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Birinchi Kumar Sarma *Editors*

Emerging Trends in Plant Pathology

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To the Legend. . . .

(Dr. Y. L. Nene)

Foreword

Plant pathology as a discipline of agricultural science has played pivotal role over the years in understanding plant diseases and in mitigating losses through cultural and technological innovations. This is despite the disease scenarios that kept on evolving and changing due to biotic, abiotic and edaphic factors. The plant pathologists have eventually contributed significantly towards food security and in ameliorating the livelihood of farmers across the globe. The science of plant pathology has been an innovative and ever-emerging discipline in its scope, importance and technologies. There have been many such innovations and advancements in each and every aspect of plant pathology starting from the identification of the pathogen, underlining the molecular mechanism of pathogenicity and resistance and also the management strategies. With the commencement of the concept of sustainable agriculture, plant disease management has become more important and has shifted from the traditional chemical-based to more eco-friendly integrated disease management strategies with more focus on the biocontrol and other green technologies. The latest innovations in the field of detection and diagnosis, host resistance, disease forecasting and plant biotechnology have helped us in better management of the diseases, but challenges are still many more.

This first edition of *Emerging Trends in Plant Pathology* edited by K. P. Singh, B. K. Sarma and Shamarao Jahagirdhar provides a comprehensive description and highlights of the latest innovation and trends in the field of plant pathology and allied fields. The focus is on understanding both the basic and applied aspects of plant pathology and plant disease management. I hope the book would be of special

interest to both academics and professionals, working in the fields of plant pathology, microbiology, biotechnology and plant breeding, as well as the plant protection sciences. This book is a comprehensive reference for all those curious to understand the latest advancements in their field of specialization.

I congratulate the editors and contributors for their dedicated effort to bring out such a classic reference book for the scientific fraternity.

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Preface

The science of plant pathology is essential for reliable food production through management of plant diseases. Dynamics in the evolution of new races of plant pathogens and changes in the global climatic scenario have made plant pathology an ever-emerging discipline in its scope, importance and technologies. During the past decade tremendous advancements and innovations took place in every aspect of plant pathology starting from identification of pathogens to their management. Additionally, with commencement of the concept of sustainable agriculture, plant disease management has become more important and disease management strategies have shifted from the traditional chemical based to more eco-friendly strategies. Further, advancements in molecular biology studies have armed the researchers to develop newer strategies for plant disease management. Therefore, recently more focus has been on the applications of biocontrol agents, development of transgenic cultivars, plant genome editing to other green technologies. Recent innovations in the field of detection and diagnosis of plant diseases, host resistance, disease forecasting and plant biotechnology have helped in developing strategies to manage the diseases better and address the challenges still on the way.

In this first edition of *Emerging Trends in Plant Pathology* we have compiled chapters to reflect on the recent trends and innovations in the field of plant pathology. Emphasis was given to understanding both basic and applied aspects of modern tools and techniques developed for detection and diagnosis of plant diseases that has helped identifying pathogens associated with a disease in a very short time. Quicker detection and diagnosis leads to identification of many new and emerging plant pathogens, and it is of great help in designing effective management strategies against them. The book has therefore also focused on the host-pathogen systems at molecular level without considering the hosts and their pathogens as separate entities. Chapters were also compiled to elaborate our understanding on host resistance to plant pathogens and the mechanisms of actions of *R* and *Avr* genes of the host and pathogen, respectively. Additionally, plant diseases and their epidemics are highly influenced by environmental conditions and crop microclimate. The book also includes chapters covering broad overviews of the recent advancements in disease forecasting, remote sensing, GIS and GPS applications that help accurate prediction of plant diseases and thereby saving crop losses from pathogens. The book also highlights the developments in the area of biological control of plant

pathogens and use of microbial consortium which have received much attention in the past decade due to promotion in organic farming and sustainable agriculture globally. Further, the new-generation fungicides are considered far more eco-friendly and very effective at low concentration and are highly target specific. In this book, we have also focused on advancements in the use of secondary metabolites from microbes and novel plant extracts as eco-friendly pesticides. We also compiled chapters on the use of transgenics, cisgenics and genome editing that are being increasingly used for plant disease management.

This book is very timely in providing essential and comprehensive source materials, as it includes most relevant areas on emerging trends in plant pathology and their role in crop protection.

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1 Emerging Plant Diseases: Research Status and Challenges

Dipannita Mitra

Abstract

Plant diseases result in significant crop destruction thereby inadequate food supply and lead to economic and post-harvest losses in the agricultural production sector throughout the world. Early detection of plant diseases and pathogens is important for maintaining sustainability for the economy of the agricultural sector. The prevention of plant disease and pathogens during the early stages aids in plant health control and yield improvement. It is also crucial to analyze the disease spread in plants for overcoming the issues related to physiological and biological states in crop protection. This chapter reviews the research status of the various emerging plant diseases responsible for a large amount of crop destruction every year all over the world and the challenges that the agricultural sector face to overcome this problem.

Keywords

Plant pathogen disease · Symptomatic stage · Remote sensing · Flow cytometry

1.1 Introduction

Agriculture plays a dominant part in the worldwide economy and is the main source of food, fiber, fuel, timber, income, and employment, thus maintaining socioeconomic stability. The major threat to agriculture is nationwide crop losses due to pathogen-induced plant diseases, which is considered to be a primary challenge for the whole scientific community. The branch of plant pathology thus largely focuses

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on increasing the fundamental understanding of host-pathogen interactions to detect plant diseases and mitigate crop losses and enhance the total agricultural yield.

It was estimated in 1994 that worldwide crop losses due to plant diseases ranged from 9.7 to 14.2% of the total yield; the figure is different in modern days due to the emergence of new pesticides with a varied target range (Zadoks 1996). But, the ability of these target pathogens to gradually develop resistance to frequently used pesticides has allowed diseases to remain persistent and is proving to be a big threat in today's world (Strange and Scott 2005). A recent article in the Food and Agriculture Organization (FAO) of the United Nations reported that there has been a loss of about 20 to 40% of the global crop production due to pest infestations.

"Each year, plant diseases cost the economy around $220 billion and the invasive pathogen around US$70 billion" as per the FAO reports. This huge amount of losses deprives more than 800 million of the total worldwide population of adequate consumable food (http://www.fao.org/news/story/en/item/1187738/icode/).

The fact that plant pathologists should be concerned with the need to minimize losses due to endemic diseases correlates with the introduction of new foreign pathogens resulting from the globalization of plants and plant products (Mack et al. 2000).These plant pathogens can rapidly spread from an infected or diseased plant to a healthy plant. The microorganisms that cause plant diseases include fungi, viruses, nematodes, bacteria, and mycoplasmas (Lucas et al. 1992).

Plant diseases can be defined as any deviation from their healthy state with symptoms and disorders mainly on their shoots, leaves, flowers, fruits, stem, and roots. Diseased plants produce poor yields in terms of both quantity and quality. Losses in the total yield in a plant can occur from the seed to the harvesting stage. Quality of a plant product is attenuated when diseased spots or blotches are present; thus monitoring plant health and detecting the pathogens early in the plant life cycle is essential to reduce the chances of disease spread and also to facilitate effective management practices.

It is a well-known fact that vegetable crops represent an important economic segment of the total global economic production. But, gradually reoccurrence of crop diseases has played havoc toward the society and for total agronomical growth. For example, the famous famine in Ireland which was started in Europe during 1845 as an epidemic was caused by the late blight of potato by *Phytophthora infestans* (Mundt et al. 2009). Similarly, plant pathogens threaten other food crops globally including citrus, banana, and grapes. In southwestern Europe, a region known for its grape cultivation, a disease caused by phytoplasmas called *Flavescence doree* is widespread and is a major cause of annual economic losses (Martinelli et al. 2016).

Similarly, ready-to-eat salads like bagged salads have gained popularity throughout the world especially in Europe since their introduction in the early 1980s, marking opportunities for the fresh food industry. As this industry is growing, the number of new diseases is growing in parallel. The past review indicates that these seasonal salads are grown massively in a highly dense region in five to six cycles annually in the same farms (Fig. 1.1); thus it lacks crop rotation and also sometimes a shortage of applicable fungicides causing the growth of many fungal diseases, e.g., downy mildew of basil and *Fusarium* wilt of lettuce (Farr and Rossman 2019).

Fig. 1.1 Multitunnel cultivation of lettuce and wild rocket in southern Italy with five to six production cycles per year. (Source: Gullino et al. 2019)

Other examples of major economic losses due to plant diseases are soybean rust which is mainly a fungal disease in soybeans, but it was reported that by removing 20% of the infection, the farmers could make a profit of $11 million (Roberts et al. 2006). It was also estimated that the crop losses due to pathogen infection in the United States could be attributed to non-native or foreign plant pathogens, e.g., chestnut blight fungus, Dutch elm diseases, and Huanglongbing citrus diseases (Pimentel et al. 2005; Sankaran et al. 2010).

Plant diseases can be spread over a larger area of cultivation land with time through the accidental introduction of vectors or through plant materials. The other route of the spread of plant pathogens can be through ornamental plants that act as hosts. Ornamental plants are always in increasing demand and are sold worldwide before these diseases could be detected.

Recent technology has made it possible to consume crops that are produced on foreign land. Thus, international import-export has resulted in new kind of plant diseases, where a very low level of seed contamination can result into rapid emergence of new diseases in a totally new geographic areas, thus resulting into severe crop losses; it can affect the biological equilibrium of that region and sometimes also start an epidemic (Gullino et al. 2019). The globalization of agricultural products has resulted in many new resistant strains of pathogens which causes diseases that are tough to detect. If these new strains migrate to a healthy area of cultivation, the crops may not be able to resist these new pathogen strain infestations, similarly like *P. infestans* in the 1840s. The problem of plant diseases is a very challenging factor in developing countries because of their limited resources to fight these pathogens through scientific research. Lack of proper resources makes the developing nations unable to efficiently identify the disease causal organisms and detect and mitigate the symptoms for crop yield loss.

In this chapter, we have focused mainly on the emerging plant diseases and strains, the risk they possess in the successful cultivation of crops worldwide, the possibility to detect these strains at an early stage by using traditional and innovative

Fig. 1.2 Aerial image of olive groves in Puglia in Italy showing olive trees infected by *Xylella fastidiosa* (left). (Source: https://www.theguardian.com/world/2019/sep/09/deadly-olive-tree-disease-spreads-france)

detection methods, and lastly the challenges that plant disease management faces in the modern era. These challenges need to be addressed through science-based cooperation on a global scale at a scientific and political level (Fig. 1.2).

1.2 Emerging Plant Diseases and Their Research Status

Our knowledge of global crop losses due to plant-pathogen infestations is very limited. There is an increase in emerging pathogen strains, and with that integrated disease management should evolved as well. In recent years, there has been a great spike in types of plant pathogens and strains especially in imported plants and plant products. Changes in climate conditions have also resulted in various new plant diseases. For example, according to recent news, the Animal and Plant Health Agency (APHA) reports some new plant diseases and new pests with their symptoms and also gave information about the countries where they can be found. Figure 1.3 and Fig. 1.4 show some examples of emerging plant diseases and plant pests.

Recent survey articles published by British Broadcasting Corporation (BBC) and *The Guardian* report the massive outbreaks caused by *Xylella fastidiosa* in European countries like Italy, France, Germany, and Spain, wiping out entire olive groves and thus causing major economic losses in these countries. This disease is also called olive quick decline syndrome and is believed to affect more than 350 plant species. This disease has also spread in the vineyards in the north and south of America. It was first detected in Puglia in Italy in the year 2013, but now it has spread almost in every corner of Europe (https://www.theguardian.com/world/2019/sep/09/deadly-olive-tree-disease-spreads-france).

The European Commission suspects it could threaten olive gardens throughout the world and thus has sought crop protection actions against the spread of *Xylella*. To help mitigate the problem, the Royal Horticultural Society of the United Kingdom has come up with some new principles for future-proofing UK gardens: (1) All imported semi-mature trees will be held for 12 months before planting them, (2) evaluation of plant health risk will be monitored according to the criteria

Fig. 1.3 Emerging new plant diseases, symptoms, and regions where they are present. (Source: https://www.rhs.org.uk/science/plant-health-in-gardens/protect-your-garden/new-pd-risks)

provided by the Royal Horticultural Society, and (3) this society will generate a list of suppliers who meet these specified criteria.

Other major factors in causing various new plant diseases are the climate changes resulting from natural and human activities. Climate change is the result of the increasing amount of global trade, agricultural system modifications, and changes in the consumer lifestyle; the global circulation of the crop also affects the global circulation of new pathogens and diseases.

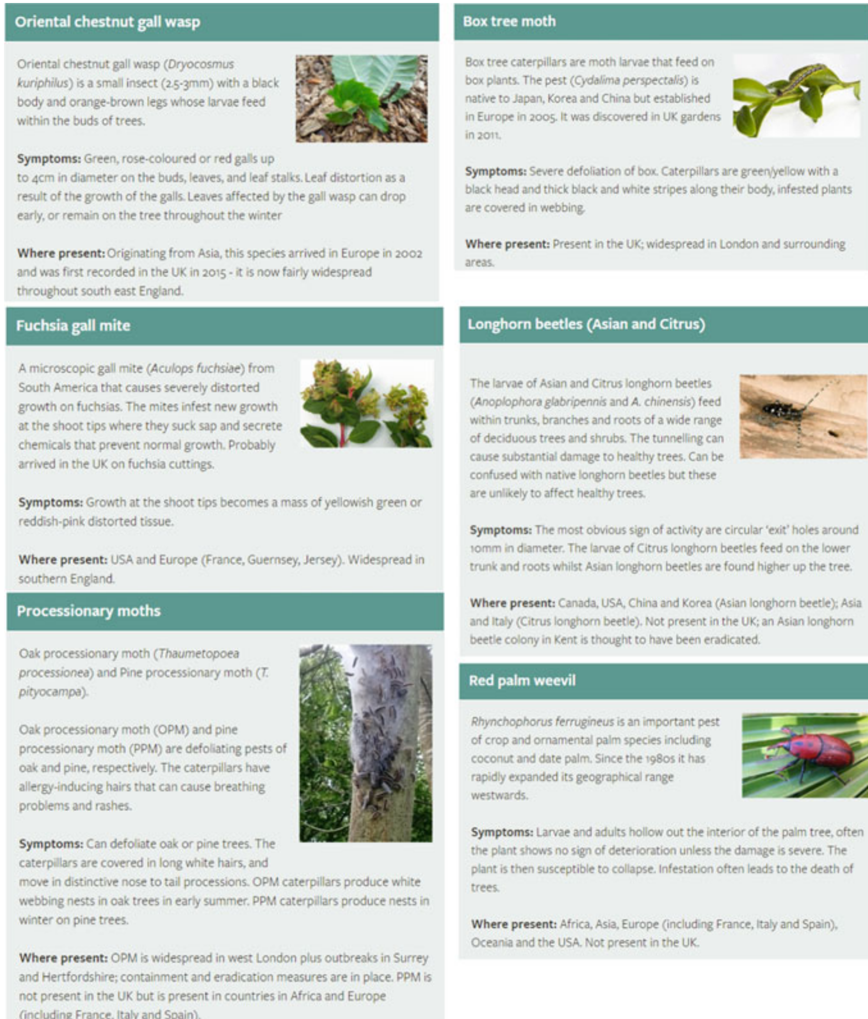

Fig. 1.4 Emerging new plant pests, symptoms, and region where they are present. (Source: https://www.rhs.org.uk/science/plant-health-in-gardens/protect-your-garden/new-pd-risks)

Plants and pathogens not only interact in isolation but also with the environment according to the "disease triangle" concept, for instance, for a disease to occur by a pathogen, a specific favorable environment is required (Fig. 1.5). Various environmental conditions affecting plant disease development include water, temperature, light, soil quality, wind speed, CO_2 concentration, and others. Though we know that plants have evolved various sophisticated defense mechanisms like PAMP-triggered immunity (PTI), effector-triggered immunity (ETI), RNA interference (RNAi), and hormonal regulation via abscisic acid, jasmonic acid, and ethylene, but new studies have shown that environmental conditions can gradually modulate these defense

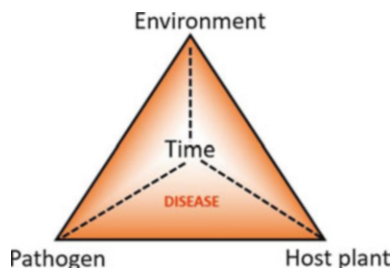

Fig. 1.5 Disease triangle showing four dimensions responsible for plant diseases, pathogen, host plant, environment, and time. (Source: http://www.ucanr.org/blogs/blogcore/postdetail.cfm?postnum=28845)

mechanisms (Couto and Zipfel 2016; Wu et al. 2019). For example, high humidity condition interferes with ETI-associated mechanism; thereby the response to *C. fulvum* Avr4 and Avr9 effectors by tomato CfR proteins is reduced when air humidity reaches more than 95% (Wang et al. 2005). Since tropical climates are prevalent in most developing countries, plant diseases are more common, causing a great part of economic loss. In contrast, cold temperate reduces the chances of rapid disease spread.

