR-Cpf1 system. Mol Plant 10:1011–1013

Wen S, Liu H, Li X, Chen X, Hong Y, Li H, Lu Q, Liang X (2018) TALEN-mediated targeted mutagenesis of fatty acid desaturase 2 (FAD2) in peanut *(Arachishypogaea L.)* promotes the accumulation of oleic acid. Plant Mol Biol 97(1–2):177–185

Wendt T, Holm PB, Starker CG, Christian M, Voytas DF, Brinch-Pedersen H, Holme IB (2013) TAL effector nucleases induce mutations at a pre-selected location in the genome of primary barley transformants. Plant Mol Biol 83(3):279–285

Woo SH, Kim HH (2016) Concepts, prospects, and potentiality in buckwheat (Fagopyrum esculentum Moench): a research perspective. In: Zhou MIK, Woo SH, Chrungoo N, Wieslander G (eds) Molecular breeding and nutritional aspects of buckwheat. Academic Press, London, pp 21–49

Wu X, Ning F, Hu X, Wang W (2017) Genetic modification for improving seed vigor is transitioning from model plants to crop plants. Front Plant Sci 8(8):00008. https://doi.org/10.3389/fpls.2017.00008

Xu R, Yang Y, Qin R, Hao L, Qiu C, Li L, Wei P, Yang J (2016) Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. J Genet Genomics 43:529–532

Xu R, Qin R, Li H, Li D, Li L, Wei P, Yang J (2017) Generation of targeted mutant rice using a CRISPR-Cpf1 system. Plant Biotechnol J 15:713–717

Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, Oost J, Regev A, Koonin EV, Zhang F (2015) Cpf1 is a single RNA-guided endonuclease of a Class 2 CRISPRCas system. Cell 163:759–771

Zhang F, Maeder ML, Unger-Wallace E, Hoshaw JP, Reyon D, Christian M, Li X, Pierick CJ, Dobbs D, Peterson T, Joung JK, Voytas DF (2010) High frequency targeted mutagenesis in Arabidopsis thaliana using zinc finger nucleases. Proc Natl Acad Sci U S A 107 (26):12028–12033

Zhang H, Gou F, Zhang J et al (2015) TALEN-mediated targeted mutagenesis produces a large variety of heritable mutations in rice. Plant Biotechnol J 14(1):186–194. https://doi.org/10.1111/12372

Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, Qiu JL, Gao C (2016a) Efficient and transgene free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. Nat Commun 7:12617

Zhang H, Gou F, Zhang J, Liu W, Li Q, Mao Y, Botella JR, Zhu ZK (2016b) TALEN-mediated targeted mutagenesis produces a large variety of heritable mutations in rice. Plant Biotechnol J 14(1):186–194

Zhang H, Zhang J, Lang Z, Botella JR, Zhu JK (2017) Genome editing—principles and applications for functional genomics research and crop improvement. Critical Rev Plant Sci 36(4):291–309

Zhang Y, Massel K, Godwin ID, Gao C (2018) Applications and potential of genome editing in crop improvement. Genome Biol 19:210

Organic Packages for Seed Production

27

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Abstract

Current farming situation incites to develop new farming techniques that are safer to the environment and sustainable from production point of view. Conventional farming seems not to be sustainable in the long run throughout the world. Sustainable farming techniques ensure the resource conservation (land, water, plant and animal genetic resources) for the future generation which is technically appropriate, economically viable and socially acceptable. Environmental concern imparted a way to reinforce the traditional farming practices in the form of organic farming and is the need of the day around the globe. Organic farming practices are easily adaptable by the farming community except for the organic seed as it is not available purely. Hence, organic package of practices is of great significance for the seed production to make it readily available to the needy farmers. This chapter mainly focuses on the organic practices that can be followed for the production of seeds.

Keywords

Conventional farming · Sustainable farming · Traditional farming practices · Organic farming · Organic seed production

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27.1 Introduction

Agriculture chemicals are acting as a source of soil and water contamination which triggers the general people, scientists and policy makers to focus on the issues arising out of the usage at global level. Conventional farming around the world seems not to be sustainable over the period. Hence, the present state of farming seeks for the development of new farming techniques to keep the production in a sustainable manner. Sustainable agriculture refers to the conservation of resources for the future generation that is capable of supplying safe and substantial food aiming to address the issues that affect present-day agriculture. It should be technically applicable, environmentally amenable, economically achievable and socially acceptable. Environmental concerns divulged the importance of organic farming and way for the same around the world which commensurate the ill-effects of conventional farming. So it is essential to comprehend the prospects and problems of organic farming to initiate an achievable outcome and impeccable production in a sustainable organic way (Somasundaram and Udhaya Nandhini 2018a).

The basic need of a mankind includes air, water and light and food, in which food plays a prominent role. Seed is a foundation to all agricultural activity which decides the food production. Food demand of any country is possible only when the seed security is attained. It carries the genetic roadmap that ensures survival, not only for the plant but for the people who will consume it. So, farmer should focus on conserving the seed which is imperative for the production. The concept of organic seed production includes land area selection which is away from conventional farming, organic inputs, organically produced seeds, local landraces, etc.

Organic seeds are seeds that are produced in an organic system preferably that is certified to grow grains, fruits and vegetables organically. Presently organic seed production taken in crops like tomato, brinjal, okra, capsicum, bottle gourd, cucumber beans and cowpea, pumpkin, amaranth, and lettuce in India is mainly done by private company which is highly valuable in global market (Annadana 2010).

27.2 Criteria for Seed Evaluation, Characterization and Multiplication

Farmers should select the seed in such a way that it should adhere to the determined characteristics that decide the yield like colour, texture, acclimatization and adaptation to dynamic climate, pest and disease resistance, nutritious value rich fodders, nitrogen giving plants (Shiva et al. 2004). The quality of a healthy and good seed is comprised of its genetic, physiological, physical and health traits. Pertaining to genetic quality, seed material should be of known origin that is produced in an isolated environment (to prevent intercrossing). When a farmer wants to select his own genetic material, he has to consider the following points in his/her mind:

- Select plants which are having vigorous growth, capable of yielding high, bearing quality fruits (shape, colour and flavour), good health, etc.
- Selected plants should be given with utmost care.
- Isolation should be properly maintained.
- Other plants which are not of selected type should be removed.
- Adjoining plants having disease incidence should be eliminated.
- When the crop attained its optimum maturity, fruits must be picked.
- Seeds should be taken out of the fruits immediately after harvest.
- Storing of seeds depends on the family:
  - Fresh tomato has to be mashed and leaved for 24–48 h for fermentation, to prevent bacterial cancrosis problems depending on ambient temperature. If the seeds get stuck with each other, the lumps should be taken apart by hand. After then the seeds should be dried and should be stored in brown paper bags with mud earth or wood ashes.
  - Grains should be sun dried before storage at low humidity, and before storage, neem oil may be sprinkled over the seeds to keep them free from pests.

Physical quality: Famers must select pure seed which is free from any foreign material. Intensive care should be given for cole crops and carrot to separate the seeds free from weeds as the separation is very difficult. It should include the smallest possible amount of inert material (remains of flowers, fruits, etc.). It should have any mechanical damage and must possess good weight and size (e.g. wild radish seeds are very sensitive, their seed cuticle being very brittle during the seed cleaning process).

Health quality: It can be achieved through growing of crops in soil that is rich in organic matter which facilitates the microbes to release more amounts of nutrients required for crop metabolic activities. Growing crops under healthy soil condition makes the plant to resist pest and diseases.

27.3 Significance of Traditional Varieties (Shiva et al. 2004)

- Farmer collects good seeds from their own field for seed purpose of ensuing season.
- Farmers usually follow the barter system for exchanging the seeds of their own which reduces the seed cost.
- Traditional seeds geared the farmer's economy in a subsistence way as they mainly grow it for food/feed and seed. They will market only the surplus.
- Traditional seeds are embedded of indigenous knowledge.
- Excellent attribute of traditional seeds is diversity.
- Traditional seeds are hardy in nature and resistance to the pests and diseases.
- They have easily acclimatized to changing local weather conditions and have high tolerance to abiotic stresses.

27.4 How the Quality of Organically Produced Seed Can Be Improved?

Training is essential to the farmers or growers who are involved in organic seed production. Training should be given in all aspects of crop improvement, production and protection mainly focusing on cross pollination, seed health and post-harvest operations for seed storage. Seed production should be combined with on-farm variety testing in order to provide as much information for farmers as possible.

27.5 Organic Seed Production Package

Package of practice for organic seed production is entirely different from conventional seed production practices involving usage of agrochemicals. Major inputs which can be used for organic seed production are organic manures, FYM, sheep manure, crop residues, poultry manure, oil cakes and other farm wastes, and compost—coir pith compost. Sunhemp, dhaincha and legumes are some of the green manures used in the organic crop production. Comprehensive package for organic seed production is discussed below (Somasundaram and Udhaya Nandhini 2018b).

27.5.1 Land Selection

Field taken for organic seed production should be organically managed. Organic seed production land has to be chosen in the upper lying area in such a way that to arrest the runoff water contamination from chemical farming system and it should be separated from live fencing or erecting by organically managed crops as buffer zone to avoid the contamination from wind. A minimum of at least 3 m should be maintained between organic and conventional farming as a buffer area. If adequate facilities are available, separate implements can be used for the organic land preparation and other intercultural operations. If not, the equipment or implements used for organic management have to be cleaned thoroughly before using. Proper crop rotation should be followed to reduce the incidence of pest and diseases. Selection of field for organic seed production should be of free from weeds.

27.5.2 Seeds and Planting Material

Organically certified seeds or traditionally grown seeds/planting material should be used. If organic seeds are not available when initiating the conversion, farmers may use untreated seeds that are conventionally grown for the first year, and for the ensuing years, organic seeds must be used. Genetically engineered seeds and plant materials are strictly prohibited in organic seed production.

27.5.3 Planting Techniques

Organically grown seeds are sown directly by making hole in the field or by transplanting. Proper spacing and depth should be followed for seed sowing in such a way that it will support for fruit and seed development by harnessing proper vegetative growth. Thereby, the movement of the pollinators will become easier and let to the proper seed set. Optimum spacing also paves the way for maintaining the microclimate, reducing the pest incidence and easier harvest operations.

27.6 Soil Management

27.6.1 Farmyard Manure (FYM)

Farmyard manure is a mixture of decomposed dung and urine of farm animals along with litter. A pit of desired size is dug, and all the available litters and roughages are mixed with soil and spread over. Then the next day, urine-soaked material along with dung is placed in a pit. When the section is filled up to a height of 45–60 cm above the ground level, the top of the heap is made into a dome and plastered with cow dung earth slurry. The FYM will be ready in 4–5 months after plastering. Generally 12.5 t/ha is recommended for field crops, and in the case of horticultural crops, it is about 20–25 t/ha.

27.6.2 Sheep and Goat Manure

Decomposed droppings of sheep contain higher amount of plant nutrients. Sheep penning also can be practised for improving the soil quality. When a field is put under fallow, wherein sheep and goats are kept overnight in the field and later is incorporated to a shallow depth which adds more nutrients to the soil.

27.6.3 Oil Cakes

Oil cakes are the resultant product after oil is extracted from the seeds or nuts which are of two types:

- Edible oil cakes which can be safely fed to livestock, e.g. groundnut cake, coconut cake, etc.
- Non-edible oil cakes which are not fit for feeding livestock, e.g. castor cake, neem cake, mahua cake, etc.

Both cakes are used as manures. Nutrients present in oil cakes, after mineralization, are made available to crops 7–10 days after application. Oil cakes need to be well powdered before application for even distribution and quicker decomposition.

27.6.4 Biofertilizers

Biofertilizers are the living cells of useful microbes which help crops in numerous ways. It can be applied through seed or soil. When applied in soil, it expedites the microbial activity that magnifies the nutrients in a readily available form to crops. They are economically viable and an alternative renewable source for chemical nutrients. Based on their function, they have been grouped under different categories.

1. Application of Biofertilizers.
   (a) Seed treatment.
   (b) Seedling dip.
   (c) Main field application.
2. Seed Treatment
   Biofertilizer (200 g) is mixed with rice gruel to make into slurry. Seeds for an acre of land are mixed to attain a uniform coating with biofertilizers and dried for 30 min. Treated/coated seeds should be sown within 24 h. One packet of the inoculant (200 g) is sufficient to treat 10 kg of seeds.
3. Seedling root dip
   Biofertilizer packet that weighs 400 g is mixed with 40 l of water. Seedlings required for an acre can be treated in this method. The root portion of the seedling is dipped in the mixture for 5–10 min and can be transplanted.
4. Main field application
   Biofertilizers around 800 g is mixed with 20 kg of well-powdered farmyard manure and can be broadcasted directly in an acre of field before transplanting.

27.6.4.1 Rhizobium

Rhizobium is applied as seed inoculants for all leguminous crops.

Rhizobial strains for various crops:

Groundnut	TNAU 14, SK-1
Soybean	Cos 1
Black gram	PMBS 47, CRU-7
Green gram	GMBS1, Coc 10
Bengal gram	CoBe 13
Red gram	CC 1

Methods to Use

- For 10 kg of seed, one packet of *Rhizobium* (200 g) is sufficient.
- Mix this *Rhizobium* in well fertile soil along with 200 ml of rice gruel (rice kanchi) and mix well.
- Shade dry for 30 min and take up sowing immediately.

27.6.4.2 Azospirillum/Azotobacter

Azospirillum can be inoculated through seed, seedling root dip and soil application methods. *Azospirillum* around 2 kg is mixed with 25 kg of well-decomposed farmyard manure or wet sand and can be broadcasted directly in a hectare of field before transplanting.

Seed Treatment

For 1 acre seeds, two packets of *Azospirillum is required*. Mix well with rice kanji and shade dry for 30 min.

For Transplanted Crop

Four packets of *Azospirillum* should be mixed with well-decomposed FYM and broadcasted over 1 acre.

Seedling Root Tip

Two packets of *Azospirillum* that weighs 400 g are mixed with 40 l of water. Seedlings required for an acre can be treated in this method. The root portion of seedling is dipped in the mixture for 20 min and can be transplanted.

For Trees

For a grown tree, 20 to 50 of *Azospirillum* should be mixed with well-decomposed farmyard manure and applied over the root zone.

27.6.4.3 Phosphobacteria

Like *Azospirillum*, it can be inoculated through seed, seedling root dip and soil application methods. *Azospirillum* around 2 kg and 2 kg of phosphobacteria should be mixed with 25 kg of well-decomposed farmyard manure or wet sand and can be broadcasted directly in a hectare of field before transplanting that helps in increasing the population of these microorganisms in the soil (Table 27.1).

27.6.4.4 Arbuscular Mycorrhiza (AM)

Arbuscular mycorrhiza colonizes almost all crops in all stages and creates resistance to entering pathogens and nematodes in plants. On the other hand, this fungus helps plants to absorb nutrients altering root anatomy, modifying root exudations and root system morphology by which plants get good health as well as less disease incidence.

AM Inoculation

Optimal spore count 60–100 spores/100 g soil.

Rate of Inoculation.

Vegetables	100 g/m^2 nursery
Fruit trees and coconut	100–200 g/tree
Other crops	10% of the seed rate
Established plants	10 g/plant
Nursery	750–1000 g m^2
Nursery poly bag	10 g/poly bag

Table 27.1 Biofertilizer recommendation (one packet—200 g)

Crop	Seed	Nursery	Seedling dip	Main field	Total requirement of packets per ha
Rice	5	10	5	10	30
Sorghum	3	–	–	10	13
Pearl millet	3	–	–	10	13
Ragi	3	–	5	10	18
Maize	3	–	–	10	13
Cotton	3	–	–	10	13
Sunflower	3	–	–	10	13
Castor	3	–	–	10	13
Sugarcane	10	–	–	36 (3 splits)	46
Turmeric	–	–	–	24 (2 splits)	24
Tobacco	1	3	–	10 g/pit	14
Papaya	2	–	–	10	–
Mandarin orange	2	–	–	10 g/pit	–
Tomato	1	–	–	10	14
Banana	–	–	5	10 g/pit	–

27.6.4.5 Algae

Growing of algae in a paddy field contributes for nitrogen and phosphorus required by the crop. *Azolla* has to spread over the field after 10 days of transplanting paddy. After 45 days, the field should be drained and algae should be incorporated in the soil. It decomposes in about 7–10 days and thereby provides nitrogen to the rice crop. For a hectare of land, 7 kg of *Azolla* is required.

27.6.4.6 Root Fungus

- Seedlings—100 g of root fungus is sufficient. Before sowing, it must be incorporated at a depth of 2–3 cm/m^2.
- For poly bag nursery seedlings—10 g/bag is sufficient.
- For a well-grown tree—200 g/tree.

27.7 Plant Growth Promoters

27.7.1 Panchagavya

Fresh cow dung	5 kg
Cow urine	3 l
Cow milk	2 l
Curd	2 l
Cow desi ghee	1 kg

27.7.1.1 Method of Preparation

Fresh cow dung and ghee have to be mixed well with hands and should be kept aside for 3 days. Then add the remaining ingredients and mix thoroughly, and allow these contents to ferment for 15 days. Stir the contents twice daily. Panchagavya at 3% can be used for seed treatment and foliar spray. Twenty litres of panchagavya is required for an acre as soil application along with irrigation water (Table 27.2).

27.7.2 Starter Solution

Cow dung	20 kg
Cow urine	20 l
Water	200 l
Jaggery	4 kg

- All ingredients are mixed together and allowed to ferment for 24 h.
- After that, the same is mixed with irrigation water as soil application at ratio of 1:10 (1 part of starter solution and 10 parts of irrigation water).

Table 27.2 Panchagavya spray schedule for various crops

Crops	:	Time schedule
Rice	:	10, 15, 30 and 50 days after transplanting
Sunflower	:	30, 45 and 60 days after sowing
Black gram	:	Rainfed: first flowering and 15 days after flowering Irrigated: 15, 25 and 40 days after sowing
Green gram	:	15, 25, 30, 40 and 50 days after sowing
Castor	:	30 and 45 days after sowing
Groundnut	:	25 and 30 days after sowing
Bhendi	:	30, 45, 60 and 75 days after sowing
Moringa	:	Before flowering and during pod formation
Tomato	:	Nursery and 40 days after transplanting, seed treatment with 1% for 12 h
Onion	:	30, 45 and 40 days after transplanting
Rose	:	At the time of pruning and budding
Jasmine	:	Bud initiation and setting
Vanilla	:	Dipping setts before planting
Fruit trees		
First time	:	A month before flowering
Second time	:	15 days after flowering
Third time	:	Fruits in pea size
Fourth time	:	After harvest once
Leafy crops		
Greens, curry leaf, tea, coffee	:	2% once weekly

- This solution is to be applied in soil at a frequency of 2–3 times for 3–4-month-old crops and twice in a month for long-duration crops.
- This makes the soil productive.

27.7.3 Cow Urine

- One litre of cow urine is mixed with 10 l of water and keep it in an earthen pot.
- Spray the dilution.

27.7.4 Fruit Solution

- At evening, collect rhizosphere soil of ten well-grown plants from the root, and pour into the plastic container.
- Add 3 kg of papaya fruit, 3 kg pumpkin, 2 desi bird egg and 1/2 kg nattu sakkarai into the container.
- Add water until the container gets filled.
- Close the lid and air tight a container.
- Keep under shade for 20 days.
- Daily just open and close the lid.
- Mix 50 ml of fruit solution with 10 l of water and spray.