To solve the problems related to the emerging plant diseases, pathogen exclusion through the plant quarantine must be the first step to combat food security in both developing and developed countries. Other methods that need to be implemented should be intercropping and crop rotation methods, use of pesticides, adequate knowledge of the molecular mechanisms of these pathogen-host interactions, and knowledge about post-harvest protection. The classically accepted phenomenon of host-pathogen interactions now will no longer be relevant for these emerging plant diseases. Thus, we must thrive to improve the traditional detection methods and focus more on new approaches for these new pathogen strains. During the last 100 years, accuracy and precision in the detection of plant diseases were based solely on the traditional methods; however, these methods are too slow and ineffective and thus need to be improved and updated.

Maintaining genetic variability in crop plants is also of major importance for better crop yield. Future breeding programs for new improved varieties of crops must incorporate the growth and biotic and abiotic resistance variability, which should favor plant immunity and not the pathogen virulence. These features are found mostly in wild-type relatives of cultivated crops and possess combined abiotic and biotic resistance over a long time. But this is not the case in the modern crop plant variety. With the help of genome-wide association study (GWAS) analysis method and various marker-assisted selection methods, these features can be introduced into the cultivated variety of plants.

1.3 Overview of Disease Detection Methods and Their Ability to Combat Emerging Diseases

Plant pathologists define "plant disease monitoring as detection i.e. deviation from a healthy state of plants to stressed state, identification i.e. diagnosis of symptoms for various diseases, and quantification i.e. measurement of disease severity for e.g. reduced chlorophyll content or reduced leaf area," etc. (Mahlein et al. 2012).

After the onset of plant disease symptoms, there are many methods applied to detect the presence of diseases, for example, two main methods used are enzyme-linked immunosorbent assay (ELISA) which is based on proteins produced by the pathogen and polymerase chain reaction (PCR), based on specific DNA sequences of the plant pathogen (Prithiviraj et al. 2004; Das 2004; Li et al. 2006; Saponari et al. 2008; Ruiz-Ruiz et al. 2009; Yvon et al. 2009).

In spite of the availability of these techniques, there is always a demand for fast, sensitive, and effective methods for the detection of plant diseases caused by varied plant pathogens. According to Sankaran et al. (2010), disease detection techniques can be broadly classified into two main groups: direct and indirect methods (Fig. 1.6).

Among the direct approaches, molecular methods and serological methods provide essential tools for accurate plant disease detection. Although DNA-based molecular methods and serological methods have improved plant disease detection, they are sometimes not very reliable, especially at the asymptomatic stage.

Other modern methods based on nucleic acid and protein analysis have been proven to be more efficient in plant disease detection (Martinelli et al. 2015). The main conclusion from a review by Martinelli et al. (2015) states

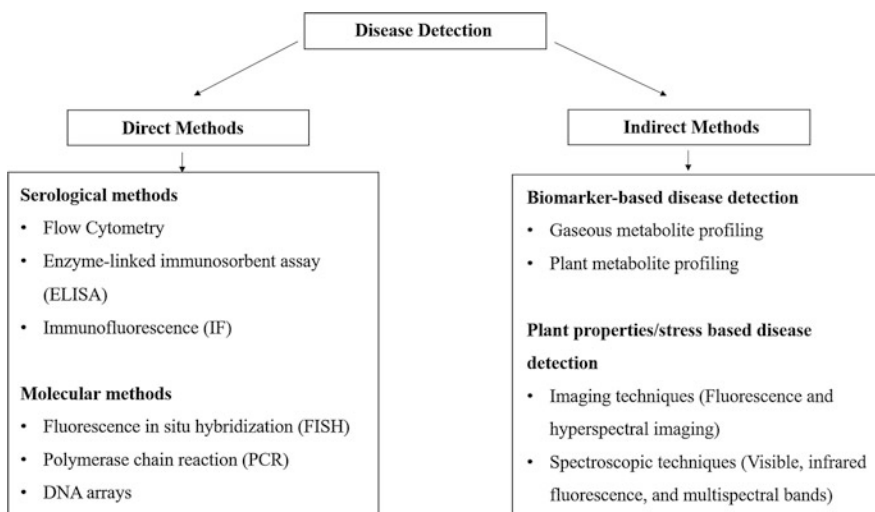

Fig. 1.6 Methods of Plant disease detection. (Source: Sankaran et al. 2010)

(1) novel sensors based on the analysis of host responses, for example, differential mobility spectrometer and lateral flow devices, deliver much more reliable and instantaneous results and can effectively detect early infections directly in the field; (2) secondly, biosensors based on phage display and biophotonics can also detect infections very fast although they can be also integrated with other systems; and lastly (3) remote sensing techniques coupled with spectroscopy-based methods allow high spatialization of the results, these techniques can prove to be very effective as a very reliable, sensitive and rapid preliminary identification of primary infections. These tools in the long run help plant disease management and complement serological and DNA-based molecular methods.

According to plant pathologists, serological and PCR-based methods are the commonly used methods to confirm plant disease detection, but volatile and biophotonic sensors provide rapid and more effective results and may be used to identify infections at asymptomatic stages. Remote sensing technologies are also very efficient tools to spatialize diagnostic results and thus provide agriculture more sustainability and safety, avoiding expensive use of pesticides for crop protection (Martinelli et al. 2015).

1.3.1 Molecular Methods for Disease Detection

Molecular methods for plant disease detection are well established. It was reported that the sensitivity of the molecular techniques for detecting bacteria ranged from 10 to 10^6 colony-forming units/mL (Lopez et al. 2003). The most commonly used molecular techniques for plant disease detection are fluorescence in situ hybridization (FISH) and PCR. As shown in Fig. 1.6, the other most commonly used methods include immunofluorescence, flow cytometry, FISH, and DNA microarrays.

Another categorization of the molecular methods can be nucleic acid-based, i.e., (1) DNA-based methods like FISH and the PCR variants nestedPCR (nPCR), cooperativePCR (Co-PCR), multiplex PCR (M-PCR), real-time PCR (RT-PCR), and DNA fingerprinting. (2) RNA-based methods include reverse transcriptase PCR, nucleic acid sequence-based amplification (NASBA), and AmpliDet RNA (Martinelli et al. 2015; Lopez et al. 2003). In the PCR method, the DNA of the pathogen is extracted, purified, and amplified. Then it is used for gel electrophoresis which if shows a specific band confirms the presence of the plant pathogen. The concept of molecular detection methods is based on the specific design of oligonucleotides and probes. Target sequences for molecular detection methods can be obtained from the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA).

DNA fingerprinting is another molecular genetic method for plant pathogen detection, where unique patterns are identified in the DNA of the plant pathogen samples also called polymorphisms. This method was first described by Alec Jeffreys in 1984. The various DNA fingerprinting methods use either PCR or restriction fragment length polymorphism (RFLP) and sometimes both to target

specific areas of DNA. Apart from these methods, DNA diagnostic microarrays are being used for plant pathogen detections.

1.3.2 Serological Assays

In the ELISA-based method of plant disease detection, the microbial protein associated with a specific disease is injected into an animal that produces the antibodies against these microbial proteins, better known as antigens. These antibodies are then extracted from the animal and are tagged with a fluorescence dye and enzymes and are used for the detection of host-target interactions. ELISA method was first used in the 1970s and is so far the most widely used immunodiagnostic technique because of its efficiency and specificity. ELISA is also a highly sensitive method, but its sensitivity depends on the samples and the volume of samples, for example, bacteria can be detected only at 100 cfu mL – 1 (Schaad et al. 2002, 2003).

Antibodies against many viruses and bacteria have been developed and are being used in numerous ELISA methods for plant disease detections globally, but due to the fact that they might show cross-reactivity, monoclonal antibodies using hybridoma technology have been developed which are more specific to the target (Nolasco et al. 2002; Holzloehner et al. 2013). To date, both polyclonal and monoclonal antibodies are available and are being used for various plant disease detections by ELISA. Pathogens like viruses, bacteria, and fungi can now be detected using these specific antibody techniques such as western blots, immuno-binding assays, and serologically specific electron microscopy (SSEM) (Alarcon et al. 1990; Caruso et al. 2002; Serological methods for detection and identification of viral and bacterial plant pathogens. A laboratory manual 1990).

However, these molecular and serological techniques have some limitations, for instance, they are time-consuming, require an elaborate sample preparation procedure, are labor-intensive, and require specific reagents to detect each specific pathogen. Also, sometimes the concentrations of seed samples, soil, water, and pathogen are below the sensitivity limit, thus hindering efficient detection by these methods.

Another issue is the occurrence of false negatives and false positives due to the degradation of target DNA sequence or poor quality of the reagents (Louws et al. 1999). Lastly, the cost of equipment, reagents, sample preparation, etc. used in molecular detection methods are very high making it less popular for most agriculture-based industries. Thus, spectroscopic techniques can be potential alternative methods for the rapid detection of new plant pathogens.

1.3.3 Spectroscopic and Imaging Techniques

Recent research developments focus on automated nondestructive methods of plant disease detection that will act as an efficient tool for disease monitoring on a large scale. With the advancement of spectroscopic methods, the detection of plant

diseases has simplified. Many spectroscopic and imaging techniques have been studied for the detection of the early and late stages of plant diseases. Some of the methods include fluorescence imaging, infrared spectroscopy, fluorescence spectroscopy, visible spectroscopy, nuclear magnetic resonance spectroscopy, etc. Spectroscopic methods can either be based on imaging or non-imaging techniques and help in crop disease monitoring because of their potential, flexible, and cost-effective role as operational instruments.

The most common imaging-based spectroscopic approaches include fluorescence spectroscopy where the fluorescence from the object of study is measured after being excited with an ultraviolet spectrum. To monitor plant stress and physiological states in plants and to monitor nutrient deficiency in plants, usage of laser-induced fluorescence is very popular (Belasque et al. 2008; Cerovic et al. 1999). Imaging spectroscopy was used and has proved to be very effective for wheat kernels for *Fusarium* head blight disease and also for weed infestations (Delwiche and Kim 2000; Okamoto et al. 2007).

Non-imaging spectroscopy methods are based on optical properties of leaf pigments, chemical components, and structural features. These spectra collected are then used for various remote sensing detection methods; this method has been used to detect winter wheat yellow rust, aphid infestation, curl mite infestation, etc. (Jacquemoud and Ustin 2001; Stilwell et al. 2013; Yuan et al. 2014; Zhang et al. 2014).

1.3.4 Other Innovative Detection Methods

During the past few years, many novel approaches were developed which are rapid, inexpensive, efficient, and reliable, for example, lateral flow microarrays (LFM) using an easily visualized colorimetric signal (Carter and Cary 2007). Metabolomics is used as well to detect plant metabolites from primary and secondary metabolism for various plant pathogens (Ibanez et al. 2014; Martinelli et al. 2016).

Volatile compounds emitted by plants for their growth, defense, and survival purposes can also be used as biomarkers to detect plant diseases in volatile compound profiling using gas chromatography-mass spectrometry (GC-MS) (Cardoza et al. 2002).

Biophotonics is also an emerging technique that has been developed for efficient plant pathogen disease detection. The main concept of this technology is based on the molecular detection of probe-target interactions based on specific peptide sequence recognition where the probe-target complex is identified using ELISA. This method uses proteins as probes, increasing the possibility of multiple epitopes for a single target present resulting in a cross-reaction. To mitigate this problem, probe size is reduced to obtain more specificity and sensitivity via various biosensors (Goulart et al. 2010).

1.3.5 Remote Sensing Method

Remote sensing method can be defined as tracking an object without any physical contact but rather by measuring the electromagnetic energy, i.e., emitted or reflected by the surface of the earth (De Jong and Meer 2004). This is an indirect detection method in which vegetation conditions from a distance are monitored and the spatial extent and patterns of vegetation characteristics and plant health are evaluated (Martinelli et al. 2015).

Plant stress or infections caused by various pathogens can be monitored by remote sensing by analyzing the change in radiation emitted and used by plants. APAR is the absorbed photosynthetic active radiation which is the total energy absorbed by the plant and can be calculated based on the plant's total leaf area and by the concentration of chlorophyll pigments since leaf chlorophyll content is reduced due to necrotic and chlorotic lesions. APAR used by a healthy plant is primarily for photochemical reactions (0–20%) and reflects the rest of the energy as heat (75–90%) and fluorescence (2–5%). Both plant physiological processes under stress conditions and plant parameters like leaf pigments, water content, and chlorophyll content can be detected by remote sensing methods (Meroni et al. 2009).

To summarize this part, early detection of pathogen infection is pivotal to avoid epidemics. Usually, primary infections begin in the growing season, and secondary infections are mostly spread by vectors leading to symptomatic disease stage with a severe loss in total yield. Some pathogens are exceptional and they remain latent and only infect the plant later in their life cycle. Thus, various detection methods can detect plant pathogens at various stages in their life cycle. Figure 1.7 obtained from Martinelli et al. (2015) represents an overview of the features of innovative methods.

1.4 Challenges of Plant Disease Management

Increasing the population with an expectation to reach nine billion by the year 2050 (Godfray et al. 2010) and changing diets and consumption patterns in the modern world suggest that the production of food must be more efficient to meet the ever-increasing demand. Figure 1.8 depicts the ever-changing percent yield change per year for maize, rice, wheat, and soybeans. However, the existence of pre- and post-harvest losses is the major hindrance to achieve this goal. The challenges of plant pathology are increasing with every passing day with depleting natural resources, a decrease in agricultural production, and an increase in epidemics of plant diseases globally (Ray et al. 2013).

Thus, in current times, greater emphasis must be given to sustainable plant disease management strategies that ensure food security and societal development. The three major components, i.e., (1) society, (2) economics, and (3) ecology, must be considered in plant disease management strategies. These strategies must focus on ensuring food security and social stability by increasing crop productivity and providing a supply of diverse and reasonable priced crops.

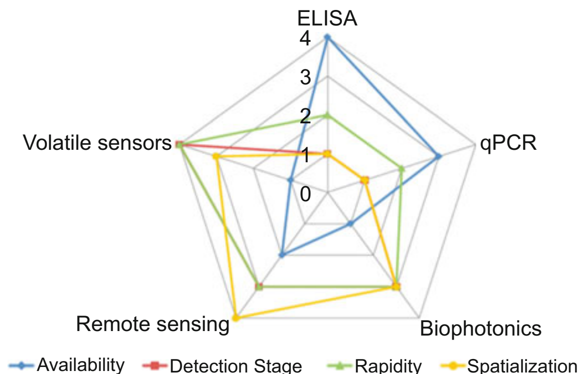

Fig. 1.7 Comparison of methods for plant disease detection. (Source: Martinelli et al. 2015) The qualitative scales indicate **1** poor, **2** fair, **3** good, and **4** very good. The categories evaluate individual techniques with respect to (i) Availability—ease of use, availability of equipment, and cost; (ii) detection stage—when infections can be detected (4 infected vectors present, 3 isolated infected plants, 2 many infected plants, and 1 symptomatic stage disease has spread over the cultivated area); (iii) speed—total time required between collection of field data and the delivery of results (thus includes sample collection, preparation, and testing); (iv) spatialization—the potential to spatialize results(4 input data already carried out in a spatialized dimension, 3 data easily spatializable, 2 data difficult to spatialize, and 1 data not subject to spatialization); and (v) reliability—effective accuracy of results

From the economic point of view, the ratio of input and output of plant disease management approaches must focus on more effective evaluation of direct and indirect economic benefits thus helping the agricultural and ecological sustainability (He et al. 2016).

Plant pathogens are considered a weapon for global terrorism and are a major issue of political challenges in all countries. Since the majority of the country's economy depends on agricultural yield, poverty, crop loss, and food security are major concerns for plant pathologists. Throughout the world, the International Agricultural Research Centers have initiated some programs in the management of plant disease, and each of these centers is responsible to handle certain crops, for example, WARDA (Africa Rice Center, CIAT (Centro Internacional de Agricultura Tropical, ICARDA (International Center for Agricultural Research in the Dry Areas), ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, and ICRAF (World Agroforestry Centre) are some of the few.

On the international level, the Food and Agriculture Organization (FAO) of the United Nations along with ISPP, i.e., International Society for Plant Pathology, plays a major role in addressing the challenges faced due to plant diseases. These organizations mainly make the farmers aware of changing food security policy, train plant pathologists in developing countries, train farmers of plant disease management techniques, and aim to mitigate this major global issue with small-scale improvements.

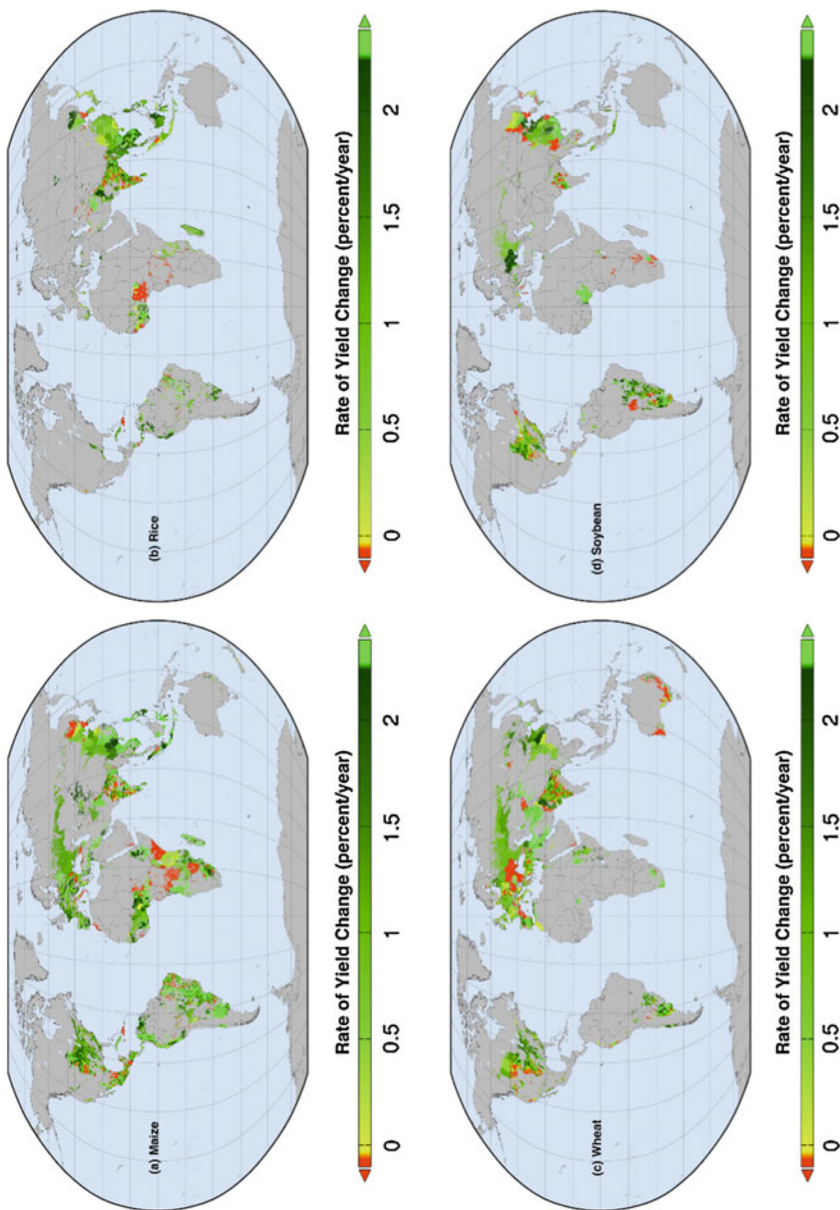

Fig. 1.8 Maps of observed rates of percent yield change per year in (1) maize (2) rice (3) wheat and (4) soybean yields. Red areas show where yields are declining whereas the fluorescent green areas show where rates of yield increase – if sustained – would double production by 2050. (Source: Ray et al. 2013)

A recent review argues the fact that to achieve sustainable plant disease management, we must understand the plant disease infestation mechanisms and strive to improve the disease management system. Global agricultural productivity and food quality can be improved and will, as a result, boost the global economy. According to He et al. (2016), to combat the recent plant disease challenges, plant pathologists worldwide should follow some strategies which are, as quoted, "(i) epidemic and evolutionary patterns of plant disease under changing climate and agricultural production concept; (ii) the role of ecological considerations in agricultural productivity and crop health; (iii) social-economic analysis of plant disease epidemics and management; and (iv) technology development for integrating management of major crop diseases with ecological principles." To conclude, our main aim should always be maintaining food security for a stable society by maintaining good crop health by regularly improving scientific approaches.

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Emerging Plant Diseases Under Changing Climate Scenario

2

Muhammad Priyadi and Pooja Upadhyay

Abstract

The effect of changing climate on plant diseases has been a point of debate since long time. This changing climate may cause imbalance in the ecosystem and directly contribute to the disease development in various crops. Different climatic conditions, e.g., change in sunlight including UV light, temperature, air, rainfall, soil nutrients, carbon dioxide, ozone gas, greenhouse gas emission, and other factors, are affecting the interaction of host plant and pathogens, e.g., fungi, bacteria, virus, nematode, viroid, phytoplasma, and spiroplasma, which are opening doors for the emergence of new diseases and pathogens worldwide. These newly emerged diseases may turn out to be an epidemic under favorable conditions if not regulated wisely as changing climatic conditions are providing favorable environment to the spread and establishment of novel pathogens into new and non-native areas. By keeping all these points into consideration, this chapter focuses on correlation between climatic conditions and disease development and impact of changing climatic conditions on disease development and emergence of new pathogens around the globe. It also puts emphasis on factors responsible for emergence of novel pathogens as well as their possible management tactic to regulate their adverse outcome on agriculture and human to sustain food security in future.

Keywords

Climate change · Emerging plant disease · Pathogen · Environment

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2.1 Introduction

Plant diseases are ubiquitous in nature and found in all the parts of the world wherever plants grow. Diseases are responsible for losses of at least 10% of global food production, representing a threat to food security (Strange and Scott 2005). Though plant diseases are found in each and every climate, primarily hot and humid condition is most favorable for development and dissemination of plant diseases caused by various pathogens, e.g., fungi, bacteria, nematode, phytoplasma, spiroplasma and virus and viroid.

Plant pathologists are considering the impact of environment influences on plant disease development since long time. The classic disease triangle emphasizes the interactions among host, pathogen, and environment for causing disease (Grulke 2011). In this triangle susceptible host, virulent pathogen and favorable environmental conditions are the essential components for disease development. If this interaction persists for a certain period of time, the disease development takes place and the triangle gets converted into disease tetrahedron. So the role of environment in pathogenesis is very important as it affects host, pathogen, as well as host pathogen complex. Thus, temperature, moisture, wind velocity, light, soil PH, soil structure, etc. are environmental factors that have considerable influence on plant diseases. The close relationship between the environment and diseases suggests that climate change may cause the emergence of new plant pathogens and new disease epidemics may take place under favorable conditions. Climate change has become a serious concern around the world in recent years. We have seen the consequences of global warming, such as melting of glaciers and raising water level, disturbed climate cycles, and extreme environmental conditions in some parts of the world. Apart from this, greenhouse gas level in the atmosphere is increasing due to various human activities (Elad and Pertot 2014). It is assumed that global temperature has increased by 0.8 °C and will increase from 0.9 °C to 3.5 °C in the next 10 years (Das et al. 2016). Changing weather affects temperature, humidity, rainfall, wind, and ecosystem balance, causing several problems such as floods, droughts, forest fires, etc. Besides, it will directly affect the support of plant ecosystems that exist in nature. Climate change can have direct or indirect impacts on plants through complex mechanisms at different places (Pautasso et al. 2012). Usually, hot weather along with high humid conditions can increase the risk of various types of plant diseases that threaten many crop commodities in every region. Thus, change in environmental conditions as increasing global temperature and disturbed rain pattern at different regions will affect the defensive ability of plants against pathogenic attack. Climate change can affect the distribution of plant diseases in large geographical areas, resistance and tolerance of plants against diseases, and the severity of plant diseases (López et al. 2012; Nazir et al. 2018; Ziska et al. 2018).

Emergence of plant pathogens in new regions can be a possible outcome of changing climate due to favorable conditions for pathogen establishment in new region. Environmental changes in a short time or a long time will have an impact on the growth, productivity, and population of microorganisms that also live around

plants, and they change the microclimate of plants permanently leading to susceptibility against new pathogens (Nurhayati 2013).

2.2 Historical Impact of Emerging Diseases

Emergence of new disease or a new strain of pathogen in a non-native place is a phenomenon which has been witnessed by humans since ages. An emerging disease is an original case or group of cases that are newly recognized or newly appeared in an area and can increase at a very fast rate in incidence and severity (Daszak et al. 2003). It represents the initial presence of a disease in a crop and, if left unchecked, can result in disease epidemics of disastrous proportions. The introduction, or arrival, of potentially hazardous plant pathogens to a new cropping area generates risks in food production. Indeed, a large number of registered plant epidemics have reduced the production of various crops in the world throughout history (Strange and Scott 2005).

There are a number of examples of epidemics witnessed by history which impacted the human life like never before. Among the most devastating cases affecting humanity were the famines in Ireland and Bengal in which more than three million people died and got migrated to different countries. The arrival of a new strain of *Phytophthora infestans* (Mont.) de Bary in Ireland and the practice of a potato monoculture caused the death of one million people (Andrivon 1996; Forbes 2004; Agrios 2005). Similarly, the shocking epidemic of leaf blight of rice caused by the fungus *Cochliobolus miyabeanus* brought hunger and serious damage on the population of Bengal state of India. Because of the epidemic, more than two million people died, especially in the cities of Calcutta and Dhaka (Padmanabhan 1973). There are many other cases about the arrival of new pathogens to a non-native area, by the addition of monoculture practices and favorable environmental conditions, which facilitated the occurrence of devastating diseases, causing human deaths because of starvation and economic losses (Ullstrup 1972; Strange and Scot 2005).

Continuous evolution of pathogens may lead to the emergence of new, dangerous strains of such plant pathogens which may spread immensely under favorable conditions as new crop species with higher qualities are constantly introduced in different countries and typically grown in large monoculture fields. Further, many varieties and cultivars are selected mainly by their productivity, without consideration for their susceptibility to pathogens which might be the major threat for the huge spread of disease. These favorable conditions suggest some plausible reasons why new diseases might emerge and flourish (Oliver and Solomon 2008).

2.3 Impact of Changing Climatic Components on Plant Disease Development

Climate change is generally called as a long-term shift in the statistics of the weather. It has been observed that last decade of the twentieth century and the beginning of the twenty-first have been the warmest period in the entire global instrumental temperature record.

2.3.1 Temperature

Increase in the globally averaged temperature is very likely due to the observed increase in greenhouse gas concentrations. This greenhouse gas concentration in the atmosphere is supposed to be increased by human activities, thus causing climate change in the form of global warming. These activities intensified after the industrial revolution at the end of the eighteenth century possibly by the intense use of natural resources such as fossil fuel burning, deforestation, and other land use activities.

Temperature affects the disease cycle of any pathogen, starting from survival, spread, penetration, development, and reproduction rates of pathogens and their vectors. Temperature plays a very important role in spread of viral diseases as this is one of the important environmental parameters in regulating the biology of insects which can act as vector or carriers of viral pathogens. Generally, behavior, distribution, reproduction, and development of insects are largely affected by climate differences in any region (Ghini et al. 2008). Therefore, it is important to maintain environmental conditions, especially temperature to avoid the establishment and dissemination of plant pathogens.

Besides, due to changes in temperature growth stage, development rate and pathogenicity of infectious agents and the physiology and resistance of the host plant may alter (Chakraborty et al. 1998; Chakraborty and Datta 2003). Change in temperature can directly affect the secondary spread of disease by affecting the survival of pathogen between two seasons. In some cases, change in temperature may favor the development of different inactive pathogens, which could induce an epidemic if it gets established and finds favorable conditions for longer period. Temperature change may impact plant diseases by combining with other factors. For example, increase in temperatures with sufficient soil moisture cause humid microclimate in crop by increasing evapotranspiration and may lead to the incidence of diseases favored under such conditions (Mina and Sinha 2008).

Temperature is one of the important factors affecting the occurrence of bacterial diseases such as *Ralstonia solanacearum, Acidovorax avenae*, *Burkholderia glumea*, etc. Thus, bacteria can proliferate in those areas where temperature-dependent diseases have not been observed before (Kudela 2009). With the increase in temperature, winter duration, growth rate, and reproduction of pathogen may be modified (Ladányi and Horváth 2010). Similarly, the incidence of vector-borne diseases will get altered up to some extent as climate can substantially influence the development and distribution of vectors of viral diseases. Changes may result in

geographical distribution, increased overwintering, changes in population growth rates, increases in the number of generations, extension of the development season, changes in interspecific interactions, and increased risk of invasion by migrant pests (Gregory et al. 2005). Because of the short life cycles of insects, mobility, reproductive potential, physiological sensitivity to temperature, and even modest climate change will have rapid impacts on the distribution and abundance of vectors. Thus, increase in temperature may result in high rate of development of insect, obtaining a greater number of insect generations per cycle. Furthermore increase in temperature could determine the distribution of areas favorable for overwintering (Garrett et al. 2006) or even more lethal zones where the insect cannot survive.

2.3.2 Carbon Dioxide Gas (CO_2)

Increased CO_2 gas in the air by climate change can encourage greater plant biomass production which is also influenced by the availability of water and nutrients from the soil (López et al. 2012). Different studies showed the impact of increased CO_2 concentration on pathogen and disease development. The effect of elevated concentrations of CO2 on the infection of barley by *Erysiphe graminis* was observed as the percentage of conidia that progressed to produce colonies was lower in such plants grown in higher CO_2 concentration (Hibberd et al. 1996). Increase in the amount of CO_2 gas can increase the production of pathogenic fungal spores (Das et al. 2016) and have impact on the severity of plant diseases. Similarly, high concentration of carbohydrates or biomass in the host tissue promotes the development of biotrophic fungi such as rust (Chakraborty et al. 2000). Increase of CO_2 concentration relate to severity level of plant disease (Debela and Tola 2018).

According to the research and experiments, elevated levels of CO_2 can directly affect the growth of pathogen. Growth of the germ tube, appressorium, and conidium of *C. gloeosporioides* fungi is slower at high concentrations of CO_2 (700 ppm) according to Chakraborty et al. (2000), However, once the pathogen infects the plant, the fungus quickly develops and achieves sporulation. In contrast, the rate of sporulation was greater at high concentrations of CO_2 (700 ppm). In another study, Hibberd et al. (1996) evaluated powdery mildew in barley and found that an acclimation of photosynthesis at elevated CO_2 caused larger reductions in plant growth also, and the percentage of conidia that progressed to produce colonies was lower in plants grown in high CO_2 (700 ppm) than in low CO_2 (350 ppm). Thus change in CO_2 concentration affects the ability of the pathogen to cause disease on its host.