27.7.5 Fermented Buttermilk and Coconut Milk Solution

- Fermented buttermilk (5 l) and coconut milk (5 l) are to be mixed in a mud pot preferably.
- Leave the contents for fermentation (7 days).
- Periodic stirring of the contents should be given.
- Mix 1 l of this solution with 10 l of water and spray.

27.8 Weed Management

Weed management in seed production is of great significance because of the two main reasons:

- Weed will compete for space, nutrients and water which may reduce the crop performance resulting in lower yield and seed quality and some cases increases the pest incidence by being a host.
- Weed seeds may contaminate the harvested crop seeds.

27.9 Cultural Practices for Preventing Weed Problems

27.9.1 Planning Steps

27.9.1.1 Know the Weeds

Identify the correct major weeds present on the field. Monitoring the fields regularly throughout the season will give idea about the major weed occurrence. Record should be maintain exclusively for the weeds to know what type of weeds are emerging at different cropping periods and the history of weed control. Knowing each weed and its natural growth, development, seed dispersal and life cycle and the mechanism by how it affects crop growth will help in deciding the controlling strategies. Proper monitoring should be given to find out the stage at which the weed can easily be controlled which will be helpful in designing a management strategy.

27.9.1.2 Design the Cropping System/Crop Rotation

Plan the crop rotation in such a way that it should avert the weed niches by space and in time dimension basis. Proper row spacing, intercropping, relay cropping, overseeding, cover crops into established vegetables or no-till management of cover crops prior to transplanting vegetables should be adopted to reduce the emergence of weeds in wide rows.

Diversified crop rotations should be followed that varies in time with respect to all cultivation aspects. In a perennial crop ecosystem, vegetable crops may be rotated for a period of 3 years to arrest the erection of annual weeds. Tillage and cultivation operations should be scheduled in such a way that they give most damage to the predominant weed species.

27.9.1.3 Planning Tools for Effective Weed Control

Control strategies should be developed to address the issues of anticipated weed occurrence in the farm. Selection of implements and tools for cultivation should be very effective in controlling of weeds between the rows or within row. Optimum row and plant spacing should be given to promote the precise cultivation; likewise irrigation methods and other cultural practices also have to be chosen in such a way that are compatible with planned weed control operations.

27.9.2 Preventive Steps During the Season

27.9.2.1 Growing of Competitive Crops

Fast-growing crop which will compete with weeds has to be established to prevent weeds problems. Varieties of our interest, must grow tall and should form more foliage to cover all open area and shade the weeds make it suffer from need of light. Nutrients and water should be applied within the row to avoid feeding weeds.

Deficiency or excess of nutrients may cause the establishment of newer weeds which has to be optimized through proper nutrient management.

27.9.2.2 Grow Cover Crops

Cover crops are grown mainly to occupy the open spaces under cropped situation. These may be of legumes or green manures which restore the soil with full nutrients, add more biomass to soil which in turn increases the organic carbon level and act as a habitat for beneficial microbes. They are capable of suppressing weeds either through competition or secreting some growth-inhibiting substance the so-called allelopathy. Field should not be put as barren, and immediately after the harvest, cover crop should be sown to arrest the weed seed bank.

27.9.2.3 Manage the Weed Seed Bank

Before flowering or at the time of flowering, weeds within the farm premises should be removed to avoid the seed set. Likewise, proliferation of rhizomes and other propagules of perennial weeds should be arrested through proper tillage mechanisms. As much as possible, farmer should avoid importing manures from off-site which may have some weed seeds, etc. Preferably before the start of sowing, follow stale seed bed technique and targeted tillage to eradicate the seed banks in the soil. Encourage the bicontrol-based insects like ground beetle and other organisms to feed the weed seeds.

27.9.3 Control Steps During the Season

27.9.3.1 Weed Control at Critical Growth Stages

Sowing of crop should be done on a clean field without any weed seeds, and weed-free situation should be maintained till the critical weed-free period is attained.

27.9.3.2 Biological Control

Livestock and geese may be allowed in a field to graze weeds and reduce the seed setting which in turn interrupts the weed cycle. Maintain high biological activity through bicontrol-based insects like ground beetle and other organisms, and provide habitat for them by erecting hedgerows, cover crops and applying mulch to encourage their effectiveness in consuming weed seeds and rhizome-like propagation materials. Commercially available biocontrol agents also can be used to reduce the weeds, but care should be taken in such a way that it should not feed on crops.

27.9.3.3 Rouging

Removal of off-types should be done to maintain the quality in seed production.

27.10 Pest and Disease Management

27.10.1 Preventive Measures

1. Choosing of varieties.
   - Select varieties that are well acclimatized and adapted to local weather conditions which makes the cop to grow healthier and stronger against incidence of disease and pest.
2. Selection of planting material.
   - Seed selection should be free from weed seeds and free from seed borne diseases.
   - Collect planting material from safe sources without any loop for incidence.
3. Cropping systems.
   - Mixed cropping: can create diverse system of beneficial insects and reduce the host population for pest which checks the insect attack.
   - Crop rotation: soil-borne pathogens will be dominated by the activation of beneficial microbes, which reduces the chances for soil-borne disease.
   - Green manuring and cover crops: enhances the soil activity with beneficial microbes.
4. Balanced nutrition.
   - Moderate fertilization: optimum fertilization results in better and steady growth making a plant less vulnerable to disease and pest attack. Excessive fertilization may result in high foliage (insect attack) and root damage paving the way for secondary infections.
   - Balanced supply of potassium contributes to the prevention of fungi and bacterial incidence.
5. Organic matter.
   - Enhances the beneficial organism population in soil and reduces the pathogenic fungi.
   - Stabilizes the soil and supplies the required nutrients properly as needed by the crop that may strengthen the plant's own protection mechanisms.
6. Soil cultivation methods.
   - Regulates the weeds and its seed bank.
   - Expedites the process of decomposition of infected plant parts.
   - Protects the microorganisms which regulate soil-borne diseases.
7. Water management.
   - Proper drainage should be facilitated to avoid water logging which encourages pathogen infections.
   - Avoid the persistence of water droplets on leaves to reduce the spread of fungal spores of water-borne disease.
8. Conservation of natural enemies.
   - Erect live barriers as fence to provide a natural habitat for natural enemies.

- Should not use any products that harm natural enemies.

9. Optimum planting time and spacing.
   - Optimum sowing/planting time should be chosen to avoid the risk of pest attack.
   - Proper distance/spacing should be maintained between the pants to reduce the spread of pathogenic fungal spores.
   - Optimum spacing results in good aeration of the plants allowing leaves to dry off faster, which hinders pathogen development and infection.
10. Sanitation measures.
    - Removal of infected parts or the plant entirely from soil is essential to prevent the spreading of disease.
    - Burn the infected plants after being removed.

27.10.2 Trap Crop

Crops rise at the border or in between the rows to attract insects and nematodes in order to provide protection. This can be achieved by:

- Preventing the insect pests from reaching the crop.
- Making them to concentrate on a certain part of the field where they can be destroyed.

27.10.3 Neem Leaf Extract

- 1 kg of green neem leaf in 5 l of water is soaked overnight.
- Then the next day, the mixture has to be crushed for extraction which is filtered.
- Add 10 ml of emulsifier (neutral pH adjuvant).

27.10.4 Neem Cake Extract

- Neem cake (100 g) is soaked overnight in 1 l of water.
- Then the next day, the mixture has to be crushed for extraction which is filtered.

27.10.5 Neem Oil Spray

- 15–30 ml neem oil is mixed with 1 l of water and stirred well.
- Add 1 ml of emulsifier to this.

27.10.6 Fermented Curd Water

- Take equal proportion of buttermilk and water.
- Keep it for 2 days in a shade place for fermentation.
- Take 1 l of this solution and mix with 4 l of water for spraying (1:4 ratio).
- Spray should be taken in the early morning.

27.10.7 Herbal Pesticide Formulation

Neem seeds	500 g
Tobacco	1000 g
Acorus calamus (sweet flag)	100 g
Asafoetida	250 g
Sapindus emarginatus seeds (soapberry)	50 g

All the ingredients are ground and the extract is sprayed for one acre cotton to control pests.

27.10.8 Neem-Cow Urine Extract

- 5 kg of neem leaves, 5 l of cow urine, 2 kg of cow dung, 100 l of water.
- Crush all ingredients for mixing and leave it to ferment for 24 h.
- Intermittent stirring should be given.
- Filter the contents and dilute with 100 l of water.

27.11 Post-harvest Processing

- Equipment should be cleaned or not contaminated.
- All the seed cleaning activities should be done at a certified organic farm or in a professional cleaning facility centre which is certified organically.
- Packaging, shipping and storage of organic seed must be clearly labelled as organic.

27.11.1 Management of Weed Seeds in Harvested Seed Lot

- Avoid weed seed contamination during the post-harvest process.
- If the seed is even a slightly different size or weight, it may be removed with standard seed cleaning practices such as screening or fanning.

27.11.2 Seed Cleaning

27.11.2.1 Cleaning Dry Seeded Crops

Dry seeds may contaminate with other plant materials such as sticks and leaves, dirt and stones. Then it is cleaned by using the method called separation in weight and size.

27.11.2.2 Separation Based on Size

Most screens have round holes (to retain large seeds on top) which execute multiple screening functions in one pass. First screening will sort out the chaffs and second lead to retain the seeds, whereas the third removes the smaller debris and small seeds.

27.11.2.3 Separation Based on Weight

- Cleaning and separation of seeds based on the weight gradient is one of the old techniques by seeking differences in specific gravity.

27.11.2.4 Cleaning Wet-Seeded Crops

Wet-seeded fruit such as tomatoes and cucumbers are cleaned in the following ways:

- Removal.
- Drying—Seeds should not reach temperatures over 95 F.
- Fermentation.

27.11.3 Organic Seed Treatment

- Seed coating with ashes, red earth and neem oil which facilitates the eradication of seed-borne pathogens or protects from soil-borne pathogens.
- Germination.

27.11.3.1 Priming

- Water is mixed with seeds to make the seeds to imbibe water.
- Upon absorption, water will dissolve the germination inhibitors, which makes the germination process quicker.

27.11.3.2 Pelleting

Seed is coated with clay mixed of nutrients, biofertilizers and biocontrol agents which streamlines the size, shape and uniformity of a small, non-round seeds to hasten the germination.

27.11.3.3 Hot Water Treatment

- Seeds should be immersed in hot water about 100 °F for 20–25 min, depending on the crop species.
- Then after cooling should be done immediately for 5 min in cold water.
- This should be followed by rapid drying.

27.12 Preventive Measures against Storage Pests and Diseases

- Timely harvesting and drying.
- Proper threshing.
- Cleaning to remove trash.
- Sorting to remove damaged ones.
- Proper packing and storage off the ground.

27.13 Factors Affecting Organic Seed Productions

- Distance between plants.
- Temperature.
- Humidity.
- Wind direction.
- Insects responsible for pollination.
- Crop variety.
- Adjacent plot.

27.14 Certification for Organic Seed Quality

Valid certification is an essential prerequisite for gaining farmer's and consumer's credence. Certification agencies have been set up in India by laying the NPOP (National Programme for Organic Production) standards to improve the certification services to the needy people. This organization involved in inspection and certification in organic seed production system, offers positive distinction for organic products and helps in market planning and lobbying.

27.15 Conclusion

Seed quality is of immense importance in achieving sustainable production. In Tamil Nadu, organically produced seeds are rare. Presently farmers are started to realize the importance of traditional varieties and their impact causing the conservation of all the locally available varieties. Government also initiated to invest more on organic agriculture which will pave a way for organic seed production by the state department itself.

References

Annadana (2010) Organic traditional vegetable seeds. Annadana seed and soil savers, seed catalog 2010

Shiva V, Pande P, Singh J (2004) Principles of organic farming: renewing the Earth's harvest. Navdanya, New Delhi

Somasundaram E, Udhaya Nandhini D (2018a) Organic agriculture: techniques to improve crop production in: eco-friendly agro-biological techniques for enhancing crop productivity. Springer Nature Singapore Pte Ltd, Gateway East, Singapore, pp 1–24

Somasundaram E, Udhaya Nandhini D (2018b) Strategies for organic seed production. In: Recent trends in seed production, post harvest handling and value addition techniques for effective seed supply chain (Selvaraju et al.). Thannambikkai Publications, P.N. Pudur, Coimbatore, pp 433–444

Effects of Prohexadione-Calcium Application on Vegetative and Generative Growth of Pepper Plants

28

Nusret Ozbay and Recai Metin

Abstract

Some crops like red pepper (*Capsicum annuum* L.) tend to stretch very early after germination, particularly when grown in low light conditions. Therefore, the height of vegetable crop seedlings must be controlled by any possible way. Using the plant growth retardant that inhibits stem elongation is the most common method for controlling plant height. This study was conducted to compare the impacts of prohexadione-calcium (Pro-Ca) doses (0, 25, 50, 75, and 100 mg L^{-1}) and three application methods (seed soaking, soil drench, and foliar spray) on growth and performance of pepper seedlings and to determine any subsequent effects in the field on vegetative growth, flowering, and yield. At field transplanting (42 days after planting), Pro-Ca concentrations higher than 25 mg L^{-1} and application methods except for seed soaking reduced seedling heights by 25–31%, leaf area size by 7–16%, shoot fresh weight by 23–32%, shoot dry weight by 19–29%, root fresh weight by 10–22%, and root dry weight by 20–24%. No delay in flowering and fruit set time were found in pepper plants grown from Pro-Ca treatments. However, the 100 mg L^{-1} Pro-Ca treatment was found to decrease yield by 22%. These results show that lower Pro-Ca levels (25 and 50 mg L^{-1}) can be used to control excessive elongation of pepper seedlings without yield loss.

Keywords

Growth retardant · Height control · Application method · *Capsicum annuum* L.

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28.1 Introduction

Production of quality crops from the transplants demands use of uniform and high-quality transplants. Ideally, the acceptable transplants should be short and stocky with thick, strong stems and deep green color (Latimer 1998). Some vegetable crops like peppers have the same tendency to early stretching as some bedding plant species do. Stretching and legginess become a problem in pepper transplants when field planting is delayed in the spring due to weather conditions. High light intensities at which seedlings are grown, together with either low levels of natural radiation during the rainy season and winter or high greenhouse temperatures during summer, often result in production of badly stretched seedlings (Kim et al. 2008). In the field, lodging plants due to excessive vegetative growth result in yield losses. Tall transplants of vegetables are also more difficult to transplant, more likely to break, and less compatible with transplanting machines than plants of more moderate size. In the greenhouse, plant stretching is an unwanted effect in plug production reducing plug quality and making it difficult to handle and perform mechanized transplanting (Magnitskiy et al. 2006). The reduction of plant height plays an important role in promoting yield and quality and reducing cost, space, and labor (Kim et al. 2010). Managing transplant height is therefore important for the vegetable transplant production in both greenhouse and field plant productions.

Commercial and experimental methods for controlling growth of pepper transplants include applying the plant growth regulators, regulating temperature during hardening of the transplants or controlling day and night temperatures, and inducing mechanical stress (Hatt Graham and Decoteau 1995). Although various cultural practices can contribute to the production of acceptable transplants, chemical plant growth regulators provided one of the most convenient and consistent management methods. Prohexadione-calcium (Pro-Ca) is a relatively new plant growth regulator that inhibits the late phases of gibberellin (GA) biosynthesis in plants, thereby reducing the plant's vegetative growth (Kim et al. 2007). The Pro-Ca has low toxicity and persistence in plants (Ilias and Rajapakse 2005). Greene and Schloemann (2010) reported that the inhibitory effects from an application of Pro-Ca lasted for 28 days. Pro-Ca is very effective in controlling the growth of many fruit crops (Palonen and Mouhu 2009) and may offer an alternative for use in vegetable transplant production.

This study was conducted to compare the effects of various Pro-Ca concentrations (0, 25, 50, 75, and 100 mg L^{-1}) and different application methods (seed soaking, soil drench, or foliar spray and) on the characteristics and quality of pepper transplants and to determine any subsequent effects in the field on vegetative growth and yield.

28.2 Material and Methods

The greenhouse and field experiments were performed at the Horticultural Research Units, Kahramanmaras Sutcu Imam University, Turkey. Seeds of "Sena" red hot pepper (*Capsicum annuum* L.) were obtained from the Agricultural Research Institute, Kahramanmaras, Turkey (latitude, 37°35'N; longitude, 36°49'E; elevation, 502 m).

28.2.1 Seed Soaking Treatment

The pepper seeds were surface disinfected in 1% NaClO solution for 10 min to eliminate possible seed-borne microorganisms, rinsed under running tap water for 1 min, and then dried at room temperature for 30 min. The seeds were placed on double layers of filter paper (Whatman #1) in covered polystyrene boxes with a length of 10 cm, width of 10 cm, and height of 4 cm. The seeds were soaked for 24 h in an aqueous solution of Pro-Ca (Regalis, BASF 125 10 W containing 10% prohexadione-calcium as the active ingredient) at 25, 50, 75, or 100 mg L^{-1}. Control seeds were similarly treated with the same amount of distilled water. After soaking, seeds were transferred to a sieve and then dried on filter paper at 20 °C for 2 h to make singulation of the seeds easier.

28.2.2 Greenhouse Experiment

The greenhouse experiment investigated the effects of Pro-Ca application methods and the concentrations on seedling growth parameters. Pepper seedlings were grown on benches in greenhouse at average day/night temperatures of 29.5/19.8 °C and 58% relative humidity. Pro-Ca-treated seeds (for seed treatment) and Pro-Ca-untreated pepper seeds (for foliar spray and soil drench application methods) were sown into 45-cell plastic trays (75 cm^3 cell volume) filled with a 4:1 ratio peat and perlite media and placed on the greenhouse benches. The seedlings were irrigated regularly and fertilized with a soluble NPK-balanced, trace element-enriched fertilizer (20–20-20 + TE) at the rate of 200 mg L^{-1}N once a week. In the second application method, at emergence of the third true leaf (21 days after sowing), pepper seedlings from Pro-Ca-untreated seeds were one-time sprayed with 0, 25, 50, 75, or 100 mg L^{-1} Pro-Ca solutions containing 0.1 percent Tween 20 (Sigma Chemical Co., St. Louis, MO) as a wetting agent. Water containing 0.1 percent Tween 20 was applied to the control plants. The pepper seedlings were sprayed on the foliage with Pro-Ca solution at five concentrations (0, 25, 50, 75, or 100 mg L^{-1}) to run-off, using a handheld sprayer. At the time of spraying, plastic trays were covered with aluminum foil to prevent the contact of growth regulator solutions with the growing substrate. In the third application method, Pro-Ca (20 mL per cell) was applied as a soil drench to the growing medium at emergence of the third true leaf. The greenhouse experiment was discontinued at 42 days after planting (DAP), and final

measurements of seedling growth were taken. Ten plants were randomly selected from each replication per treatment to determine stem diameter, seedling height, internode length, number of leaves, leaf area (with a LI-3100C leaf area meter, LI-COR, Lincoln, Nebraska, USA), shoot fresh and dry weights, and root fresh and dry weights (after drying the samples in an oven at 80 °C until constant weight was reached).