2.3.3 Light and Ultraviolet (UV)

Light has immense impact not only on the growth of plant but on diseases also. Intensity and duration of light may either increase or decrease the susceptibility of plants for infection and also the severity of disease. Light mainly causes production

of etiolated plants due to reduced light intensity which in turn increases the susceptibility of plants to non-obligate parasites but decreases the susceptibility of plants to obligate parasites. It also enhances the susceptibility of plants toward viral infections.

Ultraviolet light is part of the sun with a variety of benefits for plants. Ultraviolet has a role in inhibiting disease infections in plants because ultraviolet can increase the accumulation of plant protection pigments (López et al. 2012) and inhibit spore production from pathogens.

2.3.4 Ozone Gas

Ozone gas along with elevated carbon dioxide gas affects the plant pathogens and their activity. Tiedemann and Firsching (2000) studied the increase in the ozone (O_3) concentration in combination with CO_2 increase, for spring wheat plants infected or not with leaf rust disease (*Puccinia recondita* f. sp. *tritici*). They observed that the leaf rust disease was strongly inhibited by O_3, but unaffected by elevated CO_2. They also observed that elevated CO_2 largely equalized the negative effects of ozone gas on rate of photosynthesis, growth, and yield parameters, but was not capable of compensating the detrimental effects of fungal infection. Thus alteration in the amount of ozone may impact the pathogen as well as disease development.

2.3.5 Rainfall and Humidity

High rainfall increases the humidity of the air and soil which can be helpful factor in causing plant diseases. Besides, humidity is an ideal condition for some pathogens to emerge and develop because hot weather also increases the humidity of the environment (Das et al. 2016). Rainfall plays an important role in plant disease development. It has been observed that occurrence and severity of many diseases is directly related with amount and frequency of rainfall in that area. For example, in case of apple scab disease caused by *Venturia inaequalis*, at least 9 h continuous wetting of leaves with temperature of 18 to 23 °C is required causing primary infection by the pathogen. In powdery mildew disease, rainfall adversely impacts the infection of pathogen as presence of free moisture on the plant surface lowers the spore germination of the pathogen.

Relative humidity is very critical in fungal spore germination and the development of storage rots. Relative humidity plays a very important role in development of disease in some biotroph pathogens as well. As in case of white rust disease caused by *Albugo candida*, germination of fungal sporangia and release of zoospores take place at 16–18 °C temperature along with 80–90% relative humidity for 72 h.

In storage pathogens, *Rhizopus stolonifer*, causing soft rot of sweet potato does not cause infection if relative humidity is maintained at 85–90%, even if the storage temperature is optimum for the growth of the pathogen. Under these conditions, the sweet potato root produces corky tissues that wall off the *Rhizopus* fungus. Moisture

is generally needed for fungal spore germination, multiplication and penetration of bacteria, and initiation of infection, e.g., germination of powdery mildew spores occurs at 90–95% relative humidity.

2.3.6 Effect of Soil Moisture

Soil moisture influences the development of diseases by affecting the survival and spread of the pathogen. Soil moisture may be a limiting factor in the development of certain root rot diseases, e.g., high soil moisture levels favor development of destructive water mold fungi, such as species of *Aphanomyces*, *Pythium*, and *Phytophthora*. Clogging of the soil particles with moisture decreases the amount of oxygen and raises carbon dioxide levels in the soil which makes the roots more susceptible to root rotting organisms. Many plant diseases are more severe in low soil moisture condition, e.g., charcoal rot of corn, sorghum, and soya bean (*Macrophomina phaseolina*), take-all of cereals (*Gaeumannomyces graminis*), common scab of potato (*Streptomyces scabies*), and onion white rot (*Sclerotium cepivorum*).

2.3.7 Effect of Wind

Wind plays a very important role in dissemination and spread of pathogens in large areas sometimes from one continent to the other which is a common cause for epidemics in plant diseases. Major epidemic diseases caused by fungi, bacteria, and viruses spread either directly by wind or indirectly by insects which can travel long distances with the wind. These spread, establishment, and development of pathogen in new areas are the primary reasons of evolution of new pathogenic races of the pathogen which turn into a challenge to disease management due to their high adaptability in the new area. In case of rust diseases, fungal spores as uredospores and many conidia are transported to many kilometers by wind. Wind if accompanied with rain splashes becomes more devastating in case of bacterial diseases as it helps in spread of bacteria from the infected tissues to the healthy one.

2.3.8 Drought

Climate change causes uncertain seasons such as drought and even forest fires. Drought affects the physiology of plant species by weakening their defense system and increasing the resistance of some plant pathogens through the process of adaptation (Elad and Pertot 2014). When plants are stressed due to lack of moisture or excessive heat, they become more susceptible to those diseases which are favored by dry conditions such as charcoal rot disease of field crops, *Aspergillus* ear rot of corn, etc.

2.4 Emergence of New Diseases and Pathogens Due to Climate Change

Climate change may cause favorable environment to the emergence of new pathogens so that they may reach and survive in new host at new region as emerging pathogens have ability to infect a broad number of plants as well as new hosts altogether. The emergence of diseases is thought to be the result of various factors such as interactions among other pathogenic organisms, plant-pathogen interaction, plant-insect-pathogen interaction, and adverse environmental conditions (i.e., irregular water regime and prolonged droughts). Many authors mention that adverse factors can interact and "help" to subsequently cause complex diseases. For example, Deberdt et al. (2014) proposed that climatic factors could change the nature of microorganisms turning them into opportunistic pathogens which may lead to the emergence of new plant pathogens.

Climate change, reflected by changes in average temperatures, reduction of annual rainfall, irregular distribution of rainfall, and extended drought periods, may modify the growth or quality of crops and potentially cause plant mortality (Carnicer et al. 2011). Some authors agree that when plants become weakened or stressed by environmental factors, microorganisms can easily colonize plants thereby causing plant death (Moricca and Ragazzi 2008; Moricca et al. 2016). Indeed, global warming has contributed to the decline of trees and plants worldwide (Allen et al. 2010).

In this context, some human pathogens have been recorded affecting plants. *Enterobacter cloacae* (Jordan) is a clear example of a human pathogen exploring new hosts as plants. The bacterium has been linked to nosocomial outbreaks (Gaston 1988; Van den Berg et al. 2000) but was reported as a plant pathogen causing disease on onion (*Allium cepa* L.) in the USA. Later, this bacterium was reported to be affecting many other plant hosts including mulberry (*Morus* L.) in China (Wang et al. 2010), dragon fruit (*Hylocereus* spp.) in Malaysia (Masyahit et al. 2009), macadamia (*Macadamia integrifolia* Maiden & Betche) in Hawaii (Nishijima et al. 2007), lucerne (*Medicago sativa* L.) seeds in China, cassava (*Manihot esculenta* Crantz) in Venezuela, and chili pepper (*Capsicum annuum* L.). These events indicate that this bacterium has emerged as a plant pathogen in three continents, North America, South America, and Asia by switching its original hosts due to pathogen evolution.

In another example, bacterium *Xylella fastidiosa* which typically affects grape (*Vitis vinifera* L.) with Pierce's disease started affecting a new plant host, causing mulberry leaf scorch in California (Hernandez et al. 2006). There may be two key factors, natural host plant of *X. fastidiosa* and insect, which can possibly aid to the introduction and spread of this pathogen in to the new host and region causing new diseases. Natural host plant can serve as reservoirs for the bacterium and insects feeding upon these plants can become transmitting agent of the disease after getting infected (Hopkins and Purcell 2002).

Another example is *Tomato leaf curl New Delhi virus* (ToLCNDV), which was first described on tomatoes in 1995 in India (EPPO 2016), then other countries in

Asia found this virus on a wide range of crops. ToLCNDV was observed on courgette (*Cucurbita pepo* var. *giromontiina*) in 2012 in Spain (San Ambrosio and Fernández 2014). After Spain, it was detected in Tunisia in January 2015, causing high severity on cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), and courgette (*C. pepo* var. *giromontiina*) (Mnari-Hattab et al. 2015). The virus was transmitted in a persistent mode by the whitefly *Bemisia tabaci* (San Ambrosio and Fernández 2014) to other parts of the world. The insect vector migration could be the reason for the spread of the disease in Spain, then in Tunisia, and in Italy. As conditions became favorable for whitefly in different countries due to climate change, they could possibly spread the disease in newer regions.

One classic example of pathogen evolution and emergence in more virulent form is *Puccinia graminis* f. sp. *tritici* Ug99 which is present in Uganda, Kenya, Ethiopia, Sudan, Yemen, Iran, Tanzania, Eritrea, Rwanda, Egypt, South Africa, Zimbabwe, and Mozambique. It affects wheat causing losses up to 70% or more by causing wheat stem rust. A new virulent strain was identified in wheat fields in Uganda, and in 1999, it was designated as Ug99. This new race broke the resistance conferred by the gene Sr31 present in wheat stem rust-resistant varieties. This is a new global threat to wheat cultivation as its transmission takes place by wind or by the movement of people which spreads it immensely (Singh et al. 2011).

2.5 Possible Causes of Emergence of Plant Pathogens

There are numerous hypotheses for possible causes for emergence of new pathogenic organisms, e.g., bacteria, fungi, and virus.

1. The organism may be endemic in the crop regions but the new host discovered recently so pathogen successfully infected new host for survival.
2. After being endemic the organism became pathogenic, due to an increase in the organism's virulence or due to a decrease in the defense ability of host.
3. The organism may have been recently introduced into a new area and previously unexposed hosts, and the organism is pathogenic to novel plants (e.g., chili pepper).
4. Insect vectors exploit new plants, harboring the pathogenic organism and transmitting the organism to subsequent plants.

Besides all these factors, alterations in the host-pathogen interaction process can be a possible cause of occurrence of emerging pathogens. It is well known that pathogens use specialized secretion systems to produce proteins to infect plants or produce specialized structures or secrete toxins to invade plant cells (Doehlemann et al. 2009). To respond to this infection, plants modulate for its defense against the pathogen and pathogen may evolve in the process. This scenario of alterations in the host-pathogen relationship can be seen in the interaction between avocado (*Persea americana* Mill.) fruit and the fungus *Colletotrichum gloeosporioides* (Penz.) where the flavonoid epicatechin is synthesized by the avocado fruit to protect itself from the

laccase protein produced by *C. gloeosporioides*. The influence of geography is evident where the variability in the pathogenicity of genes within the same species is more evident in specific geographical areas. Isolates of *C. gloeosporioides* from Mexico showed increasing capabilities to metabolize epicatechin, when compared with isolates from Israel (Guetsky et al. 2005).

In case of viral diseases, the first opportunity for virus emergence is the exposure to new susceptible host plant. Successful initial infection of the host plant by a pathogenic organism is critical in establishment of the organism as an emerging pathogen or virus. The virus can remain in its initial form but is capable of genetically modifying itself to aid in exploitation of the susceptible host plant.

2.6 Actions for Mitigating Emergence of New Pathogens

Climate change is a nature's phenomenon which cannot be regulated or managed by human, but impact of climate change can be mitigated by managing the intense human activities to avoid possibility of new pathogen emergence. Some climate change mitigation strategies can be as follows:

- Renewable energy – Use of renewable energy such as wind, solar, or tidal energy can reduce our dependency on fossil fuels such as coal, oil, and natural gas. Therefore, this will reduce carbon dioxide emissions in the atmosphere.
- Carbon capture – It involves the capturing of greenhouse gases especially carbon dioxide gas from waste gases released from power stations and then storing it underground in old coal mines or gas fields. This also reduces emissions of such gases in the atmosphere.
- Afforestation – Plantation of more number of trees so that more carbon dioxide will be absorbed from the atmosphere during photosynthesis process.

These strategies may mitigate the effect of climate change up to some extent, but to avoid the impact of emerged plant pathogens, strict regulation and phytosanitary measures need to be imposed to avoid possible epidemics. Thus countries are making efforts to minimize threats by establishing regulatory measures to prevent, control, or eradicate diseases caused by pathogens potentially dangerous to crops. National and international organizations for free information about dissemination among scientists, governments, and the public would be crucial to minimize the threat of emerging pathogens.

Phytosanitary regulations are performed worldwide to prevent, combat, and eradicate pests affecting plants. For example, quarantine is an example to prevent the entry of pathogen or any material which may harbor the pathogen to the area where these pathogens do not exist. Quarantine also enables delayed introduction of pathogens into new areas until risks have been evaluated. Foreign quarantines prevent the introduction and presence of exotic pests, and domestic quarantines slow the spread and control or eradicate any pest that has been introduced to a certain country.

Phytosanitary programs include surveillance at ports, airports, and borders to avoid entry of any suspected material to avoid introduction of pathogen. These programs have generated successful results to stop the introduction of diseases from abroad. Despite similar programs and regulations, pathogens manage to arrive in new crop areas via vectors, humans, or environmental factors which may cause novel pathogen emergence.

2.7 Conclusion

Climate change is an important phenomenon that affects agricultural production significantly. By anticipating the future, we can prepare ourselves for problems caused by climate change, especially those related to agricultural activities, which generate the greatest amount of food consumed by humans. For several centuries, pests and plant diseases have played an important role in agricultural production, but global warming may modify areas affected by pests and diseases. So studies must be performed to assess pest and disease stages under the effects of climate change, determination of the magnitude of disease, and identification of the measures to minimize the risk of infection needed. Along with emergence of new pathogens and diseases, countries need to focus on minimizing the potential factors of such emergence, and because of the global distribution of some plant pathogens, researchers need to build international networks in coordination with competent authorities, to address the public policies for managing the most destructive diseases, those that are classified as quarantine diseases. So all the countries need to focus on major food threats and collaborate to design phytosanitary regulations and establish diagnostic protocols which would thereby strengthen government decisions. The protocols which are able to make accurate detection of the pathogen are the need of the hour. Protocols such as Morphological analyses, coupled with DNA sequence data, facilitate the identification of new pathogens or variants of pathogen. These diagnostic tools contribute to a rapid and accurate detection of new pathogens and should be consulted for the development of proper diagnostic protocols to manage the adverse results of newly emerged pathogen which may cause epidemic if not managed timely and carefully. Some mitigating plant need to apply for prevent, characterise and minimize the adverse of climate change.

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3 Emerging Important Nematode Problems in Field Crops and Their Management

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and Mohammad Haniph Shah

Abstract

Plant diseases are one of the major limiting factors in the production of food crops and in attaining food security and food safety. Around 40–50% of the crop produce is eaten away or destroyed by pests and pathogens, and its one fourth is attributed to plant diseases. Among various groups of plant pathogens, phytonematodes cause 5–20% yield decline in food crops, which may account to a net loss of 2–7% in crop-based food. Rice, wheat, and maize are most important food crops and are frequently attacked by plant nematodes. Generally, the crop damage caused by nematodes remains hidden to farmers because of nonappearance of discernable symptoms. In addition to direct damage, nematodes aggravate the infection of soilborne pathogens or act as vector leading to development of disease complexes. To prevent yield losses and to improve crop productivity and yield quality, it is essential to make growers realize the economic significance of plant nematodes. Adequate extension programs are needed to be implemented to demonstrate and advise appropriate nematode management methods to the crop growers. With regard to food crops, especially cereals, primary emphasis may be given to some of the most important nematode genera such as *Meloidogyne, Pratylenchus, Ditylenchus,* and *Heterodera* as these nematodes are widely distributed in agricultural fields and cause tremendous damage to food crops. Common cultural practices, viz., deep plowing, flooding, fallowing during summer, removal and burning of weeds and remnants of previous crops, use of certified and disinfested planting materials, and cultivation of non-host, resistant, or tolerant crops, may substantially prevent crop losses in cereals caused by nematodes. Further, seed priming with biopesticides of

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Trichoderma spp. or *Pseudomonas fluorescens* as a general treatment may prove quite effective in growing healthy crop with considerably higher yields.