28.2.3 Field Experiment

Pro-Ca-treated and Pro-Ca-untreated seedlings were transplanted into the field to investigate the effects of Pro-Ca on vegetative growth and yield parameters. The experimental plots were consisted of 2 rows of 10 plants each, with 20 plants per treatment with a drip irrigation pipe down the center.

Plant rows were spaced 50 cm apart on beds with 120 cm center, and plants within each row were 50 cm apart (120 × 50 × 50 cm). With standard local field production practices, plants were grown to maturity (irrigation, disease/insect management, fertilizer rates, weed control, etc.). During field experiments, the effects of Pro-Ca were observed on plant heights (measured at 60, 90, and 120 DAP), number of fruits per plant, average fruit weight, and fruit yield in pepper. During field experiments average day/night temperatures of 33.5/20.8 °C and 45% relative humidity.

28.2.4 Experimental Design and Statistical Analysis

The experiment was a 5 × 3 factorial in a completely randomized design or randomized block design with three replications per treatment. Each experiment was repeated twice and yielded similar data. Therefore, the data from two experiments have been combined and subjected to analysis of variance (ANOVA) performed with SAS 9.1 (SAS Institute Cary, NC, USA), and mean separation procedure was performed by the least significant difference (LSD) test of Fisher when F test was significant at $P \leq 0.05$.

28.3 Results and Discussion

28.3.1 Greenhouse Experiment

With application of 50, 75, and 100 mg L^{-1} Pro-Ca, seedling height was suppressed by 25%, 28%, and 31%, respectively, compared to control. There were significant differences in seedling height among Pro-Ca application methods, soil drench treatment having the highest reduction (37%). 100 mg L^{-1} Pro-Ca also effectively reduced internode length by 27% as compared to control (Table 28.1). The highest concentration of Pro-Ca (100 mg L^{-1}) resulted in reduction in shoot fresh weight and dry weight of pepper seedlings (by 32.14% and 27.72%, respectively)

Table 28.1 Effects of Pro-Ca doses and application methods on seedling height, internode length, and stem diameter of 6-week-old pepper seedlings in the greenhouse

	Seedling height (cm)				Internode length (cm)				Stem diameter (mm)			
	Application methods				Application methods				Application methods			
Pro-Ca mg L^{-1}	SS	FS	SD	Mean	SS	FS	SD	Mean	SS	FS	SD	Mean
0	11.05	9.36	9.10	9.84[a]	2.65	2.24	2.23	2.37[a]	2.74	2.65	2.50	2.63[ab]
25	10.50	10.75	7.36	9.54[a]	2.44	2.95	1.70	2.36[a]	2.66	2.59	2.80	2.68[a]
50	9.38	8.21	5.63	7.41[b]	2.43	2.17	1.53	1.91[b]	2.37	2.58	2.72	2.56[abc]
75	9.30	7.60	4.47	7.12[b]	2.67	1.97	0.95	1.86[bc]	2.37	2.48	2.75	2.53[bc]
100	9.20	7.41	3.92	6.84[b]	2.37	2.10	0.74	1.74[c]	2.49	2.30	2.56	2.45[c]
Mean	9.69[a]	8.67[b]	6.10[c]		2.51[a]	2.29[b]	1.43[c]		2.53[b]	2.52[b]	2.67[a]	
$LSD_{0.05}$	Pro-Ca = 0.568***				Pro-Ca = 0.166***				Pro-Ca = 0.127**			
	Method = 0.44***				Method = 0.128***				Method = 0.098**			
	Pro-Ca × method = 0.98***				Pro-Ca × method = 0.29***				Pro-Ca × method = 0.22**			

**Significant at $P < 0.01$, ***significant at $P < 0.001$. *SS* seed soaking, *FS* foliar spray, *SD* soil drench. Means followed by the same letter are not significantly different at the 0.05 level, using Fisher's LSD

Table 28.2 Effects of Pro-Ca doses and application methods on number of true leaves and shoot fresh and dry weights of 6-week-old pepper seedlings in the greenhouse

	Number of true leaves				Shoot fresh weight (mg)				Shoot dry weight (mg)			
	Application methods				Application methods				Application methods			
Pro-Ca mg L^{-1}	SS	FS	SD	Mean	SS	FS	SD	Mean	SS	FS	SD	Mean
0	7.00	6.33	6.70	6.68^{a}	1791	1682	1615	1696^{a}	297	245	259	267^{a}
25	6.90	6.77	6.63	6.77^{a}	1644	1655	1519	1606^{a}	275	250	250	258^{a}
50	6.13	6.33	6.00	6.15^{b}	1237	1444	1243	1308^{b}	207	210	229	215^{b}
75	6.40	6.03	5.90	6.11^{b}	1274	1209	1233	1239^{bc}	208	190	240	213^{b}
100	6.20	5.90	5.80	5.97^{b}	1379	1050	1031	1153^{c}	235	159	187	194^{b}
Mean	6.53^{a}	6.27^{ab}	6.21^{b}		1465^{a}	1408^{ab}	1328^{b}		244^{a}	211^{ab}	233^{b}	
$LSD_{0.05}$	Pro-Ca = 0.348***				Pro-Ca = 104***				Pro-Ca = 29.35***			
	Method = NS				Method = 80.78**				Method = 22.74*			
	Pro-Ca × method = NS				Pro-Ca × method =181*				Pro-Ca × method = NS			

NS non-significant, *significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$. *SS* seed soak, *FS* foliar spray, *SD* soil drench. Means followed by the same letter are not significantly different at the 0.05 level, using Fisher's LSD

(Table 28.2). It was found that all Pro-Ca doses resulted in reduction in leaf area by up to 14% compared to control plants although there was a slight difference in the leaf number between Pro-Ca treatments and control plants (Table 28.3). Root fresh and dry weights were significantly reduced by all the Pro-Ca doses with the exception of 25 mg L^{-1} (Table 28.3).

28.3.2 Field Experiment

Pro-Ca treatments resulted in 17% reduction in plant height at 60 DAP, 14% reduction in plant height at 90 DAP, and 12% reduction in plant height at 120 DAP (Table 28.4). Statistically significant differences were found in plant height among Pro-Ca application methods, soil drench treatment having the highest reduction at 60, 90, and 120 DAP (26, 23.5, and 15%, respectively). Low concentrations of Pro-Ca (25–50 mg L^{-1}) did not significantly affect the number of fruits per plant and total fruit yield. However, Pro-Ca at 100 mg L^{-1} reduced the number of fruits per plant by 17% and total yield by 21% (Table 28.5). No significant differences in mean fruit weight among Pro-Ca dosage treatments were found. Pro-Ca doses (75 and 100 mg L^{-1}) reduced yield in pepper by 17% and 21%, respectively, whereas 25 and 50 mg L^{-1} did not cause any change in yield in comparison with control.

In the present study, the application of Pro-Ca resulted in an immediate reduction in vegetative growth, most notably shown by a reduction in plant height and internode length, compared to the control treatment. This inhibitory effect of Pro-Ca decreased over time, gradually decreasing the difference between treated and control plants in plant height and internode length. These findings are consistent with some of the previous studies (Rayirath et al. 2009; Kim et al. 2010; Guak 2013). These findings also confirm Grossmann et al. (1994), who reported that treatment of seedlings with increasing Pro-Ca doses reduced plant height and fresh weight of shoots by up to 40% in hydroponically grown wheat.

By soaking of faba bean seeds in Pro-Ca solutions, shoot height and shoot fresh weight were significantly reduced. The decrease was consistent with increasing concentration of Pro-Ca (Bekheta et al. 2009). Other researchers also support the inhibitory effect of Pro-Ca on seedling height for some other species, such as cabbage (Hamano et al. 2002), impatiens and petunia (Ilias and Rajapakse 2005), okra (Ilias et al. 2007), and onion and garlic (Ouzounidou et al. 2011). Pro-Ca inhibits gibberellin biosynthesis by blocking the 3ß-hydroxylation of GA_{20} to GA_1, resulting in reduced stem elongation (Rademacher 2000). Pro-Ca treatment decreased GA_3 and IAA levels but increased levels of ABA and cytokinin in faba bean seedlings relative to their respective controls (Bekheta et al. 2009).

Through the application of exogenous GA_3, stem dry mass, leaf dry mass, and stem length of okra plants were significantly enhanced, but Pro-Ca inhibited growth (Ilias et al. 2007). Pro-Ca has a potential for effective vegetative growth control in several plant species; however, timing is very important (Ilias and Rajapakse 2005).

Table 28.3 Effects of Pro-Ca doses and application methods on leaf area and root fresh and dry weights of 6-week-old pepper seedlings in the greenhouse

	Leaf area (cm^2/plant)				Root fresh weight (mg)				Root dry weight (mg)			
	Application methods				Application methods				Application methods			
Pro-Ca mg L^{-1}	SS	FS	SD	Mean	SS	FS	SD	Mean	SS	FS	SD	Mean
0	77.45	72.34	73.72	74.50[a]	1185	1023	939	1049[a]	93	82	71	82[a]
25	76.75	65.86	66.54	69.72[b]	991	1072	788	950[b]	81	87	64	77[a]
50	74.33	65.13	52.80	64.09[c]	909	851	749	836[c]	61	70	66	66[b]
75	75.81	64.12	50.77	63.57[c]	850	715	919	828[c]	67	59	69	65[b]
100	75.63	60.42	53.17	63.07[c]	960	681	839	827[c]	73	44	70	62[b]
Mean	75.99[a]	65.57[a]	59.40[b]		979[a]	868[b]	847[b]		75[a]	68[ab]	68[b]	
$LSD_{0.05}$	Pro-Ca =4.21***				Pro-Ca = 87.82***				Pro-Ca = 8.69***			
	Method = 3.21***				Method = 2.04***				Method = NS			
	Pro-Ca × method = 7.29**				Pro-Ca × method = 152**				Pro-Ca × method = 15.06**			

NS not significant, **significant at $P < 0.01$. ***significant at $P < 0.001$. *SS* seed soak, *FS* foliar spray, *SD* soil drench. Means followed by the same letter are not significantly different at the 0.05 level, using Fisher's LSD

Table 28.4 Effects of Pro-Ca doses and application methods on plant height 60, 90, and 120 days after planting (DAP) in the field

	Plant height 60 DAP (cm)				Plant height 90 DAP (cm)				Plant height 120 DAP (cm)			
	Application methods				Application methods				Application methods			
Pro-Ca mg L^{-1}	SS	FS	SD	Mean	SS	FS	SD	Mean	SS	FS	SD	Mean
0	26.64	26.76	25.44	26.28[a]	39.17	39.14	37.05	38.45[a]	54.46	54.03	52.08	53.52[a]
25	27.16	27.38	21.02	25.19[a]	39.64	39.57	32.81	37.34[ab]	55.54	54.68	50.78	53.67[a]
50	25.97	24.78	19.81	25.38[b]	37.64	37.78	29.85	35.09[bc]	53.77	51.28	44.36	49.80[b]
75	25.63	24.30	17.23	22.39[bc]	37.78	36.70	24.70	33.06[c]	50.05	52.14	39.16	47.12[c]
100	26.19	24.85	14.39	21.81[c]	39.05	36.32	23.49	32.95[c]	49.52	52.94	38.32	46.93[c]
Mean	26.32[a]	25.61[a]	19.52[b]		38.66[a]	37.90[a]	29.58[b]		52.67[a]	53.01[ab]	44.94[b]	
$LSD_{0.05}$	Pro-Ca = 1.23***				Pro-Ca = 2.83***				Pro-Ca = 2.35***			
	Method = 0.95***				Method = 2.19***				Method = 1.82***			
	Pro-Ca × method = 2.12***				Pro-Ca × method = 4.90*				Pro-Ca × method = 4.07**			

*Significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$. *SS* seed soak, *FS* foliar spray, *SD* soil drench. Means followed by the same letter are not significantly different at the 0.05 level, using Fisher's LSD

Table 28.5 Effects of Pro-Ca doses and application methods on number of fruits per plant, mean fruit weight, and fruit yield in pepper

	Number of fruits per plant				Mean fruit weight (g)				Fruit yield (ton/ha)			
	Application methods				Application methods				Application methods			
Pro-Ca mg L^{-1}	SS	FS	SD	Mean	SS	FS	SD	Mean	SS	FS	SD	Mean
0	49.53	53.70	48.14	50.46^{ab}	9.47	9.25	8.77	9.16	16.36	17.72	15.54	16.54^{a}
25	50.58	58.93	47.79	52.43^{a}	9.55	9.11	8.64	9.10	17.37	19.18	14.44	17.00^{a}
50	54.46	59.75	42.67	52.29^{a}	8.84	8.62	8.20	8.55	17.09	18.22	12.50	15.94^{ab}
75	47.69	59.16	25.31	44.05^{ab}	9.73	8.28	8.15	8.72	16.38	17.52	7.180	13.69^{bc}
100	47.32	55.03	23.05	41.80^{b}	9.04	8.32	9.49	8.95	15.10	16.22	7.670	13.00^{c}
Mean	49.92^{b}	57.31^{a}	37.39^{c}		9.33^{a}	8.72^{b}	8.65^{b}		16.46^{a}	17.77^{a}	11.47^{b}	
$LSD_{0.05}$	Pro-Ca = 8.52*				Pro-Ca = NS				Pro-Ca = 2.35**			
	Method = 6.60***				Method = 0.59*				Method = 1.82***			
	Pro-Ca × method = NS				Pro-Ca × method = NS				Pro-Ca × method = NS			

NS not significant, *significant at $P < 0.05$, **significant at $P < 0.01$, ***significant at $P < 0.001$. *SS* seed soak, *FS* foliar spray, *SD* soil drench. Means followed by the same letter are not significantly different at the 0.05 level, using Fisher's LSD

Pro-Ca treatments (higher doses) resulted in reduction in leaf number and leaf area compared to the control plants, which agrees with previous studies on Pro-Ca and some triazole group regulators (Glen and Miller 2005; Medjdoub et al. 2007). Pro-Ca application was reported to have decreased number of leaves and leaf area of tomato plants compared to control (Altintas 2011). The reduced leaf area with the increasing treatments is most likely because of the inhibition of gibberellin biosynthesis by Pro-Ca. Kang et al. (2010) reported that with elevated Pro-Ca, endogenous bioactive GA_1 and GA_4 contents in Chinese cabbage decreased dramatically.

No delaying in flowering and fruit set time were found in pepper plants grown from Pro-Ca treatments (data not presented). These findings are consistent with Ilias et al. (2007), who reported that control and okra plants treated by Pro-Ca took the same time to bloom. In disagreement, application of Pro-Ca (200 mg L^{-1}), 45 days after planting (DAP), delayed the anthesis for up to 11 days in petunia plants (Cerny-Koenig et al. 2005). This may be because of late application of the retardant. Some yield parameters of pepper such as mean fruit weight and fruit yield were also reduced by the application of higher Pro-Ca treatments (especially with 100 mg L^{-1}) but not with the lower concentrations as mentioned previously. These findings are consistent with those reported for apple plants by Miller (2002). Pro-Ca may reduce the fruit size mainly by slowing down cell division during active period of cell division in the pepper fruits. An alternative and/or contributing factor that reduces fruit size could be the reduced leaf area, which is true in our study. On the other hand, our results are contrary to some other studies. Hytönen et al. (2009) reported that single timely treatment of Pro-Ca during the planting year leads to a significant increment of the yield in strawberry during the following season. Similarly, Greene and Schloemann (2010) found that Pro-Ca increased overall marketable yield without affecting the mean fruit size. Asín et al. (2007) determined that the Pro-Ca had no significant negative impact on yield of pear. Pro-Ca's improving or negative impact may be thought to depend on time of application, doses, environmental conditions, plant species, cultivars, and even individual plants.

28.4 Conclusion

Low Pro-Ca concentrations seem to be effective in controlling vegetative growth pepper yet seem to have nominal side effects. Based on our results, it can be stated that lower Pro-Ca concentrations can be used without any significant yield loss to control excessive elongation of pepper seedlings. It is possible to use concentration ranging from 50 to 75 mg L^{-1} to achieve a height reduction of 28%. Several growth regulators are available to control plant height by regulating gibberellin biosynthesis, but most of them are extremely persistent and have a long half-life in the plant and in the soil. In contrast, Pro-Ca is a non-toxic and a reduced risk growth regulator with a short half-life of a few weeks.

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References

Altintas S (2011) Effects of prohexadione-calcium with three rates of phosphorus and chlormequat chloride on vegetative and generative growth of tomato. Afr J Biotechnol 10(75):17142–17151

Asín L, Alegre S, Montserrat R (2007) Effects of paclobutrazol, prohexadione-Ca, deficit irrigation, summer pruning and root pruning on shoot growth, yield and return bloom, in a 'Blanquilla' pear orchard. Sci Hortic 113(2):142–148

Bekheta MA, Abdelhamid MT, El-Morsi AA (2009) Physiological response of Vicia faba to prohexadione-calcium under saline conditions. Planta Daninha 27(4):769–779

Cerny-Koenig TA, Faust JE, Rajapakse NC (2005) Role of gibberellin A(4) and gibberellin biosynthesis inhibitors on flowering and stem elongation in petunia under modified light environments. HortScience 40(1):134–137

Glen DM, Miller SS (2005) Effects of Apogee on growth and whole-canopy photosynthesis in spur delicious apple trees. HortScience 40:397–400

Greene DW, Schloemann SG (2010) Prohexadione-calcium inhibits runner formation and enhances yield of strawberry. J Am Pomol Soc 64:125–139

Grossmann K, König-Kranz S, Kwiatkowski J (1994) Phytohormonal changes in intact shoots of wheat and oilseed rape treated with the acylcyclohexanedione growth retardant prohexadione calcium. Physiol Plant 90:139–143

Guak S (2013) Effects of prohexadione-ca, ethephon, and water stress on growth and productivity of 'Golden Delicious'/M.9 Apple. Kor J Hortic Sci Technol 31(1):38–49

Hamano M, Yamato Y, Amazaki H, Miura H (2002) Endogenous gibberellins and their effects on flowering and stem elongation in cabbage (*Brassica oleracea var. capitata*). J Hortic Sci Biotechnol 77(2):220–225

Hatt Graham HA, Decoteau DR (1995) Regulation of pepper transplant growth with light and subsequent effects on fruit production. HortScience 30:487–489

Hytönen T, Mouhu K, Koivu I, Elomaa P, Junttila O (2009) Planting year prohexadione-calcium treatment increases the cropping potential and yield of strawberry. ISHS Acta Hortic 842:741–744

Ilias IF, Rajapakse N (2005) Prohexadione-calcium affects growth and flowering of petunia and impatiens grown under photoselective films. Sci Hortic 106:190–202

Ilias I, Ouzounidou G, Giannakoula A, Papadopoulou P (2007) Effects of gibberellic acid and prohexadione-calcium on growth, chlorophyll fluorescence and quality of okra plant. Biol Plant 51(3):575–578

Kang SM, Kim JT, Hamayun M, Hwang IC, Khan AL, Kim YH, Lee JC, Lee IJ (2010) Influence of prohexadione-calcium on growth and gibberellins content of Chinese cabbage grown in alpine region of South Korea. Sci Hortic 125(2):88–92

Kim HY, Lee IJ, Hamayun M, Kim JT, Won JG, Hwang IC, Kim KU (2007) Effect of prohexadione calcium on growth components and endogenous gibberellins contents of rice (Oryza sativa L.). J Agron Crop Sci 193:445–451

Kim IS, Zhang C, Kang HM, Mackay B (2008) Control of stretching of cucumber and tomato plug seedlings using supplement light. Hortic Environ Biotechnol 49:287–292

Kim YH, Khan AL, Hamayun M, Kim JT, Lee JH, Hwang IC, Yoon CS, Lee IJ (2010) Effects of prohexadione calcium on growth and gibberellins contents of *Chrysanthemum morifolium* R. cv. 'Monalisa White'. Sci Hortic 123:423–427

Latimer JG (1998) Mechanical conditioning to control height. HortTechnology 8(4):529–534

Magnitskiy SV, Pasian CC, Bennett MA, Metzger JD (2006) Effects of soaking cucumber and tomato seeds in paclobutrazol solutions on fruit weight, fruit size, and paclobutrazol level in fruits. HortScience 41(6):1446–1448

Medjdoub R, Val Falcón J, Blanco Braña A (2007) Physiological effects of prohexadione-calcium in apple trees: effects on parameters related to photoproductivity. J Hortic Sci Biotechnol 82 (1):126–132

Miller SS (2002) Prohexadione-calcium controls vegetative growth in apple. J Tree Fruit Prod 3:11–28

Ouzounidou G, Giannakoula A, Asfi M, Ilias I (2011) Differential response of onion and garlic against plant growth regulator. Pak J Bot 43(4):2051–2057

Palonen P, Mouhu K (2009) Vegetative growth and flowering of primocane raspberry 'Ariadne' as affected by prohexadione-calcium treatments. HortScience 44(2):529–531

Rademacher W (2000) Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. Annu Rev Plant Physiol Plant Mol Biol 51:501–531

Rayirath UP, Lada RR, Caldwell CD, Asiedu SK, Sibley KJ, Adams AD (2009) CCC and prohexadione-ca enhance rhizome growth and lateral bud production in rhubarb (*Rheum rhabarbarum* L.). J Plant Growth Regul 28(2):137–146

Bean Common Mosaic Virus Transmission by Bean Seed cv. Chervona Shapochka

29

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Abstract

Bean common mosaic virus (BCMV) is one of the most harmful and widespread bean viruses. The pathogen can be transmitted with seeds and pollen with a fairly high frequency. With efficient spread by vectors to susceptible crops, even a low level of seed infection can lead to an epidemic situation. In Ukraine, BCMV is widespread in all dry bean-growing areas and may cause serious crop losses. The aim of this study was to investigate the level of bean common mosaic virus seed transmission in Phaseolus vulgaris cv. Chervona shapochka. Whereas high incidence of seed transmission occurred in direct relation to virus invasion of an immature embryo, we tended to follow the virus infection in reproductive tissues of bean. To determine the possibility of vertical transmission of BCMV via pollen, detection of viral RNA in pollen grains by PCR (polymerase chain reaction) has been carried out. In the study, biological methods (mechanical inoculation of viruses, detection of virus infectivity in indicator plants) and molecular biological techniques (total RNA extraction from the plant tissues, reverse transcription polymerase chain reaction) were used. It was shown *P. vulgaris* cv. Chervona shapochka transmitted the BCMV strain in 77% of the seed produced by infected plants. The data obtained indicates a high level of seed transmission of the virus. According to the findings, virus-infected seeds

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have a great value in disease spreading. The viral RNA has been detected in plant generative organs and some components of the seed and flower. Also virus particles were found in pollen grains of *Phaseolus vulgaris* plants. In the cytoplasm and nucleus of developing embryo cells, diffuse granular viral inclusions were detected. Studies have been conducted on only one variety of beans (cv. Chervona shapochka), so whether this is a varietal feature of the beans or a characteristic of BCMV strain should be clarified in subsequent experiments.

Keywords

Bean common mosaic virus (BCMV) · Seed infection · Reverse transcription polymerase chain reaction (RT-PCR)

29.1 Introduction

The seed transmission of viruses plays a key role in dissemination and survival of many serious plant virus and viroid diseases. Infected seeds are the most important source of viruses in nature and commercial crop production. Under natural conditions, only infected plants serve as a source of infection for the secondary virus spreading by appropriate vectors. Despite the fact that viruses can infect plants belonging to different families, seed transmission is noticed only in certain virus-host combinations. The presence of virus in/on a seed does not always lead to seedling infection. Some viruses are found only on seed coat or in cotyledons or in embryos. Some of them are stored during maturation of the seed, while others are eliminated with seed maturation and drying. Currently, nearly 231 viruses and viroids can be seed transmitted. Most of them belong to the genera *Potyvirus*, *Nepovirus*, *Cryptovirus*, *Ilarvirus*, *Tobamovirus*, *Potexvirus*, *Comovirus*, *Carlavirus*, *Carmovirus*, *Cucumovirus*, *Sobemovirus*, *Furovirus*, *Bromovirus*, and *Tymovirus*. More than 70 viruses are transmitted with seeds of plants belonging to Fabaceae family (more often than with seeds of any other cultivated plants). Some viruses, with nonlegume principal hosts, are also seed transmitted in legumes (Sastry 2013).

Bean common mosaic virus (BCMV) is one of the most widespread and devastating viral diseases of common beans (Maule and Wang 1996; Kyrychenko and Kovalenko 2018). BCMV have a great potential to reduce growth and yield of food crops. Yield loss due to BCMV ranging between 6% and 98% in some fields has been reported (Varma 1988; Hampton et al. 1982). BCMV is transmitted at a high frequency through *P. vulgaris* seeds (Schmidt 1992) and can maintain its infectiveness and viability in seeds up to 38 years (Walters 1962). The percentage of infected seed varies from 0.67% to 98% (Worrall et al. 2015; Nordenstedt et al. 2017; Deligoz and Soken 2013; Hema et al. 2014). The rate of BCMV seed transmission depends on a range of factors including host cultivar, virus strain, stage of infection, and environment (Sastry 2013). It is believed that the 1977 BCMV epidemics in Europe and America were most likely initiated by seed stock

contamination and growing bean cultivars previously considered to be resistant to many BCMV strains (Worrall et al. 2015). In accordance with the epidemiological importance of pollen in virus spreading, BCMV was classified to category C (Sastry 2013). Viruses included in this category are detected directly in the pollen and/or infect plants during cross-pollination. They are vertically transmitted through pollen and lead to the derivation of infected seeds in female plants, but such virus spreading does not represent a significant epidemiological threat, since the vertical transmission of BCMV with pollen under natural conditions has not been reported in the literature.

Taking into account the importance of infected seeds in the dissemination of diseases and virus storage, the seed transmission of BCMV circulating in Ukraine in *P. vulgaris* cv. Chervona shapochka has been investigated.

29.2 Materials and Methods

29.2.1 Virus Culture

The virus culture was raised through infected seed and multiplied on healthy seedlings of susceptible cv. Pervomaisky bean plants in greenhouse conditions through mechanical sap inoculation using standard leaf rub method.

29.2.2 Mechanical Inoculation and Infectivity Test of Virus Samples

To prepare the inoculum, 1 g of virus-containing material (young beans with pre-removed seeds, mature seeds, seed coat, embryos, endosperm, and separate parts of the flower) was ground in a mortar, and 1 ml of citrate buffer (50 mM sodium citrate, 20 mM diethyldithiocarbamic acid, 2% polyvinylpyrrolidone) was added. The slurry was squeezed through muslin cloth. Sap was centrifuged at 3000 rpm for 5 min. 450 μl of citrate buffer was added to 50 μl supernatant, which is thus obtained and used for PCR analysis or biological testing (Sipahioğlu et al. 2007). Mechanical inoculation using inoculum and celite powder was done at three-leaf stage by gently rubbing leaves of bean plants. Infectivity tests were scored based on disease symptom development.

To determine transmission rates from bean seeds to seedlings, 150 mature seeds obtained from virus-infected *P. vulgaris* plant cv. Chervona shapochka were germinated in glasshouse conditions. Seedlings were raised from healthy seeds used as test plants for various experiments. Before sowing, the seeds were extracted in 4% hydrochloric acid and washed in a 10% bleach solution. The plants were kept under observation for appearance of disease symptoms and used for seed infectivity assays. Reaction of the test plants was evaluated every day for 4 weeks since the first true leaflet appeared. In addition, all plants were tested by RT-PCR. Seed transmission rate was calculated using the equation

$$P = \frac{n \cdot 100}{N},$$

where P is the seed transmission rate, n is the number of infected seedlings, and N is the number of grouped samples (Dospekhov 1985).

29.2.3 BCMV-Specific Primers

The pair of primers (forward 5′-tgtggtacaatgctgtgaagg-3′ and the reverse 5-′-gccttcatctgtgctactgct-3′) designed by us was used to amplify a 391 bp viral genome fragment corresponding to nucleotides 9267–9657 of the BCMV coat protein gene (Antipov et al. 2016).

29.2.4 RNA Extraction and RT-PCR Analysis

BCMV total RNA was isolated from samples using Ribo-Sorb DNA/RNA extraction kit (AmpliSens, Russia). PCR test kit Reverta-L-100 (AmpliSens, Russia) was used to generate cDNA according to the manufacturer's instructions. cDNA synthesis for RT-PCR amplification was carried out using 1 μg of total RNA in a final reaction volume of 20 μl. Reaction mix included 1× reaction buffer (containing 0.2 mM dNTPs and 1.5 mM $MgCl_2$) and 10–50 ng of cDNA and 0.5 U Taq polymerase in a final reaction volume of 15 μl. All primers were used at final concentration of 5 pmol per 20 μl in PCR. The amplification was performed in DNA thermocyclers "Tertsyk" TP4-PCR-01 (DNA Technology, Russia). Fragment sizes were determined by comparison with a 100 bp DNA LadderPlus (Fermentas, USA). The presence of amplicon of expected size predicted for primers used and corroborated by the positive control was considered positive by RT-PCR.

29.2.5 Detection of Viral Inclusions

Fluorescence microscopy (XS-3320 MICROmed, Micromed, Ukraine) with acridine orange staining was used for detection of virus inclusion body. The ratio of stain volume to cell volume was 10,000:1. The time of staining with fluorochrome (at pH 5.6) consists 5 min. The ovule tissue preparations were subsequently rinsed in distilled water (Worrall et al. 2015). The fluorescence of nucleic acids in the cells was quenched with 5% trichloroacetic acid (TCA). For this purpose, sections before staining with acridine were treated with TCA for 15 min in a water bath at 90 °C (Worrall et al. 2015).

29.3 Results and Discussion

Bean common mosaic virus is an important pathogen associated with the mosaic disease in different common bean cultivars. Virus is widespread in common bean-growing areas in Ukraine and causes substantial losses in bean crops. Our previous studies have shown that different bean varieties are not only listed in state register of plant varieties suitable for dissemination in Ukraine but also the bean breeding lines that are under the technical examination were infected by BCMV (Kyrychenko and Kovalenko 2018). Of the bean varieties tested, common beans cv. Chervona shapochka were found to be affected more adversely than other. In view of this, the aim of the study was to investigate seed transmission rates for BCMV in this cultivar as a possible reason for the high percent of plant infection revealed in field observations.

Phaseolus vulgaris cv. Chervona shapochka leaf samples showing symptoms like mosaic, wrinkles, blistering, vein-banding and downward rolling of the leaf margins, and reduced leaf size were harvested in bean-growing areas of the Kyiv region (Fig. 29.1). Infected plants exhibited permanent wilting and premature defoliation, become distorted, and produced no pods or few often empty pods (Fig. 29.2). Bean seeds from disease-infected bean plants were collected for further laboratory analyses.

Three replicates of 50 seeds collected randomly from the infected bean plants were sown in a greenhouse. A percentage of infected seedlings developed from collected seeds under insect-free conditions and evaluated for symptom appearance

Fig. 29.1 Symptoms produced by BCMV on *Phaseolus vulgaris* L. plant in field

Fig. 29.2 Symptoms of premature defoliation of BCMV-infected *Phaseolus vulgaris* plants

Table 29.1 Incidence of BCMV in common bean seeds

	Repeat experiment[a]		
Seedlings	I	II	III
Number of germinated seeds	48	49	49
Number of positive seedlings tested by infectivity test	39	37	37
Number of positive seedlings tested by RT-PCR	41	37	37
Range of seedling infection (%)	81	75	75

[a]50 seeds in each repeat were sown

of BCMV infection was 82%, 88%, and 82% in each repetitions, respectively. An overall incidence of infected plants was 85%. Presence of BCMV in all symptomatic plants was confirmed by PCR (Table 29.1).

The data obtained indicate high incidence of seed infection and transmission of BCMV in grow-out experiments with Chervona shapochka cultivar of *Phaseolus vulgaris* plants. Such level of seed infection may be due to varietal characteristics of plants (low resistance to BCMV) or the high pathogenicity of BCMV strains circulating in common bean in Ukraine, since seed-to-seedling transmission rate of BCMV previously determined in Ukraine consisted 12–35% (Mockovets' et al. 1971). It should be noted that the viral infection insignificantly influenced the seed germination and 96–98% of the seedlings were viable (Table 29.1).

Our previous studies *have* shown that BCMV isolates circulating in Ukraine differ from those reported early in their virulence and disease symptoms on test plants. It was suggested that the isolates belong to A serotype of BCMV – *bean common necrotic mosaic virus* (BCNMV) (Kyrychenko and Kovalenko 2018). By

Fig. 29.3 The symptoms of viral damage of dry bean plant raised from disease-infected seeds (**a**, **b**) and poor pod yield (**c**, above) compared to healthy plant (**c**, below)

the study conducted to assess the variability in BCMV infecting common bean in Ukraine, the isolate was classified as belonging to B serotype. The fact that the virus circulating in Ukraine is BCMV isolate was also confirmed by partial sequence of the coat protein region data. The results of seed transmissibility obtained in this study show other unusual properties of the BCMV isolate—high level of BCMV seed transmission that would demand a reassessment of the management and control strategies of viral disease caused by the BCMV. To determine whether such rate of seed transmission is a general property of the BCMV isolate, we are planning to test the ability of the virus to be transmitted by seeds of different common bean cultivar and varieties growing in Ukraine.

The first symptoms of virus infection on seedlings raised from infected seeds were noticed after 3–4 weeks as light green mosaic on first trifoliate (primordial) leaves. The affected leaves were deformed and roughed and became brittle. Such spotting and deformation of the first leaves indicate that the primary infection occurred through the seeds (Bos 1971). Sometimes only one primordial leaf appeared on the surface of the soil. On subsequent trifoliate leaves, there were clear symptoms of the disease—the color on affected leaves becomes mottled with light and dark green patches. The color of the leaf along the veins remained dark green (Fig. 29.3). Over time, through more intense growth of dark green areas and slow growth of chlorotic ones, the leaves become wrinkled and their edges curled downward. Such foliage distortion was accompanied by yellowing, vein-banding, and blistering symptom. Infected plants were stunted and weakly and possessed deformed spotted pods with a small amount of seeds, or pod formation did not occur at all (Fig. 29.3c). The symptoms of viral damage in plants raised from infected seeds

Table 29.2 Presence of BCMV in pods and infected seeds

Method of analysis	The source of the virus		
	Pods	Embryos	Seed coat
RT-PCR	+	+	+
Infectivity test	+	+	_

+ system mosaic on inoculated bean plants; – no symptoms

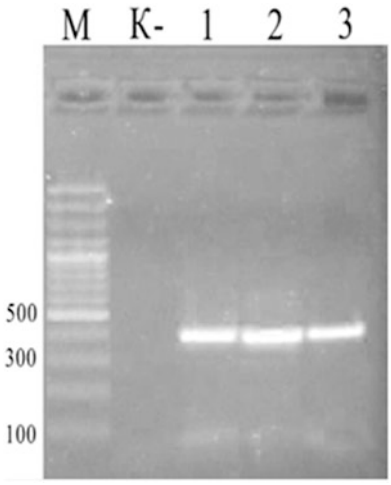

Fig. 29.4 Detection of BCMV by RT-PCR in different common bean extracts using coat protein gene-specific primers: 1, immature pods; 2, embryos; 3, seed coat; K, negative control, M, DNA length marker (GeneRuler™ 100 bp Plus DNA Ladder SM0322)

were significantly more stringent than those in plants infected mechanically under experimental conditions.

The flowers and young pods were detached from affected plants. The seed coat and germ of infected seeds were completely separated. The extracts obtained from the pods, seed coat, and embryo were used for infectivity test and total RNA extraction and reverse transcription polymerase chain reaction with virus-specific primers. Mechanical inoculation of Pervomayska bean plants to confirm extract infectivity was conducted under controlled conditions. Typical symptoms of BCMV in inoculated plants appeared in 12–16 days after inoculation with pod extracts and seed embryos. BCMV was detected by PCR in all tested samples (Table 29.2).

The absence of symptoms on plants inoculated with seed coat extracts can be explained by the low concentration of the virus on the seed surface, whereas PCR techniques reveal the presence of viral RNA in the inoculum (Fig. 29.4).

The presence of the virus in different floral parts of the infected bean was established by RT-PCR. Also, different flowers of the same plant were examined in three replicates. Thus, fragments of the BCMV genome were found in different parts of the flower (Fig. 29.5). The highest concentration of the virus was observed in the pods, calyx, and ovary. In the corolla and stamen, virus was detected at low concentrations. It was shown the presence of the viral genome in the pollen, while BCMV in bract leaf was not detected.

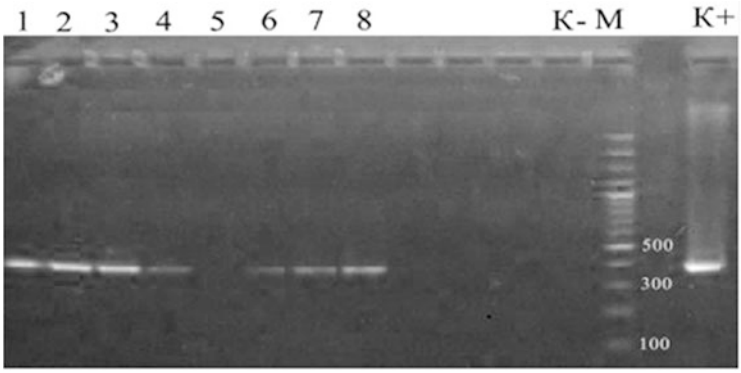

Fig. 29.5 Detection of BCMV by RT-PCR in pods and different parts of the flower using coat protein gene-specific primers: 1, pollen; 2, pods; 3, calyx; 4, corolla; 5, bract leaf; 6, pollen with stamen fragments; 7, stamen; 8, an ovary; K, negative control; K+, positive control; M, DNA length marker (GeneRuler™ 100 bp Plus DNA Ladder SM0322)

Formation of special inclusion bodies (nucleoprotein complexes) in living cells is an important diagnostic sign of the virus infection. Viral inclusions are isometric crystals 0.4–0.8 μm across or oval granular bodies with a size range of 2–6 μm in diameter (Goldin 1963). Oval granular inclusions characteristic to BCMV were detected by fluorescence microscopy in the nuclei and cell cytoplasm of the external epidermis, as well as in the middle layer of the internal integument of infected ovules of *Phaseolus vulgaris* plants (Fig. 29.6). To luminescently visualize protein inclusions, tissue treatment of the ovule with hot (90 °C) trichloroacetic acid was performed. It made possible to increase fluorescence intensity for protein inclusions and completely suppress nucleic acids glowing in the nuclei and nucleoli (Fig. 29.6a). In some cells, it has shown an accumulation of granular bodies that formed small aggregations with a size of 2.8–3.0 μm. Viral inclusions occurred less frequently in the endothelium (Fig. 29.6b). The cells of nucellus and endosperm were devoid of virions, and no inclusions were observed in these cells. The largest number of cells with viral inclusions was found in the embryo and suspension (Fig. 29.6b, d). Up to 20–27% of embryonic cells contained viral inclusions.