Keywords

Plant diseases · Cereals · Phytonematodes · Disease management · Biocontrol

3.1 Introduction

The Indian agriculture after facing several challenges has achieved self-sufficiency for the last two to three decades in numerous agricultural products particularly cereals, pulses, oilseeds, etc. (Khan and Jairajpuri 2012). During the 1950s, the indigenous production was insufficient to meet the food requirement of the people equivalent to one third of the today's population, and the country had to rely on 8–10 million tons of food import annually. The continued positive planning and policy of the governments on promotion of agriculture since independence has ultimately led to self-sufficiency in food crops and also an increase of around 20% in the net national area under crop cultivation in comparison to 1950 (Indiastat 2018). With the advancement of technology of food production and disease management, the net national productivity has increased by around 3.5 times in food grains, 1.75 times in fruits, and 2.25 times in vegetables. Presently India is self-sufficient in food grains and is able to offer food to the existing population with ease and also able to store two buffers each of 454.10 lakh MT in 2018 and 711.18 lakh MT in 2019 (FCI 2019).

The food grain cultivation in India occupies a major share in the food production. The food crops, primarily cereals, are cultivated in around 150 million hectares (mha), which is 72.2% of the total area under cultivation (Fig. 3.1). Important food crops are rice, wheat, sorghum, maize, etc., and among them rice occupies the greatest area under cultivation (43.7 mha), followed by wheat (28.15 mha Fig. 3.1). The total annual production of food crops in India is 267.5 million tons (Fig. 3.1). Rice and wheat contribute around 36 and 29.3% of the total production of food crops adding 96.43 and 78.4 million tons annually to the national food basket, respectively (Fig. 3.1).

India, after attaining self-sufficiency and food security, has focused to ensure food quality to meet the challenges of malnutrition and hunger as well as to compete in the global trade for export incentives. Pest and pathogens are important constraints in improving the crop productivity as well as the quality. In developing countries including India, around 50% of the total produce is lost quantitatively or qualitatively due to pests and diseases at pre- and post-harvest stages (Khan and Jairajpuri 2010; Fig. 3.2). Diseases caused by various pathogenic fungi, bacteria, viruses, nematodes, etc. have been found to be responsible for 25% of the total losses inflicted by pests and pathogens. Among the diseases, greatest losses are inflicted by fungi (42%) followed by bacteria (27%), viruses (18%), and nematodes (13%) of the total losses caused by the pathogens (Fig. 3.2).

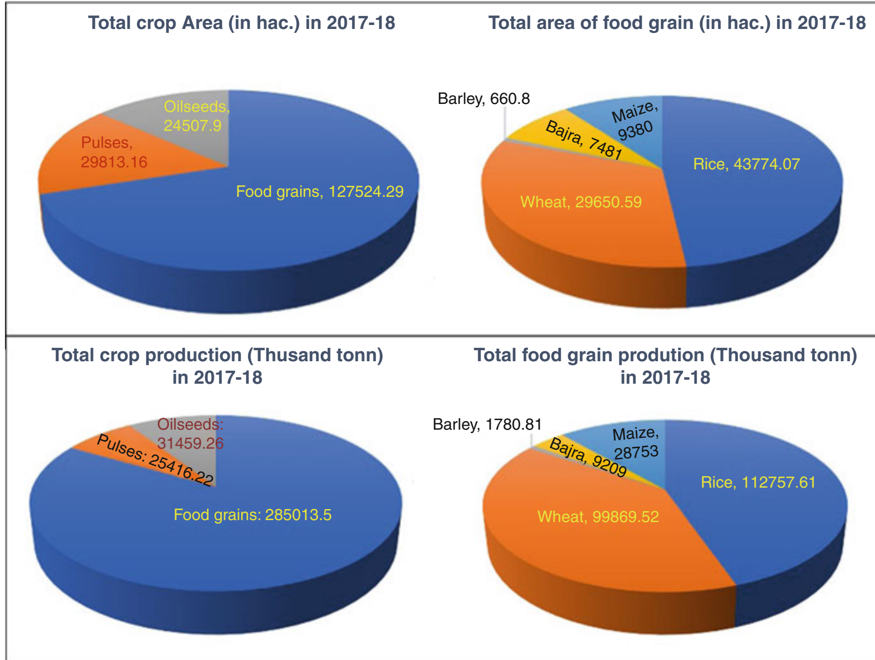

Fig. 3.1 Pie chart showing cultivation area and production of food crops in India

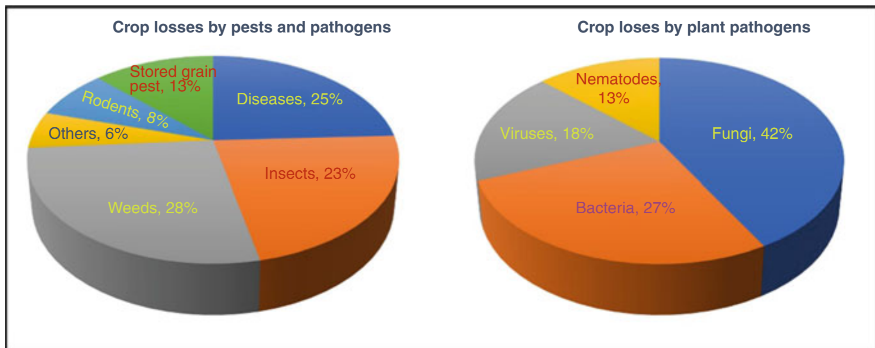

Fig. 3.2 Pie chart showing crop losses caused by pests and pathogens

3.2 Plant Parasitic Nematodes

Nematodes are vermiform or threadlike animals, and their body is thin, flexible, generally elongated (0.3–11 mm), and unsegmented and tapers at both the ends (Khan 2008; Fig. 3.3). Basically, the nematodes are aquatic animals thriving best in water, but they have adapted to terrestrial habitats. Nematodes constitute 80–90% of

Fig. 3.3 Plant parasitic nematodes in water isolated from a crop field in the faculty farm at Aligarh Muslim University, India

all the multicellular animals; fortunately, only a fraction of this number possesses ability to parasitize plants, and the rest are free-living surviving on various substrates (Khan 2008).

Plant parasitic nematodes are considered important pathogens of numerous agricultural crops. Nematodes cause damage to plants by injuring and feeding on the root hairs, epidermal cells, cortical cells, and/or stealer cells (Khan and Jairajpuri 2012). A large number of nematodes are ectoparasites feeding on root surface, e.g., *Tylenchus, Rotylenchus, Tylenchorhynchus, Belonolaimus, Hoplolaimus, Trichodorus, Longidorus,* etc. However, a considerable number of nematodes fully enter inside the host roots and are called endoparasites, such as root-knot nematodes (*Meloidogyne* spp.), cyst-forming nematodes (*Heterodera* spp.), and root-lesion nematode (*Pratylenchus* spp.). Whereas, some nematodes such as citrus nematode (*Tylenchulus semipenetrans*) and reniform nematode (*Rotylenchulus reniformis*) are semi-endoparasites as they partially enter the host tissue (Siddiqui 2005).

The most common effect of nematode parasitism is debilitation of the plant even without appearance of any symptom (Perry and Moeus 2013). In addition to direct damage, nematodes often aid or aggravate the diseases caused by fungi, bacteria, and viruses or may break resistance of cultivars to pathogens (Khan 1993). Hairy root of roses caused by *Agrobacterium rhizogenes* is of minor importance, but in the presence of *Pratylenchus vulnus* the disease becomes severe (Sitaramaiah and Pathak 1993). The *Fusarium* wilt-resistant cultivars of cotton become susceptible in the presence of root-knot nematodes (Atkinson 1982). Plant nematodes may also act as vectors for bacteria, fungi, and viruses. *Anguina tritici* carries *Clavibacter tritici* and *Dilophospora alopecuri* to shoot meristem of wheat (Khan and Dasgupta 1993). Ringspot viruses (NEPO viruses), e.g., tobacco ring spot virus, are transmitted by *Xiphinema* and *Longidorus* species. *Trichodorus* and *Paratrichodorus* species act as vector for certain tobraviruses such as tobacco rattle and pea early browning viruses (Taylor and Brown 1997).

3.3 Nematode Infestation in Food Crops and Its Management

Phytonematodes are potential pests of all kinds of food crops including cereal crops (Perry and Moens 2013). They attack root, stem, leaves, crown, inflorescence, flowers, and developing grains (Southey 1986). The crop damage depends on the plant species or cultivar, nematode species, level of soil infestation, and the prevailing environmental conditions. Nematodes usually cause severe reduction in the plant growth and yield, both quantitatively and qualitatively (Khan 2008). *Molya* of wheat (*Heterodera avenae*), *ufra* of rice (*Ditylenchus angustus*), root rot of maize (*Pratylenchus zeae*), root-knot (*Meloidogyne* spp.) etc. are some of the diseases which cause serious economic loss to cereal crops (Khan and Jairajpuri 2010). Despite a significant impact on agriculture, nematodes have not been recognized as major pests of crops in particular the cereal crops. This is probably because of the fact that the damage caused by nematodes is less obvious than that caused by fungi or other pathogens and remains hidden from the sight of farmers. Moreover, foliage of cereal crops dries at maturity, and the plants are harvested from the ground level leaving behind the roots in soil on which the nematodes cause some recognizable symptoms.

The stunting of plants and mild yellowing of foliage are the debilitation symptoms generally caused by phytonematodes and resemble with nutritional deficiency (Fig. 3.4). As a result, fertilizer in place of a nematicide is applied, which proves ineffective and noneconomic. These nonspecific or general symptoms of nematode infestation appear in the patches of plants irregularly distributed in a field and show stunted growth and sparse and dull green or pale yellow foliage (Luc et al. 2005). The infested plants show incipient wilting despite adequate moisture available in the soil during sunny days, but recover at night. Further, roots so weakened and damaged by nematodes are easily invaded by many bacteria and fungi, leading to accelerated root decay (Khan 1993). This secondary damage also does not draw immediate attention, and an incurable stage is soon reached leading to severe yield loss. However, in heavily infested fields, characteristic symptoms appear on roots or shoots. Symptoms develop more frequently on roots because mostly nematodes are root feeders (Khan 2008). Specific symptoms are root lesions, root rot, root pruning, root galls, leaf tip whitening, seed galls, cessation of panicle growth, etc. (Fig. 3.5).

Fig. 3.4 General symptoms of nematode attack in a field. Plants in patch showing stunted growth with chlorotic foliage

Fig. 3.5 Specific symptoms caused by nematodes on plants. Root knot by *Meloidogyne* on tomato (**a**), sponge gourd (**b**), and rice (**c**); dirty root by *Rotylenchulus reniformis* on vegetable (**d**); seed gall by *Anguina tritici* on wheat (**e**); and panicle chaffiness by *Aphelenchoides besseyi* (**f**)

Phytonematodes may cause about 5–20% yield loss with an average of around 13% when various crops are considered. The yield losses vary greatly depending on inoculum level and host species (Khan et al. 2009). The severe infection may result to as much as 80–90% yield decline in an individual field, and sometimes plants fail to give yield of any economic value. Crop losses due to nematodes are greater in the developing countries than the developed countries. It is probably due to unplanned agricultural practices, unawareness of the farmers about nematodes, and nonavailability of nematicides. In the developed countries where management practices are properly implemented, relatively lesser crop damage due to nematodes occurs. In the USA alone, annual monetary loss due to nematodes has been estimated above $ 6.0 billion (Agrios 2005). In India, about 10–20% crop losses occur due to nematode infestations. At high population levels, much greater losses may occur in susceptible crops. Cereal crops are considerably susceptible to nematode attack and exhibit yield decline of economic value (Table 3.1).

The researches and field-based data have shown that nematodes act as a potential factor in limiting the productivity of cereals crops in India as well as in other parts of the world. From an Indian point, the important nematode diseases in cereals are described under. These diseases cause significant yield decline in cereals; hence their management is essentially required to improve cereal productivity in the country.

Table 3.1 Crop losses caused by nematodes to food crops

Crop	Yield loss (%)
Cereals	7–22
Wheat	7–19
Rice	10–22
Maize	6–18
Barley	7–27
Oat	3–12

Fig. 3.6 Root symptoms of *Heterodera avenae* on wheat: Roots of a maturing plant with white females and infected root showing bushy rootlets

3.3.1 Wheat

3.3.1.1 Molya Disease Caused by *Heterodera avenae*

The disease is prevalent in almost all wheat-growing regions in India. The affected plants are stunted with smaller, yellow, and fewer leaves. Tillering and development of spikelets are reduced and emergence of earheads is delayed. The infected roots become elongated and have tuft of rootlets at the distal end (Fig. 3.6). The molya may lead to 15–100% reduction in the grain yield of wheat depending on the nematode population and wheat variety.

The cereal cyst nematode *H. avenae* has a narrow host range; hence, the nematode can be successfully managed by crop rotation for 2–3 years with non-hosts in heavily infested areas. The rotation with non-hosts or keeping the field fallow during Rabi reduced the cyst population by 50 and 75% and increased the wheat yield by 83 and 135% after 1 and 2 years, respectively (Smiley and Nicol 2009). Singh (1985) reported that cultivation of resistant barley and mustard crops reduced the nematode population by 57–58% and 50%, respectively. A decline of 87–100% in the population of *H. avenae* may be achieved by growing *Brassica campestris* var. *dichotoma*, *B. campestris* var. sarson, *B. campestris* var. toria, *B. juncea,* and *Eruca sativa* for one season (Singh et al. 1987). Further, the cultivation of resistant barley cvs. BH393 and fenugreek (*Trigonellafoenum-graceum*) reduced cyst population by 63 and 68%, respectively, and subsequent crop of wheat was better after cultivation of

fenugreek. Plant resistance is an effective method of nematode control which prevents nematode reproduction and shortens the rotation period without involving extra cost to the growers.

The genes imparting resistance to different populations of *H. avenae* in wheat have been identified in different countries (Montes et al. 2003; de Majnik et al. 2003; Martin et al. 2004). The genes resistant to CCN from *Aegilops variabilis* and *A. triuncialis* have been transferred to wheat (Jahier et al. 1998; Romero et al. 1998). Montes et al. (2008) reported higher level of resistance against Spanish pathotype Ha71 in wheat lines carrying Cre1, Cre4, or Cre7 genes than carrying Cre8 or Cre3 genes. The cvs. Katyil and AUS 10894 showing resistance in Australia were found susceptible to *H. avenae* in India (Dhawan 1988; Bishnoi and Bajaj 2004). The sources of resistance in wheat to Indian population of *H. avenae* have also been identified (Kanwar et al. 2001, Kaur et al. 2008) and are being incorporated in breeding programs. A resistant cv. Raj Molya Rodhak1 (Raj MR1) was released for cultivation in Rajasthan in 2002 (Mathur et al. 2004; Sharma et al. 2004). Resistance in this variety is governed by single dominant gene, which offers both pre and post-infection resistance (Kanwar and Bajaj 2009). Nevertheless, some of the *H. avenae*-resistant wheat and barley varieties are susceptible to *H. filipjevi* (Bishnoi and Bajaj 2002; Kanwar et al. 2004) suggesting separate breeding programs for the two species.

The nematicides should be used against CCN when other options of control have been exhausted. Aldicarb and carbofuran at 1.0–1.5 kg a. i./ha have been widely used against cereal cyst nematode in India, but after Bhopal gas tragedy in 1984, carbofuran is the only nematicide available in Indian market. Metam sodium has been found quite effective in suppressing the soil population of CCN (Kanwar and Bajaj 2010). Depending upon availability and feasibility, two or more control practices can be integrated for achieving effective control of *H. avenae*. For example, a combined application of nitrogenous fertilizer and nematicides significantly increased the yield of wheat and barley (Handa et al. 1978; Sakhuja et al. 1978). Bhatti et al. (1980) reported that integration of early sowing date (November) with aldicarb/Furadan at 2 kg a.i. /ha resulted in a significant enhancement in the wheat yield and reduction in cyst population. Application of neem seed powder, FYM, and *Trichoderma viride* with nematicides effectively controlled *H. avenae* infection in wheat (Pankaj et al. 2008). Soil application of *Purpureocillium lilacinum* or *T. harzianum* during the field preparation may also suppress nematode population in soil (Khan 2016).

3.3.1.2 Ear Cockle or Seed Gall Disease Caused by *Anguina tritici*

The seed gall caused by *Anguina tritici* is an important disease in several underdeveloped and developing countries including India, Pakistan, Ethiopia, Romania, Iraq, Syria, Yugoslavia, etc. In India, the ear cockle disease is reported to occur in Bihar, Haryana, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Rajasthan, and Uttar Pradesh. Its incidence is much higher in socially backward and tribal areas where certified seeds are not used (Nandal et al. 2010).