29.4 Conclusion

Thus, it has been found that 77% of common bean seeds are capable of transmitting the virus during germination. The data indicates a high level of seed transmission of BCMV in bean lines cv. Chervona shapochka. Based on the results obtained, it can be concluded that virus-diseased seeds have a great value in disease spreading. BCMV stains have a restricted host range, and their main host plants are usually annuals that do not withstand the winter, therefore seed transmission can serve as one of the main ways of virus survival from season to season and the source of

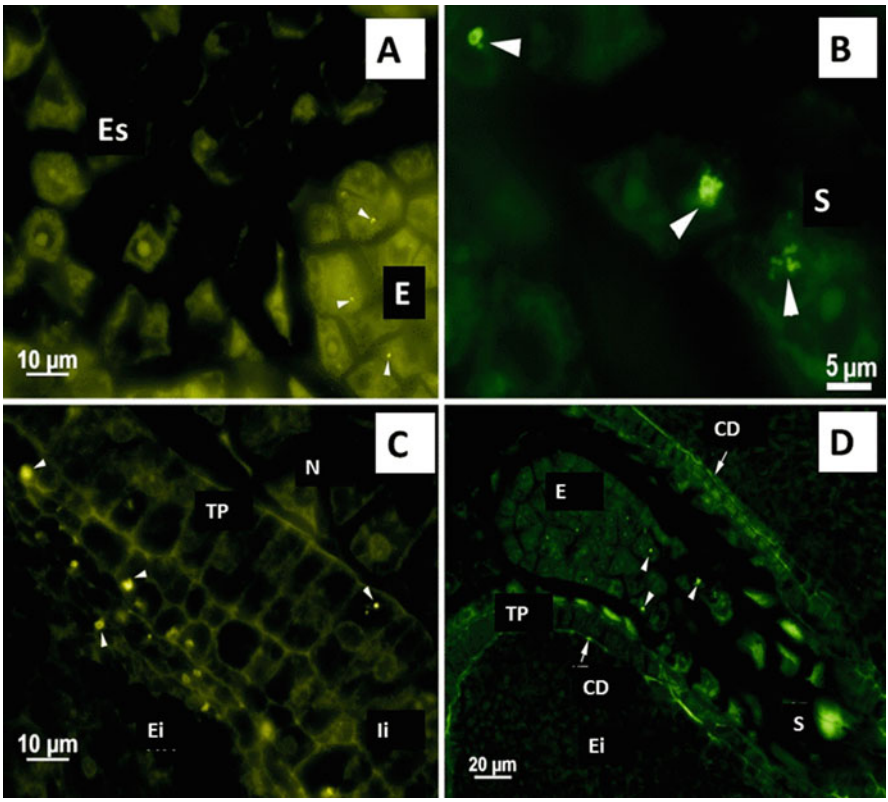

Fig. 29.6 Viral inclusions accumulated in the tissues of the embryo and ovules of *Phaseolus vulgaris*: (**a**) in the embryo; (**b**) in embryo suspensor cell; (**c**) in the outer layer of the inner integument; (**d**) cutin deposition and viral inclusion accumulation in the basal part of the embryo; *TP* integumentary tapetum, *CD* cutin deposition on the external and internal periclinal walls of the endothelium, *Ei* external integument, *Ii* inner integument, *N* nucellus, *E* embryo, *Es* endosperm, *S* suspensor. Arrows indicate the inclusion bodies. Acridine orange staining (acridine orange to a final concentration of 1:10,000)

infection for the further virus spreading in natural ecosystems The RT-PCR method revealed the presence of viral RNA in the generative organs of the plant and some parts of the seed and flower. This study shows high sensitivity of RT-PCR and selected primers that can be successfully applied for virus diagnostics in certification programs and quarantine tests. Studying the localization of viral inclusions in the tissues during seed development/maturation can be useful in establishing the likely pathways of virus transport.

As the virus is seed transmitted, planting virus-free seed can prevent primary infections. Growing cultivars with the "I" resistance gene also can provide effective protection against the BCMV, and since this gene also prevents seed transmission, it has provided valuable means of avoiding virus epidemic situation.

References

Antipov I, Hrynchuk K, Duplyak O (2016) Development of PCR systems for identification of bean common mosaic virus. Scientific herald of NULES of Ukraine. Series: biology, biotechnology, ecology 234:40–46

Bos L (1971) Bean common mosaic virus. C.M.I. A.A.B. Description of plant. Viruses 73:4

Deligoz I, Soken MA (2013) Differentiation of bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) strains infecting common bean in Samsun province. J Turk Phytopath 7:1–14

Dospekhov BA (1985) Field experience method. Agropromizdat, Moscow, 416 p

Goldin MI (1963) Viral inclusions in plant cell and the nature of viruses. Akad. Nauk USSR, Moscow, 204 p

Hampton R, Waterworth H, Goodman RM, Lee R (1982) Importance of seed-borne viruses in crop germplasm. Plant Dis 66:977–978

Hema M, Sreenivasulu P, Patil BL, Kumar PL, Reddy DVR (2014) Tropical food legumes: virus diseases of economic importance and their control. Adv Virus Res 90:431–505

Kyrychenko AN, Kovalenko AG (2018) Bean common mosaic in the Kiev region: etiology of disease and pathogen identification. Mikrobiol Z 80(4):96–107

Maule AJ, Wang W (1996) Seed transmission of plant viruses: a lesson in biological complexity. Trends Microbiol 4(4):153–158

Mockovets' SM, Kraev VG, Porembc'ka NB, Bilyk L, Baratova DF (1971) Viruses and virus diseases of legume cultures in Ukraine. Naukova dumka, Kyiv, 136 p

Nordenstedt N, Marcenaro D, Chilagane D, Mwaipopo B, Rajamaki ML, Nchimbi-Msolla S, Njau PJR, Mbanzibwa DR, Valkonen JPT (2017) Pathogenic seedborne viruses are rare but Phaseolus vulgaris endornaviruses are common in bean varieties grown in Nicaragua and Tanzania. PLoS One 12:e0178242

Sastry KS (2013) Seed-borne plant virus diseases. Springer Publishers, New Delhi, p 327

Schmidt HE (1992) Bean mosaics. In: Chaube HS, Singh US, Mukhopadhya AN, Kumar J (eds) Plant diseases of international importance, vol II. Prentice Hall, New Jersey, pp 40–73

Sipahioğlu HM, Ocak M, Usta M (2007) Comparison of three conventional extraction methods for the detection of plant virus/viroid RNAs from heat dried high-phenolic host leaves. Asian J Plant Sci 6(1):102–107

Varma A (1988) The economic impact of filamentous viruses. In: Milne RG (ed) The plant viruses: the filamentous plant viruses. Plenum Press, New York/London, pp 331–407

Walters EC (1962) Thirty eight years behind the times and still they germinate. Assoc Off Seed Anal News 36:8

Worrall EA, Wamonje FO, Mukeshimana G, Harvey JJW, Carr JP, Mitter N (2015) Bean common mosaic virus and bean common mosaic necrosis virus: relationships, biology and prospects for control. Adv Virus Res 93:1–46

Seed Biology

30

Vimlesh Rawat

Abstract

Viable seeds are living entities of the plant for the next generation. Their ability to produce new plants elevates its biological importance. A wide range of seed is consumed by human and other living being, which is the matter of its economic value. The constituents such as protein, carbohydrates and fat, which are stored in the seeds, help in the early growth and development of the plant. The quality of seed and its stored constituents directly influence the seed germination processes and the overall growth of a particular plant. It stores the genetic characteristic of a plant that determines the maintenance of that particular species in the nature. Dispersal, establishment, growth and reproduction mechanism is also based on the genetic stock provided by the seed. In short, seed is a very important stage of the plant life cycle.

Keywords

Biological importance · Economic value · Seed germination · Genetic characteristics · Genetic stock

30.1 Introduction

Plant seed acts as the reproductive unit of the seed plants and connects the successive generations. Extreme diversity is observed in the internal and external structure formation of different seeds. Seeds have numerous dispersal and germination strategies. They may also vary according to their different shapes, sizes, structures, textures and the period of viability. Plant's ability to produce seed makes them one of the most dominant components of the earth's terrestrial environment.

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Since the life begins, the humans, animals and other living beings are dependent on plants as a source of food, i.e. fruits and seeds. As fruits and seeds are consumed by humans and animals, that way they may also act as a medium of seed dispersal from one place to another via faecal matter. Wheat (*Triticum aestivum*), gram (*Cicer arietinum*), rice (*Oryza sativa*), corn (*Zea mays*), barley (*Hordeum vulgare*), etc. are the major food crops commonly used as grains worldwide. Their edible nature and nutritional values are initially recognized by the early people that encouraged them to harvest their food from such crops. They decided to grow them for food. The ability of seeds to germinate and to reproduce the plant of same genetic structure is also figured out which promotes its use to grow crops. Use of seeds ensures the massive production of new plant population. Each seed can be counted as a complete plant if it is properly grown and all the favourable conditions are given to it.

30.2 Seeds

Seeds are basically the fertilized or ripened ovules that protect the dormant embryo within and serve the functions of perennation, dispersal and reproduction of the parent plant.

30.2.1 Seed Structure

The seeds are covered by an outer seed coat and carry the embryo axis and cotyledons. The seed coat protects the embryo and the endosperm from any kind of injury and also from drying out. Seed coat might be thin and soft as in beans (*Phaseolus vulgaris*) or thick and hard as in coconut (*Cocos nucifera*). Endosperm acts as a temporary food supply that is present around the embryo in the form of cotyledons. Cotyledons are the first part that becomes visible when a seed germinates. The number of cotyledons may vary as monocotyledonous plants contain one cotyledon and dicotyledonous plants contain two cotyledons. In angiosperms, the seeds are enclosed within fruits, whereas in gymnosperms, seeds are exposed or naked.

30.2.2 Type of Seeds

The seeds are categorized into four major types:

1. Monocotyledonous albuminous seeds.
2. Monocotyledonous exalbuminous seeds.
3. Dicotyledonous albuminous seeds.
4. Dicotyledonous exalbuminous seeds.

30.2.2.1 Dicotyledonous Exalbuminous Seeds

The seed contains two cotyledons close to the embryo axis, and they are devoid of endosperm, e.g. pea, gram, etc. As in a mature green pod along the dorsal suture, the placental tissue is spread along the ventral suture, and the roundish seeds are seen arranged in two rows along the length of the pod. Each seed is attached to the placental tissue on the fruit suture by a stalk called the funicle. The funicle is narrow at the placental end but widens into a disk where it joins the seed. When the mature seed is detached, the broad end of the funicle leaves a scar on the seed called the hilum. Next to the hilum is a pinhole opening on the seed coat which is the micropyle. If the seed is soaked, wiped and then squeezed, water is seen to ooze out of this micropyle. The seed is covered by the tough seed coat which is known as testa. The delicate tegmen is completely adherent to the inner side of the testa and is not distinguishable in the mature seed. On opening the seed coat, the kernel is obtained. The kernel in dicotyledonous exalbuminous seeds is the embryo that includes two fleshy and very conspicuous cotyledons. The two cotyledons are hinged to an axis (tigellum) so that they open out like a book. The tigellum represents the axis of the future plant. One end of the tigellum is pointed and protrudes out of the cotyledons. This lies next to the micropyle and is the radicle or the rudimentary root. The protruding radicle lies under the pouch-like expansion of the seed coat and is thus visible even when the seed coat is not removed. The other end of the tigellum is the feathery plumule end which is the first apical bud of the future plant and develops into the shoot. The plumule lies in a groove inside the cotyledons. The point of attachment of the cotyledons to the tigellum is the first node on the axis, and careful observation shows the presence of the first lateral buds in the axils of the cotyledons. The portion of the tigellum just below the cotyledonary node (i.e. between radicle and node) is called the hypocotyl, and the portion just above (i.e. between node and plumule) the node is the epicotyl.

30.2.2.2 Dicotyledonous Albuminous Seeds

In this type of seeds, the food is not stored in the two cotyledons of the embryo but in the endosperm external to the embryo. It will be seen later that all embryos get their food from the endosperm which in its turn gets its food from the nucellus of the ovule. In the exalbuminous type of seeds, the embryo completely consumes the endosperm and nucellus so that they are no longer seen while the food is kept stored in the cotyledons which become swollen. In the albuminous type, the endosperm is still present, and the cotyledons are thin—acting only as food-sucking organs. Castor bean (*Ricinus communis*) is the most common example where the fruit is not a bean but a three-chambered capsule. Here, the seed coat is a hard shell of a mottled black or brown colour. The hilum is almost hidden by an outgrowth, the caruncle. The caruncle is spongy and absorbs water readily so that it may be of some use in germination: There is a distinct raphe running longitudinally down the seed from the hilum. On breaking open the shell, a white mass is found covered by a papery white membrane. This membrane is sometimes supposed to be the tegmen but has been found not to be a part of the seed coat. It is a remnant of the nucellus which has not been completely used up. Such a remnant is called the perisperm. Inside the

membrane, the whole whitish, fleshy, slightly flattened and oval mass is the endosperm which contains much oil. On cutting open the endosperm, the embryo is found to remain embedded inside. There are two thin, white cotyledons. The cotyledons show distinct vein markings like leaves. The veins leave an impression on the endosperm. The two cotyledons are hinged to the tigellum which shows the protruding radicle with a short hypocotyl behind it and the plumule hidden between the cotyledons. Among common plants, the dicotyledonous albuminous type of seed is also found in papaw (*Carica papaya*), jute, cotton, *Mirabilis*, etc.

30.2.2.3 Monocotyledonous Albuminous Seeds

Most of the monocotyledonous seeds are recognized as albuminous seeds, as they have thick, swollen endosperms that provide nourishment during the embryo development. However, it is not completely consumed during the process and acts as the nourishing tissues in seeds. Some monocotyledons such as orchids show exceptions. The large endosperm of the cereals is the most important source of starch, the principal food of all people. Rice (paddy), wheat and maize may be taken as the type seeds of this class. They are the most important cereal crops of the world. In all these, the grains are actually fruits of the caryopsis type. The rice grain is tightly covered by the husks; in wheat, the husks are loose, while in maize, the husks are short and loose so that the grains are exposed. In all the three, the outer coating of the grain is formed by the fusion of the pericarp and the seed coat. The micropyle and the hilum cannot be found because of the pericarp covering. Inside, a large endosperm forms the bulk of the grain, while a small embryo occupies a comparatively small space on one side of the base. The outermost layer of the endosperm is the aleurone layer which contains mainly protein. Of these three, maize (*Zea mays*) has the largest embryo and is the easiest to examine and dissect. Maize grains are flattened and more or less oblong.

30.2.2.4 Monocotyledonous Exalbuminous Seeds

The seeds that lack endosperm at the maturity are called exalbuminous seeds. Although all the common monocotyledonous seeds are albuminous, there are a few of the exalbuminous type. Monocotyledonous exalbuminous seeds are commonly found in the subfamily Aroideae (e.g. *Pothos* and *Amorphophallus campanulatus*) and also widely in the family Hydrocharitaceae (e.g. *Vallisneria*), Alismaceae (*Alisma plantago*), Naiadaceae, etc.

30.3 Seed Germination

The process of seed germination consists of three phases:

Phase 1: (a) Imbibition in which seed absorbs water to get hydrated
(b) Metabolic activation that includes increased respiration and protein synthesis.
Phase 2: (a) Consumption of food stock stored in seed, i.e. conversion of starch to sugars in cotyledon or endosperm

(b) Translocation of sugars for embryonic growth.
Phase 3: Cell division and growth enhancement of seedling.

Seed germination starts with the embryonic activation processes such as flow of food reserves and initiation of cell division and elongation. The embryo radicle turns into the root system, and the epicotyl converts into the shoot system of the seedling plants. Initial germination process starts with the imbibitions of water. Dry seeds that contain comparatively less amount of water (about 5–10%) need to be soaked well for 8–12 h or more as per need, so that they can absorb water properly. After imbibition, the stored food that is packaged into cytoplasmic organelles called protein bodies, lipid bodies and amyloplast gets activated and is immediately released to convert the stored food into smaller molecules. These smaller molecules can be then easily transported and converted into the energy required for the proper growth. Swollen radical is generally considered as the first indication that germination has begun. It imbibes water rapidly that encouraged the bursting of the seed coat and other such coverings that may be present. Downward growth into the soil stars with it.

The economic and ecologic importance of any seed is completely based on the successful seed germination and establishment of a normal seedling for the propagation of the plant species. Germination is considered as the most critical phase as the risk of injury, diseases and environmental stress are high during this phase of plant life cycle. Germination is a complex process during which the imbibed mature seed must quickly shift from a maturation- to a germination-driven programme of development and prepare for seedling growth (Nonogaki et al. 2010). The seed results from double fertilization of the ovule by the pollen grain. It houses both a zygotic embryo that will form the new plant and a storage tissue to supply nutrients that support seedling growth following germination. This latter storage tissue is usually triploid, e.g. the endosperms of cereal grains; however, in some species, the storage tissue may derive only from the maternal nucellus, such as the perisperm in sugar beet.

Seeds can be divided into two categories:

1. Orthodox seeds: these are long-lived seeds that can be dried to moisture contents as low as 5% without any injury. They can tolerate freezing. Ex situ conservation of these seeds for a long period is also convenient. This kind of seeds is the most commonly used in agriculture.
2. Recalcitrant seeds or unorthodox seeds: such type of seeds are not fit for ex situ conservation (Roberts 1973), as they cannot tolerate the extreme environmental conditions like drying or freezing. Long-term storage of these seeds is extremely tough, e.g. avocado, mango, lychee, cocoa, coffee, citrus, rubber, etc.

Orthodox seeds are important to study because a simple imbibition is enough for them to perform all metabolic activities without any interruption. In such seeds, preservation of embryonic cell viability in the dry state can extend over centuries (Farrant and Moore 2011; Shen-Miller 2002; Walters et al. 2010). This indicates the presence of specific mechanisms to maintain the state of metabolic quiescence in mature dry seeds while preserving their integrity to ensure that cell metabolism is

activated and restarted during germination. The mature dry seeds of most species require a period of dry storage known as after-ripening to release them from dormancy (Iglesias-Fernandez et al. 2011). Seed germination process of many plants can be influenced by various environmental factors; however internal factors of seed are also important. The knowledge of such factors and the seed biology of indigenous trees; production of seedlings from seeds would be difficult to incorporate (McDonald 2000; Hartmann et al. 1997).