Fig. 3.7 *Anguina tritici* symptoms on wheat plant (**a**), seed galls (**b**), and healthy seeds (**c**)

The diseased plants show basal swelling of stem; crinkling, curling, twisting, and rolling of the leaves; profused tillering; and stunted growth. The diseased earheads are smaller and broader than the normal ones with or without awns on them, and in such earheads instead of formation of grains, cockles (seed galls) are formed. The cockles are brown to black at maturity and contain large number of second-stage juveniles of *Anguina tritici* in a quiescent state (Fig. 3.7). The nematode also aids a bacterium, *Clavibacter tritici*, and a fungus, *Dilophospora alopicrozi*, causing yellow slime (tundu) and twist diseases, respectively. Average incidence of the diseases and decline in the yield of wheat caused by this nematode in India has been reported as up to 50%.

Since *A. tritici* is a strict seed borne in nature, use of cockle-free seeds is the best approach to control the seed gall disease as nematode cannot survive in soil or in seed galls lying in the field (Popov et al. 2006; Paruthi et al. 2009). Seeds can be cleaned by separating the cockles from the healthy seeds using the mechanical and physical methods. Winnowing the contaminated seeds by winnowing basket in an open area is a common practice in several parts of India to separate the cockles from the wheat grains. Sieving the contaminated wheat seeds with sieves of definite mesh has been a common method to achieve gall eradication in several countries (Nandal et al. 2010). But complete removal of galls is not achieved by this method. Putting the nematode-infested wheat seeds into a drum containing water and stirring it well with some wooden stick or hand for some time help in removing cockles from the infested seed lot. The galls because of lighter in weight float on the surface of water and are removed by ordinary sieves and buried deep or burnt. This method is relatively more effective but still 10–15% cockles remain with healthy seeds. Use of brine by putting the seed in 10–30% concentrations of salt solution is another method of removing the cockles from the grains. Addition of salt increases the range and duration of floating of cockles, and 100% recovery of cockles is attainable with this method (Paruthi and Bhatti 1992). The seeds are required to be washed in water several times before sowing to avoid injurious effect of salt on germination of seeds (Nandal et al. 2010).

3.3.2 Rice

Rice is a susceptible host to a number of nematodes, viz., *Ditylenchus angustus, Hirschmanniella oryzae, H. gracilis, H. mucronata*, *M. incognita, M. javanica, M. arenaria, M. graminicola, M. triticoryzae, M. oryzae,* and *M. salasi*, which cause following disease in paddy.

3.3.2.1 Root-Knot Disease Caused by *Meloidogyne graminicola*

Among *Meloidogyne* spp., *M. graminicola* is the most serious and emerging threat to the rice cultivation in various rice-growing regions of the country. Earlier this nematode was prevalent in upland rice, but during the last 25–30 years, the nematode has rapidly spread to deepwater and irrigated rice in India. Unlike other *Meloidogyne* spp., *M. graminicola* incites galls on the tip of lateral roots (terminal galls) which are spiral or hook-like (Fig. 3.7; Khan and Anwer 2011; Salalia et al. 2017). The nematode attacks rice in the nursery as well as in the main field and causes substantial yield loss to the crop. The rice root-knot nematode is found on rice mainly in South and Southeast Asia, but also in South Africa, the USA, Colombia, and Brazil. Now it is a potential pest of upland, lowland, irrigated, and deepwater rice throughout the world including India (Haque et al. 2019). In India, the nematode has been reported from all states where rice is cultivated (Prasad et al. 2010; Khan and Ahamad 2019). Common weeds of rice fields and rotation crops such as wheat, onion, cabbage, and tomato are also susceptible to the nematode. MacGowan and Langdon (1989) reported 100 host plants of *M. graminicola*, which includes food, fodder, fruits, ornamentals, and weeds. The symptoms produced due to the infection of *M. graminicola* are manifested in the form of characteristic hook- or spindle-like terminal galls on the roots (Fig. 3.8). Due to formations of galls, the absorption capacity of roots is impaired, leading to stunting of plant growth and yellowing of leaves. These symptoms appear on the plants in patches within a nursery or main field (Fig. 3.8).

The rice root-knot nematode causes significant yield decline in upland rice and rainfed lowland rice (Prasad et al. 2010). It has frequently been observed in irrigated

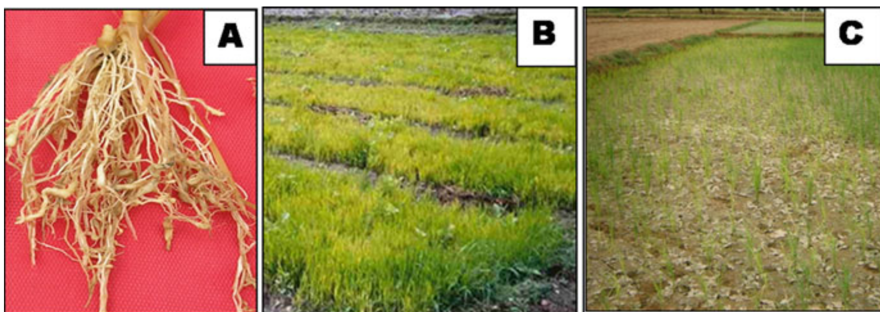

Fig. 3.8 Root-knot nematode, *Meloidogyne graminicola* infecting rice. Root galls on a plant (**a**), yellowing of nursery (**b**), and severely suppressed growth of young plants in a field (**c**)

rice (Prot and Matias 1995; Khan and Jairajpuri 2010). The yield losses are greater under flooded conditions (Kinh et al. 1982; Prot et al. 1994) than in upland rice and are responsible for the poor plant growth and yield in aerobic rice (Krreye et al. 2009). The ability of *M. graminicola* to attack oats and cereals in addition to rice in Southeast Asia makes this nematode a potential threat to small grain production (Dutta et al. 2012). The impact of *M. graminicola* on rice yield has been well established, with yield losses up to 20–90%. In India, *M. graminicola* is responsible for 20–35% yield decline in rice due to poorly filled kernels (Prasad et al. 2010; Dutta et al. 2012; Khan et al. 2012, 2014; Khan and Ahamad 2019). Khan et al. (2012) have reported 20–31% yield decline at 1000 J_2/kg soil. The basmati rice has been found highly susceptible to the rice root nematode, posing a major economic threat to the growers. The root-knot in rice can be managed using the following methods depending on the circumstances.

Sesbania rostrata is a very good host for *M. graminicola* when grown in non-flooded soils. Hence its cultivation as a green manure trap crop before rice in non-flooded soils infested by *M. graminicola* may reduce nematode population in the field. However, care in the time of plowing should be taken as a delay may lead to increase in the population. However, under rainfed conditions *S. rostrata* should not be used. Growing *Tagetes* sp. and *Crotalaria* spp. (*C. incana* and *C. mucronata*) may lead to reduction in *M. graminicola.*

Crop rotation with non-host crops like jute, mustard, and chickpea and rice-resistant varieties (TKM-6, Patna 6, Dumai, Ch 47, Hamsa) has been found effective in reducing *M. graminicola* infestation. Soil amendments with water hyacinth compost (300 or 600 g/4.5 kg soil) reduced root-knot nematode infestation and increased plant growth (Roy 1976). Rice-mustard-rice crop sequence, followed by rice-maize-rice, and rice-fallow-rice were reported to be effective in reducing nematode development (Kalita and Phukan 1990; Reddy 2018). Crop rotation with non-host crops, viz., sweet potato, cowpea, sesamum, castor, sunflower, soybean, onion, turnip, and cauliflower, may inhibit nematode development (Rao et al. 1984; Rao 1985; Soriano and Reversat 2003; Mantelin et al. 2017).

Good puddling before transplanting helps in improving water retention in soil which reduces aeration and also reduces nematode movement and invasion of fresh rice roots by infective juveniles of *M. graminicola* and *M. triticoryzae* (Garg et al. 1995). The population of *Meloidogyne* spp. decreased drastically when the field was puddled every year over a 10-year period (Gaur 2003). In Bangladesh widespread infestation of *M. graminicola* was found when direct-seeded rice was grown instead of puddled rice (Padgham et al. 2004).

Biocontrol agents especially *Trichoderma harzianum* (Bokhari and Fardos 2009), *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*; Kiewnick and Sikora 2006), *Pochonia chlamydosporia* (Khan et al. 2005; Niu et al. 2010), *Aspergillus niger* (Khan and Anwer 2008; Ashoub et al. 2009), *Pseudomonas fluorescens* (Khan and Haque 2011), *P. putida* (Haque et al. 2019), and *Bacillus subtilis* (Dawar et al. 2008) have shown great potential in suppressing the infection of *Meloidogyne* spp. (Khan 2016; Khan et al. 2019) on different crops. The BCAs can be applied through seed treatment (Santos et al. 1992), root-dip (Khan et al. 2005), or soil application (Zeinat

et al. 2010) depending on the crop and convenience of the grower. However, the degree of effectiveness of the BCA may vary with the method of application (Khan 2007).

The soil solarization in nursery bed may effectively reduce the nematode population. Prior to sowing, the nursery bed is covered with a plastic polyethylene tarp for 3–4 weeks in summer, and nursery bed treatment with carbofuran or phorate at 2 kg a.i./ha was found very effective in reducing root-knot nematode infestation in rice nursery (Das et al. 2018). Carbofuran at 2.4, 4.8, and 7.2 g a.i./kg seed reduced galls of *M. oryzae* by 57, 79, and 80%, respectively (Segeren et al. 1985). Seed soaking with 0.1–0.2% carbofuran for 12 h was found to be effective in reducing *M. graminicola* population (Rahman and Das 1994). Basamid at 40 g/m^2 was found to be most effective in reducing rice root-knot nematode population (Singh 2017). Soil application of Furadan, phorate, isazophos, cartap, carbosulfan, or quinalphos at 0.5, 1.0, and 2.0 kg/ha reduced 82% galling of *M. graminicola* at 2 kg (Khan et al. 2016). The nursery bed treatment with carbofuran (3G) at 0.3 g a.i./m^2 followed by the main field treatment with carbofuran (3G) at 1 kg a.i./ha 40 days after transplanting is effective to reduce nematode population (Walia and Khan 2018). A combination of *P. fluorescens* at 20 g/m^2 + *T. harzianum* at 20 g/m^2 + carbofuran effectively controlled rice root knot in rice (Narasimhamurthy et al. 2017). Combined application of neem cake + vermicompost + *Trichoderma* spp. in field resulted in almost gall-free plants (1–2 galls/root systems, Kumar et al. 2017). Das et al. (2018) reported that an application of carbofuran at 0.3 g a.i/m^2 in the nursery bed combined with field application of *Trichoderma viride* at 2.5 kg /ha pre-incubated with 25 kg FYM at 45 days after transplanting proved quite effective and economical for the management of *M. graminicola* in the field. Field application of neem cake+vermicompost+*Trichoderma* spp. was also found effective against *M. graminicola* (Kumar et al. 2017).

3.3.2.2 Ufra Disease Caused by *Ditylenchus angustus*

The *ufra* is an important prevalent disease of rice in Malaya, Malaysia, Burma, the Philippines, Egypt, Southern Thailand, Madagascar, parts of West Coast, Vietnam (Bora and Rahman 2010), and India. The nematode attacks rice in Assam, West Bengal, Maharashtra, Andhra Pradesh, and U.P. The *ufra* is characterized by mosaic-like discoloration arranged in a splash pattern or chlorosis or white streaks on young leaves and sheaths. The entire leaf may become twisted or severely malformed, or the basal portion of the young leaf becomes wrinkled and whitish green (Fig. 3.9). Under severe infestation, the leaf margin crinkles and leaf tip gets twisted. The emerging panicle becomes distorted, bears sterile or empty grains at the base, and produces normal grains only near the tip (Bora and Rahman 2010). Yield losses due to *ufra* have been reported as 5–100% depending on the rice cultivar and nematode population. Management of *D. angustus* is difficult because of the nature of the deepwater rice ecosystem. However, following methods may prove effective against the nematode.

Burning of diseased stubbles, followed by plowing may substantially reduce the incidence of *ufra* disease in the next crop (Ou 1972; Walia and Khan 2018). Stubble

Fig. 3.9 The deformed and underdeveloped panicles due to infection by *Ditylenchus angustus*

burning should be carried out in an organized way to avoid remaining of infested patches unburnt, but this may cause air pollution. Instead of burning the stubble may be collected in a big pit, and some *Trichoderma* formulation be sprayed on the stubble and be allowed to decompose to use this as a biopesticide compost for next crop. This may give additional earning to the farmer as the decomposed stubble produced from 1 ha may be sufficient for 4–5 ha. For root stubbles, plowing should be carried out soon after harvest of the crop so as to give sufficient time to decompose the stubbles and to expose the nematodes to the sun. The nematode does not survive in the quiescent state for longer period of exposure to sun and desiccation. The field should be kept free from weeds and ratoons. Removal of infected leaves along with upper portion of rice plant also reduces the nematode population in the field. Rotation of deepwater rice with non-host crop such as jute (*Oletorious* spp.) and mustard (*Brassica* sp.) reduces the *ufra* disease in the next crop. In areas with good irrigation facilities, cultivation of autumn rice *(boro* rice) followed by summer rice (*ahu* rice) will result in control of this disease, as this nematode will find no host during its active period. However, this practice may not be feasible in most of the deepwater rice-growing areas, where floodwater rises to a high level, in which cultivation of *ahu* rice may not be possible. Cultivation of late cultivars (Padmapani) before the flood and earlier harvesting shall escape the nematode attack (Mac Geachie and Rahman 1983). The rice cvs. Bazail-65, Jalamagna, Basudev, AR-9(C), IR 13437-20-4E-PI, IR 17643-4, Lakhi, Karkati, BR 308-3-3-2, Rayada 16-011, Rayada 16-013, Rayada 16-05, Rayada 16-06, Rayada 16-07, Ba Tuc, etc. are quite effective in tolerating the nematode attack (Plowright et al. 2002; Bora and Rahman 2010). The wild rice *Oryza subulata* is also resistant to *D. angustus* (Miah and Bakr 1977); Walia and Khan (2018). Rahman and MacGeachie (1982) found three promising rice entries, viz., CN 540, NC 493, and TCA 55, completely free from *ufra* infection. Two rice entries, namely, Fukuhonami and Matsuhonami, were found highly resistant and, seven, namely, Rayeda, Bazail, Hunenwase, Shinanokogane, Kinonishiki, Aokazi, and Koshinihsini, were resistant against ufra (Haque and Latif 2011).

Chemical control is also an option, difficult to apply in deepwater rice. The disease generally manifests under flooded condition, making almost impossible to

implement curative measure. This necessitates higher dose of chemical which may cause serious health and environmental hazards and harm the fish fauna and other aquatic resources. Among the pesticides, carbofuran has been found most effective in controlling the disease (Bora and Rahman 2010). When carbofuran was applied at the time of transplanting, maximum yield and minimum *ufra* infestation were recorded (Rahman 1993; Islam et al. 2013). Latif et al. (2011) reported that the ufra infestation was significantly reduced when 1 kg ai/ha Furadan 5G was applied 20 days before transplanting of infested seedlings in the field. Walia and Khan (2018) reported that the seed treatment with carbosulfan (25EC) at 3% a.i. (w/w) and foliar spray with carbosulfan 20.2% a.i. at 40 ml and 120 days after transplanting is helpful in reducing disease incidence. Haque et al. (2013) reported that application of neem seed kernel extract, Furadan 5G, and Bavistin 85WP was applied singly and in different combinations to develop an integrated management package against ufra. Single spray of neem seed kernel extract at 10% concentration in combination with application of Furadan 5G at 10 kg/ha or Bavistin 85 WP at 1.5 kg/ha ensured lower ufra incidence and maximized rice yield.

3.3.2.3 Root Yellowing and Rotting Disease Caused by *Hirschmanniella* spp

Another important nematode disease in rice is caused by *Hirschmanniella oryzae*, which has been reported from countries like Thailand, India, China, Pakistan, the Philippines, Bangladesh, Cuba, Madagascar, Sri Lanka, the USA, East Asia, Taiwan, New Zealand, Nigeria, Vietnam, Europe, and Japan. In India, the nematode attacks rice in Andhra Pradesh, Kerala, and Tamil Nadu (Ghosh and Manna 2010a, b). The infested plants are stunted with fewer and shorter tillers. Foliar growth is retarded and flowering is delayed. Necrotic areas develop on the roots (Fig. 3.10). Infestation with *Hirschmanniella* spp. may result in 10–70% yield loss.

Application of neem cake at 1 ton/ha and press mud at 10 ton/ha (Johnathan and Pandiarajan 1991) and castor oil cake and mustard oil cake (Khan and Shaukat 1998; Lakshmy 2014) significantly reduced *Hirschmanniella oryzae* populations. Incorporation of *T. erecta* whole plant ranked first among the organic amendments

Fig. 3.10 Symptoms caused by *Hirschmanniella* spp. in rice (**a**) aboveground and (**b**) underground. (https://images.app.goo.gl/nQqeM6UjysLS9pwq6, https://images.app.goo.gl/R2xpQLDpJVX59AdB6)

(Lakshmy 2014). Rotations of rice with cabbage and tobacco reduced populations of *H. oryzae* by 83–88% in paddy fields (Gao Xue Biao et al. 1998; Sikora et al. 2018). The cropping sequences following Pankoj Paddy→Jalmasta jute (*Hibiscus sabdariffa* L.) → 2-month fallow → Pankoj Paddy or Pankoj Paddy → 1-month fallow → wheat (*Triticum aestivum*) → Disimasta jute (*Corchorus olitorius) → 1*-month fallow → Pankaj Paddy have been found effective against the nematode (Ghosh 2001; Ghosh and Buddhadeb 2008). During cultivation of Jalmasta jute, the population of *H. oryzae* decreased more than 50% (Chen et al. 2012).

Rice cultivars belonging to the Japonica group are found to be more susceptible to *H. oryzae* than the other groups (Youssef 1999). Significantly higher populations of *H. oryzae* were found in Pakistan, Basmati and Basmati 370, while Basmati 385 supported the lowest population (Randhawa et al. 1992). Walia and Khan (2018) reported that the rice cultivars TKM 9, CR 142-3-2, CR 52, N 136, and W 136 were resistant to *H. oryzae*. Wild rice species, *Porteresia coarctata*, showed the highest degree of resistance to *H. mucronata* (Panigrahi and Mishra 1995). *Oryza collina* and *O. nivara* were moderately resistant to *H. oryzae***.**

Application of phosphamidon and chlorpyrifos as root dip at 0.02% for 20 min before planting reduced *Hirschmanniella oryzae* population to 0.83/2 g root 30 days after transplanting (Ramakrishnan et al. 1984). Bare root dip treatment with carbosulfan and phosphamidon at 0.3% reduced *H. oryzae* population by 46.2 and 40% and increased grain yield by 35.1 and 34.7 q/ha, respectively, over untreated control (Lahan et al. 1999). Nursery bed treatment with carbofuran at 0.3 g a.i./m^2 followed by field application of carbofuran at 1 kg a.i./ha 40 days after transplanting in endemic spots at farmers' field has been recommended for management of root-knot nematode (*Meloidogyne graminicola*) and rice root nematode (*Hirschmanniella oryzae*) in rice (Khan et al. 2010).

3.3.2.4 White Tip Caused by *Aphelenchoides besseyi*

This disease is known by different names, viz., white tip of rice, "black grain" disease of rice, "ear blight" of Italian millet, and "heart blight" of rice. *A. besseyi* is widely distributed in Andhra Pradesh, Assam, Bihar, Himachal Pradesh, Kerala, Maharashtra, Meghalaya, Orissa, Punjab, Tamil Nadu, Tripura, and West Bengal causing chaffiness of panicles and whitening of leaf tip (Fig. 3.11; Khan 2010). The nematode also plays an important role in the development of rice disease complex, synergizing *Acrocylindrium* (=*Sarocladium*) *oryzae*, *Curvularia* spp., *Fusarium* spp., etc. (Rao and Prakash 2002). Yield loss varies from 10 to 71% in nematode-affected rice fields.

The infested plants show white tip or whiplike malformation of the top third of the leaf blade (Fig. 3.9). At the flowering stage, chaffiness and abnormal elongation of glumes in some spikelets, rachii, and rachillae occur. Infected plants show reduced vigor, height, and weight of spikelets and number of grains. Abnormal elongation of the panicles (Rao 1978) and chaffiness or scattered chaffiness in the florets also occur in case of severe infestations (Fig. 3.9; Prasad et al. 2007). In some rice cultivars, A. besseyi *may produce only the symptoms of small grains and*

Fig. 3.11 White tip nematode damage in leaves, ovary, and spikelets. (Source: https://images.app.goo.gl/GPbhQkZjWCCcf4sSA)

erect panicles, but not the typical leaf white tip. The disease can be controlled using the following methods.

Physical methods such as storage of *A. besseyi*-infested seeds in regulated gas medium (97.5% nitrogen and 2.5% oxygen) for 10 days at 25 °C kill the quiescent larvae. The treatments with hot water at 53–54 °C for 15 min may also eliminate the infestation in seeds (Youssef 2014; Pashi et al. 2017a, b). A combination of seed treatment (0.3% by seed weight) and spraying with benomyl (2.5 g/dm^3 at 1 or 15 days after transplanting) may fully protect rice plants from seed-borne infestation of *A. besseyi*. The seed treatment with hot water or chemical treatment if adopted at community level, infestation of *A. besseyi* in rice, can be effectively controlled. Amin and Al-Shalaby (2005) found that soaking of rice seeds infected with *A. besseyi* in hot water at 70 °C for 15 minutes and hot air-drying treatment at 70 °C for 24 hours showed best results in controlling white tip nematode without any effect on sprouting.

Growing of tolerant or resistant cultivars may reduce *A. besseyi* population in rice and thus prevents yield losses. A few important rice varieties tolerant/resistant to *A. besseyi* are Bluebonnet, Bluebonnet 50, and Starbonnet (Papova et al. 1994), IR 841, IAC 435, IAC 120, IAC 899 (Rao et al. 1986; Sivakumar 1988), AUS 15854 (Baheti and Verma 2001), Binam, Domsiah, Khazar and Hassansarayi, Sepidroud, Kadus, Hassani and Ramezani, Hashemi, Deilamani (Jamali and Mousanejad 2011), and Asahi (Tulek et al. 2015).

Seed treatment with *carbofuran, aldicarb, sulfone, or methomyl at 0.1% WP* has been found highly effective in controlling *A. besseyi, but seed germination may get 11–29% low* (Kuriyan 1995). Wang et al. (2006) reported soaking of seeds in 16% cartap and prochloraz cartap solution for 48–60 h before sowing satisfactorily controlled white tip in rice. Application of carbofuran 3G treatment before transplanting or seed disinfection followed by carbofuran 3G water surface treatment at the early stage of injury provided effective control of the disease (Khan et al. 2006). Application of monocrotophos 36 SL at 0.075% through seed treatment

before sowing and foliar spray monocrotophos at 20 and 50 DAS (days after sowing) was found to the most effective and reduced the foliar distortion and boot leaf stage nematode infestation (Prasad and Varaprasad 1992; Pathak and Khan 2010; Pashi et al. 2017a, b).

Islam et al. (2015) reported that the integrated treatment with brine solution, hot water, and Furadan 3G significantly enhanced different parameters of the plant and yield and reduced the disease incidence. Khan et al. (2006) reported that the integration of NeemAzal and pongamia oil with chemicals as well as hot water treatment was found more effective in managing whit tip disease. Hot water treatment of rice seeds for 10 min at 50–55 °C followed by foliar spray with carbosulfan (25 EC) at 0.1% 40 days after transplanting reduced the infestation of white tip nematode (Khan et al. 2010).

3.3.2.5 Leaf Curl and Wilt Disease Caused by *Hoplolaimus indicus*

The disease is caused by *Hoplolaimus indicus* which frequently occurs in all the rice-growing states in the country. The general symptoms are stunting, chlorosis, curling of young leaves, and wilting of tip of old leaves in early stages of crop growth (Fig. 3.12; Rahman 2010). The nematode damages the roots internally leading to distortion in the arrangement of vascular bundles. Crop loss depends on the nematode populations. Infestation with *H. indicus* is common in upland areas where other cereals are grown in a rice-based cropping system, causing up to 18% losses in rice. Following measures may help in suppressing the nematode attack.

Crop rotation may effectively reduce the *H. indicus* population in soil. The paddy-gram rotation for 2 consecutive years followed by paddy-mustard or paddy-garlic significantly decreased the *H. indicus* population in the field (Haider et al. 2001). The okra-cowpea-cabbage, okra-brinjal-okra, and okra-cucumber-mustard sequences were found to be most effective and reduced the population of *H. indicus* (Chandra and Khan 2011). The nematode population was considerably lower in plots under mustard, sunhemp, and *Tagetes* cultivation (Haque and Prasad 1983). In another crop rotation schedule, the fallowing proved effective against most of the plant parasitic nematodes. A fallow period of 3 months greatly reduced the

Fig. 3.12 Leaf curling and wilt caused by *Hoplolaimus indicus*. (Source: https://images.app.goo.gl/GPbhQkZjWCCcf4sSA)

population of *H. indicus* (Khan and Chawla 1975). Deep plowing may prove more suppressive to the nematode than the normal plowing. Deep plowing before the wheat-chili followed by fallow and thereafter lentil-cowpea-mung cropping sequence significantly reduced the total population of *H. indicus* (Wani 2005).

Soil application with diazinon at 250 ppm, dimethoate at 500 ppm, or DBCP at 1000 ppm has been recorded to be quite effective against *H. indicus* in rice (Biswas and Rao 1971). The root dip treatment of rice nursery at 500 ppm diazinon and DBCP for 10 min reduced the invasion and development of the nematode inside the rice roots. Nemaphos and Terracur P were found highly effective against endoparasitic stage of *H. indicus*. The phorate, fensulfothion, and dimethoate were also found highly effective in reducing the population of *H. indicus* and other nematodes and increasing plant weight and yield (Alam et al. 1981). About 82% reduction in population of *H. indicus* was recorded due to application of phenomiphos at 10.0 kg/ha in soil (Sethi and Meher 1991).

3.3.3 Maize

Numerous nematode species, viz., *Heterodera zeae, H. avenae, Pratylenchus zeae,* and *Meloidogyne* spp. attack the maize crop, among them. *Heterodera* spp. are of immense economic importance. In India, *H. zeae* occurs in major maize-growing regions in Himachal Pradesh, Rajasthan, Gujarat, Punjab, Haryana, Uttar Pradesh, Uttaranchal, Bihar, Jharkhand, Madhya Pradesh, and Maharashtra (Parihar and Siddiqui 2010). Aboveground symptoms are nonspecific. The infected plants tassel earlier but bear smaller cobs with relatively fewer grains. The presence of cysts of *H. zeae* on the root surface is the most important and characteristic symptom of the nematode infection in maize (Fig.3.13). Root system of the infected maize plant becomes bushy and is poorly developed. The yield loss due to *H. zeae* and *H. avenae* may be up to 40%, and the nematode infestation in maize can be minimized by adopting the following strategies.

There is good scope of using crop rotation as a control strategy for *H. zeae*, since the nematode has a limited host range. Monoculture of maize and other host crops in the same field should be avoided (Parihar and Siddiqi 2010). Two-year rotation with non-host crops like vegetables, pulses, and oilseeds can be fruitful, as it would bring

Fig. 3.13 Nematode damage showing in maize field by *H. zeae*. (Source: https://images.app.goo.gl/zFm5CkoU18mzJy5YA)

down the nematode populations below economic threshold levels (Srivastava and Chawla 2005). Two to three deep plowings at 10–15 days interval during April–May in hot summer may also reduce the nematode population densities to a considerable extent (Srivastava and Chawla 2005). The maize cultivars Ageti-76 and Karnal-1 have been found to be moderately resistant to *H. zeae* in India (Kali Ram 1989).

Chemicals offer effective control of *H. zeae* in maize. Carbofuran (3G) and phorate (10G) at 2 kg a.i./ha have been found to reduce the nematode population and subsequent increase in the maize yield. Carbosulfan (25 ST) used as seed treatment at 2–3% (w/w) also provided drastic decline in the soil population of *H. zeae* (Srivastava and Chawla 2005; Kaushal et al. 2007). Soil application of cadusafos at 1 kg ai/ha at the time of sowing or in the split dose significantly enhanced the plant growth and reduced cyst population (Srivastava and Lal 2007). The seed treatment of acephate (2%w/w) suppressed the soil population of *H. zeae* and enhanced the plant growth and yield of maize (Baheti et al. 2015).

Seed treatment and soil application on maize with carbosulfan 25 ST 2% in combination with carbofuran at 1 kg a.i./ha increased cob weight, stalk weight, and grain yield of maize and reduced the final cyst population by 80% (Singh et al. 1997). Biocontrol agents have also been found effective against *H. zeae*. The seed treatment with biocontrol agents, *Purpureocillium lilacinum, Pochonia chlamydosporia,* and *Trichoderma viride* (1, 2, and 4% w/w), suppressed the cyst nematode and enhanced the yield. The treatment with *P. lilacinum* (4%)was found most effective in reducing the infection of *H. zeae,*followed by *P. chlamydosporia* (4; Bahetiet al. 2015). Baheti et al. reported that the seed treatment (2% w/w) with *P. lilacinum*, *P. chlamydosporia,* and *T. viride* and soil application (2 g/plant) of leaf powder of neem, karanj, and lantana effectively controlled the maize cyst nematode.

3.3.4 Barley

The nematodes of economic importance that infest barley include *Heterodera* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Anguina tritici,* and *Ditylenchus dipsaci*. *Heterodera avenae* is the principal species on temperate cereals and has been detected in India (Bishnoi and Pankaj 2010). The disease is characterized by patchy growth of stunted and yellow plants (Fig.3.14). Infested plants bear thin and narrow leaves, reduced number of tillers, delayed emergence of ears, and reduced number of spikelets and grains, ears, if formed, and have very few grains. Root system becomes bushy, bunchy, and shallow due to its proliferation and has slight swelling near the root tip. Such plants can be easily pulled out of the ground.

Cultural methods have been successful in managing the *H. avenae* population, since this nematode possesses narrow host range being specific to cereals. Growing non-host crops may prove much effective (Bishnoi and Pankaj 2010). Handa (1983) found that nematode population decreased by 70% with continued rotation of mustard, carrot, fenugreek, and gram or by fallowing. This led to 87% increase in the barley yield. Singh et al. (2009a, b) and Shekhawat et al. (2017) observed

Fig. 3.14 Aboveground symptoms caused by *Heterodera avenae* on barley

decrease in the population of cereal cyst nematode with summer plowings and subsequent increase in the yield of cereals. Growing of non-hosts and resistant varieties such as barley cv. Morocco, Maroccaine, PI 253826, PL 101, C-164, and Raj Kiran (Walia and Khan 2018) offers resistance against a wide range of pathotypes of *H. avenae s.s* in India, but not effective against *H. filipjevi* of Punjab and Ambala populations. Out of hundreds of lines showing resistance, one line having local number RD 387 was found agronomically superior in yield and showed complete resistance to prevailing pathotypes of CCN except Ambala population (Bishnoi and Bajaj 2004).

A wide range of chemicals have been evaluated to control CCN in barley. Soil application of DD, DBCP (EC), aldicarb, and carbofuran has shown high degree of effectiveness against *H. avenae* in barley (Mathur et al. 1986; Handa et al. 1985; Sharma 2003). Soil drill of carbofuran and aldicarb at 1.0 kg a.i/ha effectively reduced the nematode population even when Pi was as high as >5 juveniles/g soil. Indra and Sharma (2000) reported that soil application of Sebufos (cadusafos) 10G (1 kg a.i./ha), Padan (cartap) 10G (2.0 kg/ha), and carbofuran 3G and seed treatment with neem-based Achook (10 and 20%) suppressed the *H. avenae* attack on barley. All treatments significantly increased grain and fodder yields and reduced the number of cysts per plant.