30.4 Environmental Factors Affecting Seed Germination

Seed germination is widely affected by the environmental factors of a particular area. The seed performance is directly affected by the soil fertility, water content, temperature and availability of light.

30.4.1 Soil Fertility

It is mainly dependent on the availability of three major elements N, P and K. Well-fertilized plants that have received three major elements N, P and K produce more seeds than the less fertilized one. Increased nutrient availability results in an increase in seed size that might be due to the enhanced seed development rate. According to Copeland and McDonald (2001), when the effects of individual fertilization elements on seed development are considered, nitrogen has the greatest influence on seed size, seed germination and vigour. Soil pH is an edaphic factor that can influence the distribution of plants, and some plant species germinate in a wide pH range (Yazdi et al. 2013; Rezvani and Yazdi 2013), while for others, it can be a limiting factor (Amini et al. 2015).

30.4.2 Water Content

Availability of water determines the metabolic growth and seed development. Its deficits reduce the overall growth of the plant. Several studies reveal that it causes a decrease in germination percentage, leaf area and photosynthetic rate and promotes flower abortion that negatively influences the seed development and yield. Reduction in photosynthetic rate directly affects the carbohydrate content of the seeds. Long-term drought and less soil water availability result in decreased seed size and seed number (Copeland and McDonald 2001). Water stress is a key environmental factor that can cause hindrance in seed germination and other such processes.

30.4.3 Temperature

Temperature is the important one as high temperature during seed growth produces smaller seeds, while low temperature depresses seed development. Exposure to low temperature during seed growth adversely affects the seed germination and vigour. High temperatures are recognized as the main factor for the forced maturation in some plants. This phenomenon is also applicable on water-deficit plants where inappropriate timing during maturation is observed. The occurrence of greenish seeds is undesirable because this abnormality results in decreases in seed germination and vigour (Copeland and McDonald 2001). Temperature is known to be one of the most influential environmental factors for seed germination (Finch-Savage and Leubner-Metzger 2006). Temperature requirement of different plants might be different as some need high temperatures, while others need specific low temperatures. However, germination of some plants increases with the alteration in temperature (Bazzaz 1979). Temperature also affects germination by altering the gibberellins and abscisic acids in the seed (Finch-Savage and Leubner-Metzger 2006).

30.4.4 Light

Solar radiation and its distribution are a fundamental factor that assures the complete plant growth. In short, reduction in sunlight results in reduced plant growth and comparatively smaller seeds (Copeland and McDonald 2001).

30.5 Seed Dormancy

It is an important phase of the seed cycle where a seed lacks the germination process even though all the required factors such as humidity, temperature, light and oxygen are provided (Bonner 1984). Seeds that have hard seed coats or lack of supply and enzyme activity (internal dormancy) tend to show dormancy for germination. The production of many seed crops is controlled by the seed dormancy. Various physical and chemical methods are applied to pre-treat the seeds to overcome dormancy. Dormancy is an important trait of seeds that might be gained during evolution to deal with adverse or stress conditions such as heat, cold, less water and saline conditions. Seed dormancy allows plant species to adapt to different geographical regions that have wide variations in precipitation and temperature. Dormancy has a significant role in the development of new species and the successful dispersal of existing species (Baskin and Baskin 1998). Seed dormancy can be mainly categorized into three types:

30.5.1 Quiescent Seed Dormancy

This is a unique trait of quiescent or non-dormant seeds, which tend to go back to the dormant state if any kind of adversity is observed (Bradford et al. 2000). It is one of the most desired qualities of the seeds when they have to survive in wild conditions (Hartmann et al. 1997).

30.5.2 Structural Dormancy

This type of seed dormancy might be due to the hard seed coat structure that prevents the inward flow of water and air and outward dispersal of endogenous germination inhibitors. It can also be due to the inactivity of internal enzymes or inhibiting chemicals inside the seed. California lilac (*Ceanothus*), sumac (*Rhus*), manzanita (*Arctostaphylos*) and members of the legume family are some common examples of seed coat dormancy. Trees such as *Podocarpus falcatus*, *Olea europaea* and various *Acacia* species have seeds with hard and impervious seed coats (Hartmann et al. 1997). Seed dormancy of such seeds is cracked by some mechanical methods that help in seed germination. Seeds of *Prunus africana* germinate by the seed coat scarification (Negash 1995). Scarification, hot water, dry heat, fire, acid and other chemicals, mulch and light are the methods used for breaking seed coat dormancy (Emery 1987).

30.5.3 Physiological Dormancy or Embryo Dormancy

In this type of seed dormancy, the embryo growth and seed germination are prevented until the chemical changes occur in the seed. Physiological dormancy is indicated when an increase in germination rate occurs after an application of gibberellic acid (GA3) or after dry after-ripening or dry storage. Abscisic acid (ABA) is the most common inhibitor. This kind of dormancy can be broken by some special treatment to induce the active growth. In some seeds, a cold temperature (1–7 °C) application is enough to break the dormancy (Bradford et al. 2000). In some plant species, dormancy can be broken by a specific quality of light, i.e. germination induction is observed in lettuce seeds when they are exposed to red light (about 660 nm), and it shows inhibition when exposed to red light (730 nm) (Negash 2004). Species such as sugar maple (Enu-Kwesi and Dumbroff 1978), Norway maple (*Acer platanoides* L.) (Tillberg and Pinfield 1982), planetree maple (*Acer pseudoplatanus*) (Webb and Wareing 1972), European hazel (*Corylus avellana* L.) (Williams et al. 1973), white ash (*Fraxinus americana* L.) (Sondheimer et al. 1968), apple (*Malus pumila* Mill.) (Singh and Browning 1991) and northern red (*Quercus rubra* L.) and English oaks (Szczotka 1977) have abscisic acid (ABA) as an internal inhibitor.

30.6 Fruit and Type of Fruits

Fruits are the outcome of the transformation of an ovary either in dry and hardened or enlarged and fleshy form. This process is commonly induced by hormonal activities along with seed formation. The shape, size and structure of a fruit in a particular plant usually depend on the nature of the ovary from which it develops. A fruit can be classified on the basis of several features. In case of the composition, a fruit is called the true fruit if it develops from ovary tissue only. If the fruit includes modified tissues of other floral parts, it will be termed as accessory fruit. A simple fruit is originated from the single ovary; an aggregate fruit, i.e. raspberry, strawberry, etc., develops from several ovaries of a multipistillate flower; a multiple fruit, e.g. pineapple, forms from the ovaries of several different flowers within an inflorescence. The texture of fruit also varies as some fruits are juicy and fleshy, whereas others are dry in nature.

30.7 Seed Dispersal

Numerous mechanisms are present in nature and are well adapted to enhance the seed dispersal (Ridley 1930; Van der Pijl 1982). The structure of seed is the key to interpret the most favourable mode of dispersal. The structure of seed is the key to interpret the most favourable mode of dispersal; structures like flotation devices help them to travel by rivers or waterbodies, diaspores with wing or plumes are dispersed by air, seeds with hooks or sticky surfaces able to hitchhike on the fur of mammals, explosive opening of fruits that project their own seeds, or seeds with a nutritive reward that attract foraging animals which in turn transport the seeds in their gut.

Geographical conditions, climatic conditions and type of ecosystem play an important role to decide the different dispersal guilds, worldwide. In temperate regions, birds and mammals plays an important role in seed dispersal (Herrera 1995), while in tropical and subtropical regions, reptiles plays an important role in seed dispersal (Olesen and Valido 2003; Nogales et al. 2005) because large mammals are commonly absent there (Williamson 1983; Whittaker and Fernandez-Palacios 2007). The practice of seed dispersal is basically habitat dependent, i.e. in wet tropical forests, most of the seed dispersal is carried out by vertebrates as their proportion is high in that region (Willson et al. 1989); similarly in sclerophyllous biomes in southern hemisphere, the high frequency of dispersal is caused by various ant species (Rice and Westoby 1986).

The plant habit differently affects the ability of a particular dispersal mechanism, e.g. seeds of taller tress are more frequently adapted to wind dispersal, while small plants often choose ballistic mechanisms (Thompson and Rabinowitz 1989; Willson et al. 1990). Seed dispersal is a sequential transportation by several steps, which have remarkable effect on the final result. A phenomenon known as diplochory or secondary seed dispersal (reviewed in Vander Wall and Longland 2004) is considerably less studied than ‘traditional’ single-vector dispersal, due to some challenges; it seems to be essential in the reproduction of some plants (Chambers and

Macmahon 1994). Secondary seed dispersal systems are widely based on the potential combinations of various possible abiotic and biotic dispersal agents.

30.8 Conclusions

The development of seed represents a significant transformation for photosynthetic organisms. Its shape, size and structure widely depend on the nature of the ovary from where it develops. The external covering and the internal structure define the dormacy characteristics of a particular plant species. Dispersal mechanism is also based on the structure of the seed, and mode is mainly dependent on geographical conditions. The process of seed germination is a very important phase and is affected by multiple factors such as the type of seed, its structure, state of dormancy, etc. In short the basic characteristics of seeds define their way to germination and development and even for production.

References

Amini V, Zaefarian F, Rezvani M (2015) Interspecific variations in seed germination and seedling emergence of three *Setaria* species. Rev Bras Bot 38:539–545

Baskin CC, Baskin JM (1998) Germination ecology of seeds with nondeep physiological dormancy. In: Baskin CC, Baskin JM (eds) Seeds: ecology, biography, and evolution of dormancy and germination. Academic Press, San Diego, CA, pp 57–64

Bazzaz FA (1979) The physiological ecology of plant succession. Annu Rev Ecol Syst 10:351–371

Bonner FT (1984) Glossary of seed germination terms for tree seed workers. Gen. Tech. Rep. SO-49. USDA Forest Service, Southern Forest Experiment Station, New Orleans, p 4

Bradford KJ, Chen F, Cooley MB (2000) Gene expression prior to radicle emergence in imbibed tomato seeds. In: Black M, Bradford KJ, Vazquez-Ramos J (eds) Seed biology advances and applications. Proceedings of the sixth international workshop on seeds, Mérida, México, 1999. CABI Publishing, pp 18–30

Chambers JC, Macmahon JA (1994) A day in the life of a seed - movements and fates of seeds and their implications for natural and managed systems. Annu Rev Ecol Syst 25:263–292

Copeland LO, McDonald MB (2001) Principles of seed science and technology, 4th edn. Kluwer Academic Publishers, Boston, 467p

Emery DE (1987) Seed propagation of native California plants. Santa Barbara Botanic Garden, Santa Barbara, CA

Enu-Kwesi L, Dumbroff EB (1978) Changes in abscisic acid in the embryo and covering structures of *Acer saccharum* during stratification. Z Pflanzenphysiol 86(4):371–377

Farrant JM, Moore JP (2011) Programming desiccation-tolerance: from plants to seeds to resurrection plants. Curr Opin Plant Biol 14:340–345

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523

Hartmann HT, Kester DE, Davies FT et al (1997) Plant propogation: principles and practices. 6th edn, Prentice Hall, Englewood Cliffs, NJ

Herrera CM (1995) Plant-vertebrate seed dispersal systems in the Mediterranean: ecological, evolutionary, and historical determinants. Annu Rev Ecol Syst 26:705–727

Iglesias-Fernandez R, Rodriguez-Gacio MC, Matilla AJ (2011) Progress in research on dry afterripening. Seed Sci Res 21:69–80

McDonald MB (2000) Seed priming. In: Black M, Bewley JD (eds) Seed technology and its biological basis. Plenum Press, New York
Negash L (1995) Indigenous trees of Ethiopia: biology, uses and propagation techniques. SLU Reprocentralen, Umeå, 285p
Negash L (2004) Rapid seed-based propagation method for the threatened African cherry (*Prunus africana*). New For 27(3):215–227
Nogales M, Nieves C, Illera JC, Padilla DP, Traveset A (2005) Effect of native and alien vertebrate frugivores on seed viability and germination patterns of *Rubia fruticosa* (Rubiaceae) in the eastern Canary Islands. Funct Ecol 19:429–436
Nonogaki H, Bassel GW, Bewley JD (2010) Germination—still a mystery. Plant Sci 179:574–581
Olesen J, Valido A (2003) Lizards as pollinators and seed dispersers: an island phenomenon. Trends Ecol Evol 18:177–181
Rezvani M, Yazdi SA (2013) Factors affecting seed germination of black nightshade (*Solanum nigrum*). Acta Bot Hungar 55:397–408
Rice B, Westoby M (1986) Evidence against the hypothesis that ant-dispersed seeds reach nutrient-enriched microsites. Ecology 67:1270–1274
Ridley HN (1930) The dispersal of plants throughout the world. L. Reeve & Co., Kent, UK
Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Technol 1:499–514
Shen-Miller J (2002) Sacred lotus, the long-living fruits of *China Antique*. Seed Sci Res 12:131–143
Singh Z, Browning G (1991) The role of ABA in the control of apple seed dormancy re-appraised by combined gas chromatography-mass spectrometry. J Exp Bot 42:269–275
Sondheimer E, Tzou DS, Galson EC (1968) Abscisic acid levels and seed dormancy. Plant Physiol 43:1443–1447
Szczotka Z (1977) Changes in the activity of indole acetic acid and abscisic acid in the embryo axes and cotyledons of *Quercus borealis* Michx. and *Quercus robur* L. acorns stored under controlled conditions. Arboretum Kornickie 22:257–273
Thompson K, Rabinowitz D (1989) Do big plants have big seeds? Am Nat 133:722–728
Tillberg E, Pinfield NJ (1982) Changes in abscisic acid levels during, after-ripening and germination of *Acer platanoides* L. seeds. New Phytol 92:167–172
Van der Pijl L (1982) Principles of dispersal in higher plants. Springer-Verlag, Berlin
Vander Wall SB, Longland WS (2004) Diplochory: are two seed dispersers better than one? Trends Ecol Evol 19:155–161
Walters C, Ballesteros D, Vertucci VA (2010) Structural mechanics of seed deterioration: standing the test of time. Plant Sci 179:565–573
Webb DP, Wareing PF (1972) Seed dormancy in Acer: endogenous germination inhibitors and dormancy in *Acer pseudoplatanus* L. Planta 104:115–125
Whittaker RJ, Fernandez-Palacios JM (2007) Island biogeography: ecology, evolution, and conservation. Oxford University Press, Oxford
Williams PM, Ross JD, Bradbeer JW (1973) Studies in seed dormancy: 4. The abscisic acid content of the seeds and fruits of *Corylus avellana* L. Planta 110:303–310
Williamson M (1983) Island populations. Oxford University Press, Oxford
Willson MF, Irvine AK, Walsh NG (1989) Vertebrate dispersal syndromes in some Australian and New Zealand plant communities, with geographical comparisons. Biotropica 21:133–147
Willson MF, Michaels HJ, Bertin RI, Benner B, Rice S, Lee TD, Hartgerink AP (1990) Intraspecific variation in seed packaging. Am Midl Nat 123:179–185
Yazdi SAF, Rezvani M, Mohassel MHR, Ghanizadeh H (2013) Factors affecting seed germination and seedling emergence of sheep sorrel (*Rumex acetosella*). Romanian Agric Res 30:373–380

Links

http://www.biologydiscussion.com/seed/types-seed/types-of-seed-4-important-types-with-diagram/13129
https://www.cabrillo.edu/~ncrane/bio1c/botPDFs/SeedsandFruitskey.pdf
http://ag.arizona.edu/pubs/garden/mg/botany/seeds.html
http://en.wikipedia.org/wiki/Seed

Seedborne Diseases and Its Management

31

Sandhya Rai, Amod Kumar, Indrakant Kumar Singh, and Archana Singh

Abstract

Among all the crop diseases, seedborne diseases occur most commonly. Seedborne diseases are one of the major issues leading to low crop yield. A number of pathogens like viruses, bacteria, and fungi are the main causative agents of seedborne diseases. There are various modes of seed infection either at the dormant stage or at maturity stage that reasons abnormalities in seeds. These pathogens are continually transferred from one generation to another with seeds and grounds for crop loss every season. The most effective way for protection from seedborne diseases at an early stage is by detecting and controlling pathogens. Disease-free seeds are very important for high yield. Across the world, most of the cereals are grown from seeds. Seeds are traded across the countries, which lead to the transmission of seedborne disease and an efficient way to plant disease dispersal. Therefore, strong seed managements will be very important to cope up with increased food demand. These seed diseases are mostly controlled by physical and chemical treatments, but use of chemicals is hazardous to seeds and to the environment. Therefore, nowadays, biocontrol agents are recommended for controlling seedborne diseases. New methods are constantly being developed to make disease-free seeds.

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Seedborne diseases · Physical control · Chemical control · Biocontrol · Detection of seedborne diseases

31.1 Seedborne Diseases

31.1.1 Introduction

Seed plays an indispensable role in the production of healthy crops. Seed is the "unit of reproduction," is an "embryonic plant" enclosed within protective layers, and is capable of growing into another such angiospermic plant. History tells us that early man started domesticating certain plants of his choice around him, and today, we witness that seeds are an indispensable part of human society, be it as food or a means of propagation. About 90% of all the food crops are propagated through seeds, and thus, good-quality seeds are the backbone of proliferating agrarian economies. Seeds are often associated with microorganisms which projects high risks for the health of the seed as well as the plant grown from them that causes negative influence on the yield potential leading to severe economic losses. Seedborne pathogens cause disease at any stage of plant development pathway from germination to crop maturity.

Uncontaminated seed is the prime prerequisite in starting crop production as advised by the agricultural scientists as well as the plant pathologists, and it seems logical too, yet it is the most difficult target to achieve (Dutta et al. 2014). Seeds provide an efficient means for the transfer of plant pathogens across vast distances, natural barriers, and geo-political borders leading to the introduction of pathogens to new areas as well as re-emergence of past diseases. Paul Neergaard and Mary Noble coined the term "seed pathology" in the 1940s (Nameth 1998). Paul Neergaard is regarded as the father of seed pathology. He authored books named *Seed Pathology* which is the standard textbook of seed pathology which is referred all across the world in which the author detailed vast number of subject-related issues (Neergard 1977). Seed pathology is the study of various seedborne pathogens and diseases caused by them.

During the middle of the eighteenth century, many experimental evidences were produced which proved the farmers' suspicions of "seeds" being the carrier of various disease agents to be true (Baker and Smith 1966). Hellwig (1931) first demonstrated a seed carrying pathogen while working on *Claviceps purpurea* of rye. Micheli (1723) showed seed transmission of a pathogen of bean, *Orobanche minor*. Internal seed transport was demonstrated by Needham (1745) with *Anguina tritici* on wheat, while Frank (1883) gave evidence of internal seed transmission of *Colletotrichum lindemuthianum* on bean. Tillet (1755) studied external seed transmission of *Tilletia* sp. in wheat.

31.2 Seed-Infecting Pathogens

A seedborne pathogen is a disease-causing agent associated with the seed and results in the buildup of symptoms and signs of the disease in any stage of the plant growth. The disease of a seed is thus a result of the immunologic interaction of a susceptible host, a potential pathogen, and suitable environmental conditions. Seedborne pathogen includes bacteria, fungi, viruses, etc. The pathogen can be carried on or within the seeds. Some pathogens are externally seedborne as they are present on the outer surface of the functional part of the seed like *Ustilago segetum* var. avenae and *U. segetum* var. segetum are located on the outer surface of barley and oats, respectively. On the other hand, some pathogens are internally seedborne as they invade the internal functional part of the seeds like *U. segetum* var. tritici that is localized inside the barley seeds. Some can be both externally and internally seedborne like the pathogen of downy mildew of pearl millet, *Sclerospora graminicola* (Agarwal and Sinclair 1997). Many seedborne infecting pathogens initiate the infection when the seeds are sown, and this drastically reduces the germination potential and crop yield. It causes seed decay as well as the damping off during pre- and post-emergence.

The seed primordium may be infected (1) directly from the plant surface or from the infected plant through seed stalk or fruit stalk or flower stalk, or (2) infection may be introduced from outside through ovary wall or stigma and the fruit stalk and then through the seed coat, or (3) seed infestation or concomitant contamination may occur during harvesting, thrashing, processing, transportation, or other storage procedures. The extent of damage is responsible on various factors like the type of pathogen, environmental conditions, and storage conditions (Fig. 31.1). Christensen and Kaufmann (1965) had divided the plant pathogens into two types:

1. When infection occurs during any of the development stage of the crop plant before harvesting, then it is called as field pathogens. Symptoms of the infection can be unnoticeable till the time of the harvest.
2. When infection occurs during storage, then it is called as storage pathogen. This infection can be caused from the same field pathogen or any other one.

31.2.1 Bacteria

A number of seedborne bacterial pathogens have been studied, but the study cannot outnumber that of the fungal pathogen. That's why strategies for bacterial pathogen management are inadequate and, thus, seedborne bacterial pathogens are of particular concern (Gitaitis and Walcott 2007) (Table 31.1). Historically, the seedborne infecting nature of *Xanthomonas campestris* pv. *phaseoli* was proved by Beach (1892) in the bean seeds. Skoric (1927) did the classic experimentation of bacterial seed infection while studying bacterial blight of pea (*Pseudomonas syringae*

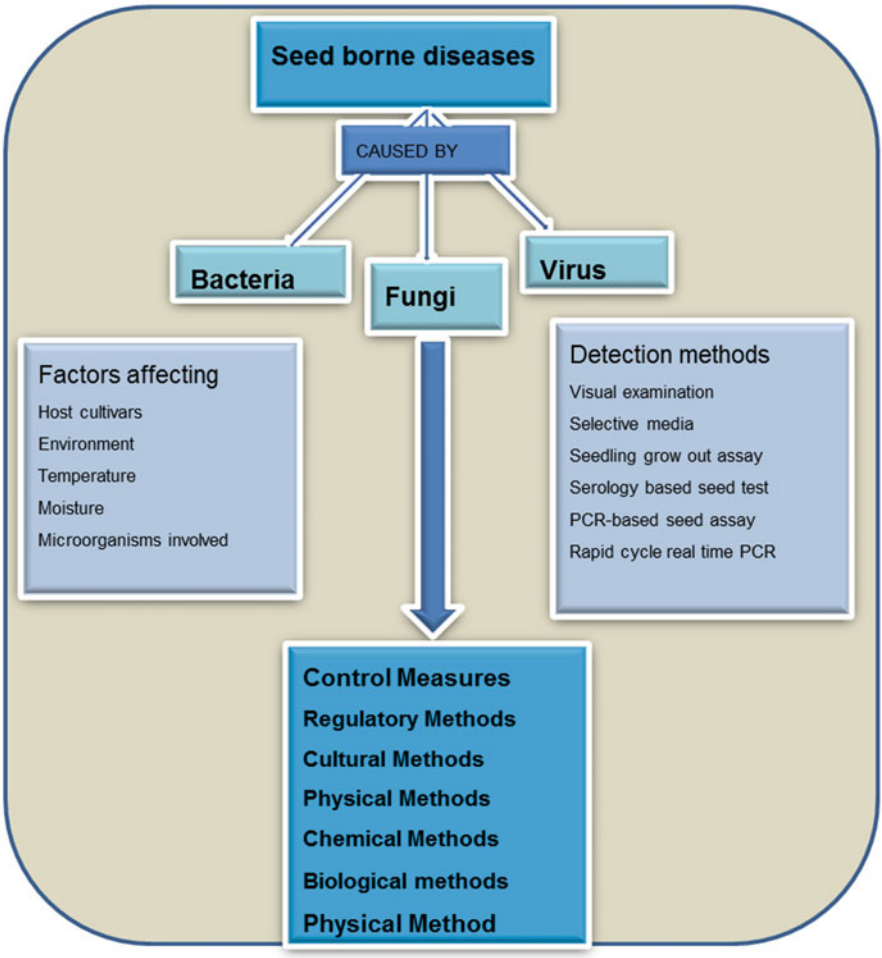

Fig. 31.1 Seedborne diseases: causative agents, factors influencing the efficacy of the disease, detection methods, and control measures

pv. *pisi*). He inoculated the flowers developing into pods with the aqueous suspensions of bacteria and investigated if seeds could carry the bacterial infections. As a result, it was found that bacteria had moved into the seed coat where cavities containing large number of bacteria were formed. A fruitful functional management strategy can be proposed if the location of the pathogen within the seed is known (Verma and Agrawal 2018). The location of seedborne bacterial pathogens depends not only on the environmental conditions but also on the host cultivar and mode of infection.

Bacteria panicle blight caused by *Burkholderia glumae* (primary cause) or *B. gladioli* is one of the most serious diseases that has affected more than 18 countries

Table 31.1 Seeds and their pathogens

Seed	Pathogen	Reference
Bacterial pathogens		
Bean seeds	*Xanthomonas campestris* pv. *phaseoli*	Beach (1892)
Pea	*Pseudomonas syringae* pv. *pisi*	Skoric (1927)
Rice	*Burkholderia glumae* or *B. gladioli*	Zhou (2019), Zhou-qi et al. (2016)
Potato, tomato, alfalfa, maize, and beans	*C. michiganensis* subsp. *michiganensis* *C. michiganensis* subsp. *sepedonicus* *C. michiganensis* subsp. *insidiosus* *C. michiganensis* subsp. *nebraskensis* *C. michiganensis* subsp. *tessellarius* *C. michiganensis* subsp. *phaseoli*	Li et al. (2017)
Cereal grains	*Pseudomonas fuscovaginae*	Quibod et al. (2015)
Fungal pathogens		
Wheat	*Tilletia indica*	Warham et al. (1990)
Rice	*Alternaria padwickii* *Alternaria zinniae*	Garnbogi et al. (1976)
Maize	*Fusarium* sp. *Aspergillus* sp.	Tsedaley and Adugna (2016)
Cereals	*Aspergillus*	Begum et al. (2013)

of Asia, Africa, and North and South America and, thus, is a global disease in rice. It is a case of shifting of disease from minor plant disease to major one due to the changed environmental conditions. High temperature as well as high humidity or frequent rain leads to infection and disease. The disease is highly destructive leading to around 75% loss in yield as well as the milling quality (Zhou 2019; Zhou-qi et al. 2016). Five out of seven subspecies of *Clavibacter michiganensis* are well-known pathogens, viz., *C. michiganensis* subsp. *michiganensis* (causing bacterial canker and wilt in tomato); *C. michiganensis* subsp. *sepedonicus* (causing bacterial ring rot in potato); *C. michiganensis* subsp. *insidiosus* (causing wilt and stunting in alfalfa); *C. michiganensis* subsp. *nebraskensis* (causing wilt and blight in maize); and *C. michiganensis* subsp. *tessellarius* (causing leaf freckles and spots in wheat) with a recent addition of *C. michiganensis* subsp. *phaseoli* (causing bacterial yellowing of leaves in bean) (Li et al. 2017). Movement across the geo-political borders may lead to the access of much more favorable environmental conditions for the pathogen to sustain and proliferate like *Pseudomonas fuscovaginae*, a gram-negative bacterium causing sheath brown rot along with grain discoloration (Quibod et al. 2015).

31.2.2 Fungi

Halfon-Meiri (1978) reviewed the consequences of seedborne fungi on germination. The causative agent of Karnal bunt in wheat is *Tilletia indica*; the infected seeds have lower survival rate in storage than the noninfected one (Warham et al. 1990). *Alternaria padwickii* leads to the decay of rice seeds and death of the young seedlings. Superficial seed infection of host plants by *Alternaria zinniae* causes post-emergence disease, while severe infection can lead to pre-emergence death (Garnbogi et al. 1976). Around 11% of total maize production faces grain loss due to several diseases caused by fungi like *Fusarium* sp. and *Aspergillus* sp. (Tsedaley and Adugna 2016).

Christensen and Kaufmann (1965) had categorized seedborne infecting fungi into two distinct categories: (1) field fungi and (2) storage fungi. Few examples of field fungi are *Fusarium* sp., *Alternaria* sp., *Cladosporium* sp., and *Verticillium* sp. Fungal invasion deteriorates the quality of the seeds as it may lead to seed discoloration, reduction of the germination potential, and development of disease of the seedlings. Storage fungi invade grains or seeds during storage. Storage fungi can be already present on the seeds going for storage or can invade on spilled seeds during harvesting, transporting, or storage structures. The most common fungi causing seedborne infections are *Aspergillus*, *Penicillium*, *Fusarium*, *Tilletia*, etc. Additionally, some fungi are associated with another spoilage problem as they also produce toxin substances called as mycotoxin (Judd et al. 2002). A wide variety of seedborne fungi produce dangerous mycotoxin in some part of their life cycle. *Aspergillus*, a common fungus pathogen, causes aflatoxin contamination in poorly stored seeds and grains like cereals, cottonseeds, and groundnut in tropical and subtropical areas across the world (Begum et al. 2013). Sometimes, insect-damaged seed samples showed increased levels of mycotoxins as Sinha and Sinha (1992) showed comparatively increased occurrence of *Aspergillus flavus* as well as high levels of aflatoxins in insect-damaged maize samples than the undamaged ones (Table 31.1).

31.2.3 Viruses

Apart from bacterial and fungal seedborne pathogens, viruses have also established themselves as potential disease-causing organisms in plants. Seedborne viruses are very small, infectious particles composed of two basic virus structure subcomponents: a protein coat and a nucleic acid (Table 31.2). Different factors like host cultivar, environmental factor, vectors, and their interactions as well as the type of virus play an important role in the disease establishment (Sastry 2013). More than 231 plant viruses and viroid diseases are reported worldwide.

Out of 140 viruses infecting cowpea, at least 15 have been studied as seedborne possessing RNA genomes (Salem et al. 2010), which are distributed worldwide and responsible for great loss of the crop. A majority of these viruses uses insect as the vectors. Many viruses like *bean common mosaic virus* (BCMV), *peanut stunt virus*

Table 31.2 Seed-infecting viruses and their hosts

Virus	Vector	Seed	Reference
Cowpea aphid-borne mosaic virus (CABMV), *bean common mosaic virus* (BCMV), *cucumber mosaic virus* (CMV), and peanut stunt virus (PSV)	Aphid	*Vigna unguiculata*	Salem et al. (2010)
Cowpea mild mottle carlavirus (CPMMV)	Whiteflies	*Vigna unguiculata*	Brunt and Kenten (1973), Jeyanandarajah and Brunt (1993), Salem et al. (2010), Naidu et al. (1998)
Cowpea mosaic virus (CPMV), *cowpea severe mosaic virus* (CPSMV), *southern bean mosaic virus* (SBMV), and *cowpea mottle virus* (CPMoV)	Beetle	*Vigna unguiculata*	Salem et al. (2010)
Bean common mosaic virus (BCMV) Bean common mosaic necrosis virus (BCMV)	Aphid	*Phaseolus vulgaris*	Nordenstedt et al. (2017)
Southern bean mosaic virus (SBMV)	Beetle	*Phaseolus vulgaris*	Nordenstedt et al. (2017)

(PSV), *cucumber mosaic virus* (CMV), and *cowpea aphid-borne mosaic virus* (CABMV) are aphid-transmitted, while *cowpea mottle virus* (CPMoV) and *southern bean mosaic virus* (SBMV) are beetle-transmitted (Salem et al. 2010).

31.3 Factors Affecting Seedborne Infection

Seedborne infecting pathogens can infect in the field itself; however, the development of the disease as well as the expression of the symptoms can occur at any stage before the final consumption. Prior to the consumption, chances of the exposure of seedborne pathogens are high in all the steps of harvesting, extraction, threshing, selection, packing, transportation, or storage. Microbial infections are responsible for great loss of the quality as well as quantity of the stored food. Infections can be partial or total and, sometimes, can lead to the accumulation of toxin substance secreted by the microorganisms also. There are two types of seedborne pathogen: (1) adhering to the outer covering of the seed and (2) borne inside the seed. A severe infection by the seedborne pathogen significantly decreases the germination potential of the seeds as well as the growth of the seedlings, which finally leads to the overall decline in the yield of the crops.

Many of the seedborne pathogens have been recognized as fungi (Wallen and Sutton 1965). Christensen and Kaufmann (1965) categorized seedborne infecting fungi into two types: field and storage fungi (1). When infection occurs during any of the development stage of the crop plant before harvesting, then it is called as field pathogen. Symptoms of the infection can be unnoticeable till the time of the harvest

(2). When infection occurs during storage, then it is called as storage pathogen. This infection can be caused from the same field pathogen or any other one. Fungus usually attacks and establishes as a complex successional species. Among them, drought-resistant (xerophilic) species play an important role as pioneer species that raises the moisture content of the surrounding with its metabolic activities which further creates right environmental conditions for the growth of hydrophilic fungi as well as other microorganisms. This also includes various mycotoxin-producing fungal species (Hocking, 2003).

Around 1500 seedborne microorganisms and viruses have been observed in about 600 genera of crop plants (Agarwal and Sinclair 1997). One of the important epidemiological steps is the successful establishment of the seedborne infecting pathogen on the seeds which may be in the filed or during transit and storage. Several environmental factors apart from the intrinsic changes in the seeds are responsible for initiation and development of seedborne diseases. The factors contributing to the establishment of seedborne inoculum are:

31.3.1 Host Cultivars

Every gram of seed may be bearing millions of microorganisms including bacteria, actinomycetes, yeast, and fungi (Hyde 1950). Out of these, majority of microorganisms live on the surface of the seed, and only few can invade the seed internally. The initiation of seedborne infection in the field depends on the cultivar, which means whether the host cultivar is resistant (infection may not occur) or susceptible (infection may occur). For example, the formation of loose smut by pathogen, *Ustilago tritici*, may not occur in resistant wheat cultivars due to embryo resistance (Agarwal and Sinclair 1997). *Tilletia caries* (causes bunt in wheat) penetrates both susceptible and resistant seedlings but does not develop infection in the resistant cultivars beyond epidermal cells. The causative organisms of bacterial blight of soybean is *Pseudomonas syringae* pv. *glycinea* which shows an excellent example of pre-emergence host-pathogen specificity, and it was reported that larger populations grow on germinating seeds of a susceptible host cultivar than a resistant one.

31.3.2 Environment

Moisture and temperature are the prime environmental factors not only for seed germination but also for the establishment of seedborne pathogen in the host plant, the infection process, and the increase in population density for further spreading. After harvest, seeds are stored under dry conditions, so no free moisture is available. Most of the pathogens require relatively high moisture content to grow and multiply, but, seeds and grains have very low moisture content (12–14%). The normal requirement of relative humidity for bacteria and fungi is 90% and 75%, respectively. Fungi are active under varied temperature conditions. However, fungi such as

Fusarium, *Cladosporium* and *Alternaria spp*. infect seeds on growing crops but they cannot grow under storage conditions, since the moisture content is very low. During storage or transportation, seeds are attacked by different species of *Penicillium* and *Aspergillus*, which can thrive well at moisture content of 70–90%. The higher the moisture and temperature, the more rapid is the deterioration of the quality of seeds (Harman 2000) since the conditions induce microbial growth.

A number of storage fungi including many species of *Aspergillus* infect the embryo reducing the germination potential noticeably. The deterioration of stored food sometimes increases the temperature drastically up to 70 °C which further create the suitable environmental conditions for many thermotolerant and thermophilic seedborne fungi species. Apart from fungi, various bacteria and virus species also found suitable growing conditions. The favorable growth conditions of temperature and relative humidity encourage enormous multiplication of the pathogens. Some fungi are also known to produce mycotoxins, which are harmful not only for the plant but also for the animals as well as humans. Wind-blown rain is also an essential component of the ecological need for the spread of some pathogens like *Pseudomonas syringae* pv. *glycinea* responsible for bacterial blight of soybeans (Daft and Leben 1972).

31.3.3 Microorganisms Involved

Although microbial growth in dried stored seeds with very less moisture content does not occur and infecting pathogen remain in the quiescent stage (Harman 2000), but, xerophilic fungi such as *Aspergillus halophilicus* can grow even in such environment. The metabolic activities of the pioneer xerophilic fungi can increase the moisture content which further becomes the conducive environment for *A. candidus*, *A. restrictus*, *A. glaucus*, *A. flavus*, *Penicillium* sp., etc. Shortly after arrival at the seeds or grain stores, the seeds are very much prone to the infection unless the relative humidity of the stored seeds is maintained very low. Insects also play their part in bringing inoculum to the stored seeds.

31.4 Detection of Seedborne Pathogens in the Stored Food

Visual examination: It is a 5–10-min process which requires an experienced observation and identification of infected seeds having visual symptoms like rotting, discoloration, shriveling, deviation from normal size, etc. However, microorganism-infected seeds may show no visible symptoms of the developing disease, so this decreases the detection sensitivity of the method to very low. The conventional standard blotter method (for fungi) and agar-plate method have their own importance in detecting seedborne fungi. *Selective media*: The surface-sterilized seeds or seed-wash liquid is plated directly on the selective media and incubated for the required time period. It takes around 2–15 days to observe any pathogen growth on the media, and then it is isolated and identified. King et al.

(1956) reported the production of bluish green fluorescent pigments by florescent *Pseudomonas* sp. and that of dark and muriform conidia by fungus *Alternaria* sp. both in the King's B medium. The method has low to moderate sensitivity. However, the seedling grow-out assay takes time (2–3 weeks), but it is highly accurate. The seedling grow-out assay is highly accurate but it is time consuming (takes 2–3 weeks) and precise identification of microbes may not be possible.

Another powerful and quick (few hours) method is serology-based seed test in which antibodies are generated against specific antigens. Currently, this is the most effective method for detecting seedborne viruses (Walcott 2003). Polymerase chain reaction (PCR)-based seed assays are highly specific and less time taking; however, the DNA extraction methodology also extracts various PCR inhibitors, and the disadvantage of using harmful chemicals to remove these inhibitors led to the use of more specialized PCR protocols in order to tap the full potential of the robust methodology of highly specific technique. These include BIO-PCR (target cell enrichment prior to PCR), Immunomagnetic separation PCR (IMS-PCR) (involves the immunomagnetic separation of target cell from the heterogenous cell suspension followed by DNA extraction for PCR) (Widjojoatmodjo et al. 1992), and MCH-PCR (magnetic capture-hybridization PCR involving the use of single-stranded DNA probes to capture pathogen-specific DNA fragments which further serve as the template DNA for PCR) (Jacobsen 1995). Rapid cycle real-time PCR (coupled protocols of DNA amplification and real-time PCR) displays fluorescent signals that increase according to the numbers of the target amplicons. DNA chip or microarray technology is also a recent addition to the detection of seedborne pathogens.

31.5 Control Measures

The following control measures against the seedborne pathogens are recommended:

31.5.1 Regulatory Method

The import and spread of pathogens across national as well as international borders are prevented by imposing strict regulatory laws for cultivation of crops and their distribution between states and countries. These include quarantines, crop field as well as storehouses' inspection, and if needed discretionary or mandatory eradication of specific host plants.

(a) Quarantine: Quarantine serves as the filter against the introduction of harmful pathogens. There are two basic principles of disease management: exclusion and eradication. The entry of plants or its parts or products is checked, inspected, regulated, and if needed restricted at the national as well as international levels through Plant Quarantine Acts, which were initiated since 1870.

(b) Inspection/Certification: Growers interested in selling materials like seeds, seedling stock, etc. have to get through the inspection of their crop in field or storage by the regulatory agency in order to obtain the certification.
(c) Use of pathogen-free seeds.

31.5.2 Cultural Method

The initiation and development of seedborne pathogenic disease requires suitable environmental conditions during the contact between host and pathogen. Modifications through the cultural practices can alter the suitable environmental conditions that stop the disease to develop. It had been an age-old practice with the farmers, and in today's scenario, these techniques are coupled with the use of resistant cultivars as well as for the chemically protected crops. These methods include traditional procedures of crop rotation, removal of alternate or collateral hosts, mixed cropping, roughing, hot weather plowing, manure and fertilizer management, sowing time, soil amendments, irrigation, etc.

31.5.3 Physical Method

A number of physical methods are employed for the removal or reduction of seedborne pathogens:

(a) Hot water treatment of seeds: The seeds are presoaked for around 4 h for 20–30 °C which allows the dormant mycelium to initiate the metabolic activities and, thus, become vulnerable to exposure to hot water (50–52 °C) for few minutes. The method has limitations of maintaining the exact temperature for specific time period of treatment, and only a small batch of seeds can be treated at a time.
(b) Hot air treatment: Seeds are exposed to hot air treatment at 54 °C for around 8 h which effectively removes the pathogen without interfering with the germination potential. It is less injurious to the seeds, easy to operate, but less effective than hot water treatment.
(c) Solar heat treatment: This method effectively controls the seedborne pathogens, like the loose smut of wheat caused by *Ustilago nuda tritici*. The seeds are presoaked in water for around 4 h (preferably morning hours) on a bright summer day and then sun dried for 4 h (afternoon time).

31.5.4 Chemical Method

It involves application of chemicals having bactericidal or fungicidal properties to the seeds, which effectively prevent the infection by seedborne pathogen. Chemical seed disinfection was in vogue for a long time. Historically, sulfur, copper, and

mercury compounds had been found to have potent fungicidal properties. For example, copper sulfate was used for seed treatment against spores of bunt fungi, while sulfur dust was used against smuts in cereal seeds. Today, other synthetic compounds like mancozeb, carbendazim, and a mixture of both are used to control seedborne fungal infection. However, the indiscriminate use of chemicals in agriculture has negatively impacted the ecological balance of nature, which has led to the use of nonchemical biological methods alone or in combination with the chemical ones. Garlic tablet and hot water treatment is recommended against seedborne fungi in sorghum (Masum et al. 2009). The ecofriendly neem leaf extract seed treatment is also highly effective and recommended.

31.5.5 Biological Method

Biological method includes the use of biological control agents of the disease-causing pathogens using other organisms. Biological control agents such as predators, parasites etc., are the natural enemies of the pathogens and has tremendous potential to be used as antimicrobial agent (Table 31.3). Biocontrol agent can be the exotic species or nonpathogenic isolate of microorganisms (Schouten et al.

Table 31.3 A summary of seeds, pathogens, and biological control agents

Pathogen	Crop	Biological control agent	Nature of the natural enemy	Reference
Colletotrichum truncatum	Soybean	*Pseudomonas aeruginosa*	Antagonist	Begum et al. (2010)
Colletotrichum truncatum	Soybean	*Trichoderma harzianum*	Parasite	Begum et al. (2010)
Colletotrichum truncatum	Soybean	*Trichoderma virens*	Parasite	Begum et al. (2010)
Aspergillus flavus	Cereal grains, legumes, etc.	*Trichoderma* sp.	Parasite	Calistru et al. (1997)
Fusarium moniliforme	Maize	*Trichoderma* sp.	Parasite	Calistru et al. (1997)
Rhizoctonia solani	Rice, soybean	*Pseudomonas fluorescens*	Antagonist	Vidhyasekaran and Muthamilan (1999), Nagarajkumar et al. (2004)
Xanthomonas campestris pv. *vinae*	Pigeon pea	*Trichoderma harzianum*, *T. viride*	Antagonist	Singh et al. (2014)
Botrytis cineraria	Bean	*Trichoderma harzianum*	Antagonist, antibiosis	Woo et al. (1999)
Pyricularia oryzae	Rice	*Cryptosporiopsis quercina*	Antimycotic	Li et al. (2000)

2014). The first commercial use of an organism to control a disease-causing agent was that of nonpathogenic isolates of *Agrobacterium radiobacter* var. *tumefaciens* (strain 84) against crown gall in rosaceous plants (Kerr 1980). Strain 84 had been reported to reduce galling by nearly 100% in many countries (Kerr 1980). Many biocontrol commercial products are available as seed treatment against a number of seedborne pathogens, and they are very effective in major crops like rice, wheat, cotton, maize, etc. Antagonistic fungi and bacteria have been used to control few post-harvest fungal diseases (Heydari and Pessarakli 2010). There are two major strategies of biological methods: (1) ecological approach and (2) augmentative approach. Ecological approach is the classical biocontrol method involving only one time introduction of the biocontrol agent whereas augmentative approach involves control for limited time period that generally coincides with the cropping period (Knudsen et al. 1997).

Amid the environmental impact of agrochemical, nowadays biocontrol methods got attention among plant growers as an alternative to save their cultivar without further increase in the load of chemicals to the environment. Bio-priming is a process of biological seed treatment that refers to the combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organism to protect seed. It is an ecological approach using selected fungal antagonists against the soil and seedborne pathogens. Biological seed treatments may provide an alternative to chemical control.

Different *Trichoderma* spp. produce many antibiotic toxins like trichodermin, gliotoxin, trichobrachin, trichovirin, viridin, peptide antibiotics, etc. (Monte 2001; Kumar and Ashraf 2017). These chemicals supposed to damage fungal cell wall and cause decay in mycelium and cytoplasm leakage (Hou et al. 1972). Many bacterial species like *Pseudomonas* sp., *Xanthomonas* sp., *Agrobacterium radiobacter*, etc. also produce antagonists in the form of 2,4-diacetylphloroglucinol (2,4-DAPG or Phl) (Bonsall et al. 1997; Umesha et al. 2005; Gitaitis and Walcott 2007) and phenazines (Phz) and its derivatives (phenazine-1-carboxylic acid (PCA); phenazine-1-carboxamide (PCN)) (Haas and Keel 2003; Mavrodi et al. 2006). Pushpalatha et al. (2013) reported the efficacy of *Trichococcus* spp. as biocontrol measures of many fungal infection.

References

Agarwal VK, Sinclair JB (1997) Principles of seed pathology, 2nd edn. Boca Raton Publications, 538 pp

Baker KF, Smith SH (1966) Dynamics of seed transmission of plant pathogens. Annu Rev Phytopathol 4:311–334

Beach SA (1892) Some bean diseases. New York State Agric Expt Stn Bull 48:305–333

Begum S, Saxena B, Goyal M, Ranjan R, Joshi VB, Rao CV, Krishnamurhty S, Sahai M (2010) Study of anti-inflammatory, analgesic and antipyretic activities of seeds of *Hyoscyamus niger* and isolation of a new coumarinolignan. Fitoterapia 81:178–184

Begum M, Venudevan B, Jayanthi M (2013) Storage fungi in groundnut and the associate seed quality deterioration. Plant Pathol J 12(3):127–134

Bonsall RF, Weller DM, Thomashow LS (1997) Quantification of 2,4-diacetylphloroglucinol produced by fluorescent Pseudomonas spp. in vitro and in the rhizosphere of wheat. Appl Environ Microbiol 63:951–955

Brunt AA, Kenten RH (1973) Cowpea mild mottle, a newly recognized virus infecting cowpea (*Vigna unguiculata*) in Ghana. Ann Appl Biol 74:67–74

Calistru C, McLean M, Berjak P (1997) In vitro studies on the potential for biological control of *Aspergillus flavus* and *Fusarium moniliforme* by *Trichoderma* species. A study of the production of extracellular metabolites by *Trichoderma* species. Mycopathologia 137:115–124

Christensen CM, Kaufmann HH (1965) Deterioration of stored grains by fungi. Annu Rev Phytopathol 3:69–84

Daft GC, Leben C (1972) Bacterial blight of soybeans: seedling infection during and after emergence. Phytopathology 62:1167–1170

Dutta B, Gitaitis R, Smith S, Langston D Jr (2014) Interactions of seedborne bacterial pathogens with host and non-host plants in relation to seed infestation and seedling transmission. PLoS One 9(6):e99215. https://doi.org/10.1371/journal.pone.0099215

Frank B (1883) Ueber emlge neue und weniger bekannte Pflanzenkrankheiten. 1. Die leckenkrankheit der Bohnen, veranlasst durch Gl oeosporillm Lindemuthianum Sacco et Magnus. Landwirtsch Jahrb 12(5):11–23

Garnbogi P, Triolo E, Vannacci G (1976) Experiments on the behavior of the seed borne fungus Alternaria zinniae. Seed Sci Technol 4:333

Gitaitis R, Walcott R (2007) The epidemiology and management of seedborne bacterial diseases. Annu Rev Phytopathol 45:371–397

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41:117–153

Halfon-Meiri A (1978) The effect of seed-borne fungi on germination of seeds. In: Gupta US (ed) Crop physiology. Oxford and IBH, New Delhi

Harman GE (2000) Myths and dogmas of biocontrol changes in perceptions derived from research on Trichoderma harzianum T-22. Plant Dis 84(4):377–393

Hellwig LC (1931) Kurzes Sendschreiben wegen des sogenannten Honigthaues, welcher sich am heurigen Korn sehen lassen, und die grossen schwarzen Korner, insgemein Mutterkorn genannt, hervorgebracht, was davon zu halten, wovon es entstanden, und ob es niitzlich oder scM.dlich sey. (Langensalze, 1699. Cited by G. Barger, Ergot and Ergotism)

Heydari A, Pessarakli M (2010) A review on biological control of fungal plant pathogens using microbial antagonists. J Biol Sci 10:273–290

Hocking AD (2003) Microbiological facts and fictions in grain storage. In: Wright EJ, Webb MC, Highley E (eds) Stored grain in Australia. Proceedings of the Australian postharvest technical conference. CSIRO, Canberra, pp 55–58

Hou CT, Ciegler A, Hesseltine CW (1972) New mycotoxin, trichotoxin A, from *Trichoderma viride* isolated from southern leaf blight-infected corn. Appl Microbiol 23(1):183–185

Hyde MB (1950) The subepidermal fungi of cereal grains. I. A survey of the world distribution of fungal mycelium in wheat. Ann Appl Biol 37:179–186

Jacobsen CS (1995) Microscale detection of specific bacterial DNA in soil with a magnetic capture hybridization and PCR amplification assay. Appl Environ Microbiol 61:3347–3352

Jeyanandarajah P, Brunt A (1993) The natural occurrence, transmission, properties and possible affinities of cowpea mild mottle virus. J Phytopathol 137:148–156

Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ (2002) Plant systematics: a phylogenetic approach. Sinauer Associates, Sunderland, MA

Kerr A (1980) Biological control of crown gall through production of agrocin 84. Plant Dis 64:25–30

King EO, Ward MK, Raney DE (1956) Two simple media for the demonstration of pyocyanin and fluorescein. J Clin Microbiol Med 44:301–307

Knudsen I, Hockenhull J, Jensen DF, Gerhardson B, Hokeberg M, Tahvonen R, Teperi E, Sundheim L, Henriksen B (1997) Selection of biological control agents for controlling soil and seed-borne diseases in the field. Eur J Plant Pathol 103:775–784

Kumar M, Ashraf S (2017) Role of *Trichoderma* spp. as a biocontrol agent of fungal plant pathogens. In: Kumar V, Kumar M, Sharma S, Prasad R (eds) Probiotics and plant health. Springer, Singapore

Li JY, Strobel GA, Harper JK, Lobkovsky E, Clardy J (2000) Cryptocin, a potent tetramic acid antimycotic from the endophytic fungus *Cryptosporiopsis* cf. quercina. Org Lett 2:767–770

Li X, Tambong J, Yuan KX, Chen W, Xu H, Lévesque CA, De Boer SH (2017) Re-classification of Clavibacter michiganensis subspecies on the basis of whole-genome and multi-locus sequence analyses. Int J Syst Evol Microbiol 68:234–240

Masum MM, Islam SMM, Fakir MGA (2009) Effect of seed treatment practices in controlling of seed-borne fungi in sorghum. Sci Res Essay 4(1):22–27

Mavrodi DM, Blankenfeldt W, Thomashow LS (2006) Phenazine compounds in fluorescent Pseudomonas spp. biosynthesis and regulation. Annu Rev Phytopathol 44:417–445

Micheli PA (1723) Relazione dell'erba delta Ia'Botanici Orobanche e volgarmente sueeiamele, jiamma, e maId' occhio. (S.A.R.P. Tartini, Florence, 47 pp)

Monte E (2001) Understanding Trichoderma: between biotechnology and microbial ecology. Int Microbiol 4:1–4

Nagarajkumar M, Bhaskaran R, Velazhahan R (2004) Involvement of secondary metabolites and extracellular lytic enzymes produced by Pseudomonas fluorescens in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Microbiol Res 159:73–81

Naidu RA, Gowda S, Satyanarayana T, Boyko V, Reddy AS, Dawson WO, Reddy DVR (1998) Evidence that whitefly-transmitted cowpea mild mottle virus belongs to the genus *Carlavirus*. Arch Virol 143:769–780

Nameth ST (1998) Priorities in seed pathology research. Proceedings of Seed Biology Symposium, Piracicaba, Sao-Paulo, Brazil, 24–27 Aug 1998. Scientia-Agricola, 55: SPECIAL, pp 94–97

Needham T (1745) New microscopical discoveries (F. Needham, Halbom, England, 126 pp, 1 45)

Neergard P (1977) Seed pathology. Wiley, New York, 839p

Nordenstedt N, Marcenaro D, Chilagane D, Mwaipopo B, Rajamaki ML, Nchimbi-Msolla S, Njau PJR, Mbanzibwa DR, Valkonen JPT (2017) Pathogenic seedborne viruses are rare but *Phaseolus vulgaris* endornaviruses are common in bean varieties grown in Nicaragua and Tanzania. PLoS One 12:e0178242. https://doi.org/10.1371/journal.pone.0178242

Pushpalatha HG, Sudisha J, Shetty SH (2013) Cellulysin induces downy mildew disease resistance in pearl millet driven through defense response. Eur J Plant Pathol 137:707–717

Quibod IL, Grande G, Oreiro EG, Borja FN, Dossa GS, Mauleon R, Cruz CV, Oliva R (2015) Rice-infecting Pseudomonas genomes are highly accessorized and arbor multiple putative virulence mechanisms to cause sheath brown rot. PLoS One 10(9):e0139256

Salem NM, Ehlersb JD, Robertsband PA, Ng JCK (2010) Biological and molecular diagnosis of seedborne viruses in cowpea germplasm of geographically diverse sub-Saharan origins. Plant Pathol 59:773–784

Sastry KS (2013) Seed-borne plant virus diseases. Springer, New Delhi, pp 10–30

Schouten HJ, Brinkhuis J, Burgh A, Schaart JG, Groenwold R, Broggini GAL, Gessler C (2014) Cloning and functional characterization of the Rvi15 (Vr2) gene for apple scab resistance. Tree Genet Genomes 10:251–260

Singh D, Raghavendra BT, Rathaur PS, Singh H, Raghuwanshi R, Singh RP (2014) Detection of black rot disease causing pathogen Xanthomonas campestris pv. campestris by bio-PCR from seeds and plant parts of cole crops. Seed Sci Technol 42:36–46

Sinha KK, Sinha AK (1992) Impact of stored grain pests on seed deterioration and aflatoxin contamination in maize. J Stored Prod Res 28:211–219

Skoric V (1927) Bacterial blight of pea: overwintering, dissemination and pathological history. Phytopathology 17:611–627

Tillet M (1755) Dissertation on the cause of the corruption and smutting of the kernels of wheat in the head, and on the means of preventing these untoward circumstances. Bordeaux. 150 pp (English translation by H.B. Humphrey. 1937. Phytopath. Classics 5:1–191)

Tsedaley B, Adugna G (2016) Detection of fungi infecting maize (*Zea mays*) seeds in different storages around Jimma, southwestern Ethiopia. J Plant Pathol Microbiol 7:338

Umesha S, Kavitha R, Shetty HS (2005) Transmission of seedborne infection of chilli by Burkholderia solanacearum and effect of biological seed treatment on disease incidence. Arch Phytopathol Plant Prot 38(4):281–293

Verma AK, Agrawal K (2018) Location and histopathology of seed-borne bacterial pathogen *Pseudomonas syringae* pv. pisi carried by pea seeds. J Appl Biol Biotechnol 6(1):20–22. https://doi.org/10.7324/JABB.2018.60104

Vidhyasekaran P, Muthamilan M (1999) Evaluation of a powder formulation of Pseudomonas fluorescens Pf1 for control of rice sheath blight. Biocontrol Sci Tech 9:67–74

Walcott R (2003) Detection of seedborne pathogens. Horttechnology 13:40–47. https://doi.org/10.21273/HORTTECH.13.1.0040

Wallen V, Sutton M (1965) *Xanthomonas phaseoli* var. fuscans (Burkh.) Starr & Burkh. on field bean in Ontario. Can J Bot 43:437–446

Warham EJ, Butler LD, Sutton BC (1990) Seed testing of maize and wheat: a laboratory guide. CYMMYT, El Batan, 84 pp

Widjojoatmodjo MN, Fluit AC, Torensma R, Verdonk GPHT, Verhoef J (1992) The magnetic immunopolymerase chain reaction assay for direct detection of Salmonella in fecal samples. J Clin Microbiol 30:3195–3199

Woo SL, Donzelli B, Scala F, Mach RL, Harman GE, Kubicek CP, Sorbo D, Lorito GM (1999) Disruption of the ech42 (endochitinase-encoding) gene affects biocontrol activity in *Trichoderma harzianum* P1. Mol Plant-Microbe Interact 12:419–429

Zhou XG (2019) Sustainable strategies for managing bacterial panicle blight in rice [Online First] IntechOpen, https://doi.org/10.5772/intechopen.84882., https://www.intechopen.com/online-first/sustainable-strategies-for-managing-bacterial-panicle-blight-in-rice

Zhou-qi C, Bo Z, Guan-lin X, Bin L, Shi-wen H (2016) Research status and prospect of *Burkholderia glumae*, the pathogen causing bacterial panicle blight. Rice Sci 23:111–118