Catenaria vermicola has been found parasitizing cyst, juveniles, and egg of *H. avenae*, *H. cajani*, *H. zeae*, *H. graminis*, *H. mothi*, and *H. sorghi* in India (Bhatti and Paruthi 2009), while *Verticillium uniseptum* parsitized the eggs of *H. avenae* (Choudhary and Kaushal 1984). Several other fungi and like *Catenaria auxiliaris*, *Nematopthora gynophila*, *Verticillium chlamydosporium*, *Tarichium auxiliare*, and *Cylindrocarpon destructans* are also known to parasitize *H. avenae* in Britain (Bhatti and Paruthi 2009). Bhattacharya and Swarup (1988) reported that *Bacillus penetrans*, *Glomus fasciculatum*, and *Pasteuria penetrans* were found to be the most destructive against *H. avenae*. Walia and Khan (2018) reported that seed

treatment with *Azotobactor chrococcum* (strain HT 54) may effectively reduce the soil population of cereal cyst nematode and improve the grain yield.

3.3.5 Sorghum

Sorghum cyst nematode, *Heterodera sorghi*, *Tylenchorhynchus vulgaris*, *Hoplolaimus* spp., *Meloidogyne* spp., and *Rotylenchus* spp. are important phytonematodes associated with sorghum crop in India. Cyst nematode disease of sorghum (*H. sorghi*) is quite prevalent in Haryana, Punjab, Delhi, Uttarakhand, Madhya Pradesh, Himachal Pradesh, Jammu and Kashmir, Andhra Pradesh, and Maharashtra. Most common symptoms of the disease are presence of light to dark brown cysts on roots. The root-knot nematode, *M. incognita*, is another important disease in sorghum (Srivastava and Chowla 2010). The nematode causes chlorosis and stunting of foliage and galls on the roots. Root lesion disease caused by *P. zeae* inflicts considerable yield loss to sorghum in India. The infested plants are stunted and become yellowish. On roots, necrotic lesions are formed. *Tylenchorhynchus vulgaris* is also an important nematode that feeds on the roots of sorghum. The nematode causes decay and tip swelling of feeder roots.

Crop rotation with sugar beet, peas, beans, crucifers, and potatoes may suppress the population of *Pratylenchus crenatus* in soil (Srivastava and Chawla 2010). Two to three deep summers plowing in hot months of May/June followed by fallowing can reduce the soil population of *Pratylenchus* and *Meloidogyne* spp. Some maize cultivars like Nab Elgamal, Early American, Giza, and Balady have shown moderate level of tolerance to *P. zeae* and can be cultivated in the areas/field with high nematode infestation level. The maize cv. Kanchan, P128, possesses remarkable degree of resistance against *M. incognita, M. javanica,* and *M. arenaria* (Khan et al. 1994) and may be recommended for cultivation in hot zones. Growing antagonistic plants such as mustard, marigold, *Asparagus* sp., *Crotalaria* sp., and sesame can reduce the population of the nematodes (Srivastava and Chawla 2010).

Soil application of Temik and Furadan at 2.5 kg/ha controlled *P. delattrei*. Bergeson (1978) observed that application of carbofuran at 4.5 kg a.i./ha reduced *Pratylenchus* population up to 84% and significantly increased the maize yield. Kutche and Rossner (1978) recorded up to 90% control of *Pratylenchus* sp. with different formulations of carbofuran. Walia and Khan (2018) recommended soil application of carbofuran (3G) at 2 kg a.i./ha to control *P. thornei*.

Biocontrol strategy may also prove effective against nematode infection in sorghum. *Trichoderma harzianum* at 25 kg/ha along with *Pochonia chlamydosporia* at 10 kg/ha 1 week prior to sowing effectively managed lesion nematode, *Pratylenchus thornei* (Abd-Elgawad and Askary 2018). Dias-Arieira et al. (2018) evaluated the effect of *Purpureocillium lilacinum* and *T. harzianum*, either alone or in combination, along with a bioactivator (mass) to control *Pratylenchus brachyurus.* Both fungi were efficient in controlling the nematode when they were applied alone, whereas the combination of the two did not improve nematode control. However, the addition of moss to the combination of fungi (*P. lilacinum* + *T.*

harzianum) generally provided better control of the nematode and increased the plant yield.

3.4 Conclusion and Future Prospects

The crop losses inflicted by phytonematodes to agriculture on a worldwide basis appear enormous, as the data generated on this aspect has covered relatively limited crops as well as countries neglecting by and large the Asian and African countries. In this situation, the estimate of $100 billion annually appears to be unrealistic (Khan 2008). Hence to develop efficient management technology, it becomes mandatory to undertake extensive survey and extension work to assess the actual enormity of the problem. Although India is self-sufficient in food grain production, the national average productivity is considerably lower than the global average productivity in most of the crops. Hence, there is a lot of scope for improvising food grain production from the area under present cultivation in India. This can be achieved by implementing pest and disease management programs including plant nematodes at the grassroot level.

The literature has revealed that several nematode species infest cereal crops in India, but relatively a much smaller proportion of nematode community constitutes potential plant parasitic genera and species that cause economic damage and yield decline to food crops. Hence, special attention in a phase manner should be given to reduce the population of five most economically important nematode genera, viz., *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus,* and *Aphelenchoides*. The most simple way of controlling the nematodes is crop rotation by growing non-host resistant or tolerant crops. Since chemical application (nematicides) creates several hazards, eco-friendly methods should be preferred. Deep plowing and fallowing coupled with solarization should be taken as an integral strategy of disease management in Indian farming system. Field sanitation especially the removal of root stubs which support nematode survival and multiplication after harvesting the cereals may prove effective in reducing the soil population of nematodes for subsequent crops. Overflooding of water from the adjoining infested fields should be avoided. In addition, certified and disinfested seeds/planting material coupled with seed dressing with biocontrol agents such as *Trichoderma* spp. or *Pseudomonas fluorescens* should be used. This package of practices shall help in reducing the nematode populations in the field and shall prevent spread and introduction of initial population densities of phytonematode inocula.

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4 Modern Tools for Detection and Diagnosis of Plant Pathogens

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Abstract

Plant diseases contribute to an estimated annual crop loss of $60 billion worldwide, threatening the food security and thereby the survival of humankind. Plant pathogens are an important component of the widely accepted disease triangle concept where they cause diseases in susceptible hosts under favorable environmental conditions. Under such favorable environmental conditions, pathogens often spread rapidly to new hosts. Therefore, early and accurate detection of a disease and diagnosis of its causal agent is highly important for disease control and sustainable agricultural production. This chapter provides information on an array of methods actively being used to detect and diagnose diseases. We include discussion of established methods such as microscopy and serology, indexing, and PCR as well as methods that are actively under development. An elaborate account of information is provided on promising diagnostic methods based on programmable nucleic acid-binding proteins such as clustered regularly interspaced palindromic repeat (CRISPR)-associated (CAS) proteins, zinc finger (ZnF) proteins, and transcription activator-like effector (TALE) proteins. Finally, we discuss indirect pathogen detection technologies that utilize optical sensors to identify changes in the host plant properties due to a disease.

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4.1 Introduction

The impact of exogenous forces on plant phenotype has long been observed among both wild and cultivated plants. These exogenous forces include both biotic and abiotic causes, but the importance of biotic factors was first identified by Robert Hooke (1665) who illustrated in a book, Micrographia, that a plant pathogenic microfungus, *Phragmidium mucronatum*, causes rose rust disease.

Although plant disease had been observed previously, plant pathology truly began as a field of inquiry in the wake of the Irish potato famine of 1845. With the death of over 1 million people and the migration of 1 million more, late blight of potato altered world history. However, when Anton de Bary (1861) identified the cause as *Phytophthora infestans*, he opened the door to routes of treatment, control, and management previously unavailable.

Mirroring history, the first steps of any modern phytopathological investigation are the detection and diagnosis of the causative pathogen. Moreover, given the impact of plant pathogens there is substantial motivation to detect and diagnose quickly, sensitively, and specifically.

To address these needs, methods have developed from simple observation of disease symptoms on plants to rather sophisticated methods to detect portions of the pathogen, by-products, or impacts of the pathogen. In this chapter, we review established diagnostic methods such as microscopy, indexing, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), real-time PCR, and other nucleic acid amplification methods. Further, we review progress on newer methods actively under development. Specifically, we review the detection of nucleic acids with nucleic acid-binding proteins, as well as indirect signatures of pathogen presence using metabolomic, volatile organic compound (VOC) data, and optical biosensors.

4.2 Established Methods

4.2.1 Microscopy and Serology

Beyond visual inspection of disease symptoms, microscopy was the first approach utilized to identify the presence and identify of plant pathogens (Hooke 1665). Technological advances in microscopy led to ever-increasing sensitivity and specificity. Ultimately, electron microscopy has allowed plant pathologists to determine the shape and dimensions of different viruses, and the effects on host cells. For

example, potyvirus-infected plants develop characteristic pinwheel inclusion bodies only observable by electron microscopy (Edwardson 1974).

Some pathogens evaded early microscopy efforts. Early on plant pathologists identified transmissible agents of disease that were smaller than bacteria. Beale (1928) first identified something of their chemical nature—tobacco mosaic-infected plants possessed an antigen (Beale 1928). Gratia (1933) then showed that plants infected with different viruses possessed different specific antigens. Stanley (1935) reported that the virus could be isolated in a crystalline state, and Bawden et al. (1936) detected the presence of a nucleic acid. X-ray crystallography indicated that TMV is rod-shaped (Bernal and Fankuchen 1937). This was soon confirmed by early electron microscopy (Kausche et al. 1939).

Through the 1950s research showed that the nucleic acid component was more important than initially thought (Mathews 1970). Nevertheless, it was the protein components that received attention from a diagnostic standpoint. Several assays were developed to help identify viruses with antisera raised in various mammals, but especially rabbits. These included agglutination assays and gel precipitation assays, especially the double diffusion test which could detect strain differences (Ouchterlony 1962).

Eventually, serology and microscopy were brought together. One technique is to apply antiserum to the particles on the grids; in the presence of the capsid protein, the antiserum adheres and gives the particles a fuzzy appearance (Derrick 1973). Another technique uses gold-labeled antisera (van Lent and Verduin 1985) or fluorescent labeling (Nagaraj 1965) and has been useful in identifying the location of the virus particles in ultrathin sections of plant cells. Antisera produced by injecting purified virus into a mammal are composed of a mixture of antibodies, each produced in response to a different site on the protein. Köhler and Milstein (1975) developed a technique to produce antibodies specific to one antigenic site (monoclonal antibodies) that proved useful in the detection of specific strains of viruses.

Enzyme-linked immunosorbent assays (ELISA), developed by Clark and Adams (1977), are done in microplates coated with antisera to trap any virus particles in the sample. Trapped viruses are detected by adding alkaline phosphatase-linked antisera along with the enzyme's colorless substrate which the enzyme catalyzes into a visible yellow product. This visible signal can be quantified with a spectrophotometer.

Different variations of ELISA have been developed to improve speed, accuracy, and quantification such as lateral flow devices that have been used for diagnostics of bacterial pathogens (Alvarez 2004). Alternative serological approaches involve separating viral proteins by polyacrylamide gel electrophoresis, transferring the proteins to a cellulose membrane (a technique known as western blotting), and then detecting the protein bands with nonspecific staining or specific immunoassay (O'Donnell et al. 1982; Rybicki and von Wechmar 1982).

4.2.2 Pathogen Indexing

While there are several early illustrations and descriptions of viral infections of plants (e.g., tulip color breaking), it was not until the demonstration that tobacco mosaic could be mechanically transmitted to healthy plants (Mayer 1886), and that the agent was able to pass through a bacterial filter (Iwanowski 1892), that a pathogen other than a fungus or a bacterium could cause a plant disease. The first diagnostic assays to be used for plant viruses were biological indicator plants. Holmes (1929) was able to quantify *Tobacco mosaic virus* (TMV) by inoculating a local lesion host. Smith (1931) was able to show that some potato diseases were caused by mixtures of viruses. He found that *Potato virus Y* (PVY) could be transmitted by the aphid, *Myzus persicae*, while another, *Potato virus X* (PVX) could not. Also, PVY could not be mechanically transmitted to *Datura stramonium*, while PVX could. Indicator plants remain a widely used diagnostic tool today.

4.2.3 Nucleic Acid-Based Detection

Since the advent of PCR (Mullis et al. 1986) nucleic acids have become a staple of molecular diagnostics. They represent a signal for direct detection that is highly specific and highly adaptable.

4.2.3.1 PCR

PCR has been widely used for the amplification of specific target nucleic acid molecules, a key step in nucleic acid detection assays, present in low quantity in various sources (Craw and Balachandran 2012; Zanoli and Spoto 2013). Kuzdralinski et al. (2017) discussed on the basic steps involved in designing and developing a specific PCR assay such as selection of DNA extraction and amplification methods, identification of appropriate target gene, in silico analysis, and optimization of PCR conditions by validation using environmental samples and appropriate number of strains.

PCR is one of the most cost-effective nucleic acid-based diagnostic tools. A significant drop in the cost of sequencing offers an advantage to obtain nucleotide sequences for a large number of PCR amplicons and utilize them in accurately identifying different closely related pathogen species or strains (Henson and French 1993). Another PCR variant, nested PCR (Kawada et al. 2004) reported to be more sensitive with reduced impact of PCR inhibitors and is obviously a desirable assay when the pathogen is in small amounts.

Despite a wide adoption of PCR in diverse fields, the requirement of a thermocycler for PCR limits its application where the resource is limited (Zhao et al. 2015; Zeng et al. 2019). Since 1990s, many novel DNA amplification techniques that can be conducted at a constant temperature, called isothermal amplification, have been developed to amplify nucleic acids without the need of thermocycler (Zanoli and Spoto 2013; Zhao et al. 2015; Qi et al. 2018). These include, for example, rolling circle amplification (RCA) (Walter and Strunk 1994),

helicase-dependent amplification (HDA) (Vincent et al. 2004), loop-mediated amplification (LAMP) (Notomi et al. 2000), nucleic acid sequence-based amplification (NASBA) (Compton 1991), recombinase polymerase amplification (RPA) (Piepenburg et al. 2006), and multiple displacement amplification (MDA) (Dean et al. 2002; Zanoli and Spoto 2013).

Development of these isothermal DNA amplification techniques has opened the door to the development of simple, fast diagnostic methods in a form of point-of-care testing (POCT). Some of these are available as commercial kits in portable devices (Chang et al. 2012; Craw and Balachandran 2012; Zanoli and Spoto 2013; Zhao et al. 2015; Zeng et al. 2019). Zhao et al. (2015), divided the various types of isothermal amplification techniques into three groups based on the kinetics of the reaction: exponential amplification, linear amplification, and cascade amplification. Among these groups, exponential isothermal amplification techniques are known to provide higher amplification and detection sensitivity compared to other isothermal techniques (Zhao et al. 2015; Qi et al. 2018). In this section, we focused on the exponential isothermal amplification and discussed their application as a diagnostic tool for the detection of target nucleic acids.

4.2.3.2 Nucleic Acid Sequence-Based Amplification (NASBA)

NASBA was developed in 1991 as a method that can specifically amplify single-stranded RNA or DNA molecules (Compton 1991). NASBA is similar to a self-sustained sequence replication (3SR), a transcription-based amplification system (TAS) that mimics retrovirus replication by incorporating reverse transcriptase, RNase H, and DNA-dependent RNA polymerase (Kwoh et al. 1989; Guatelli et al. 1990; Compton 1991; Blais et al. 1997; Deiman et al. 2002; Zhao et al. 2015). NASBA is mediated by first, a conversion of single-stranded RNA into double-stranded heteroduplex composed of the original RNA molecule and its complementary DNA (cDNA) by reverse transcription with a primer that has a promoter sequence (e.g., T7) at its 5′ portion. Subsequently, the RNA strand from the RNA: cDNA heteroduplex is removed by RNase H leaving a single-stranded cDNA with a promoter sequence at its 5′ end. The single-stranded cDNA is then converted into double-strand DNA (dsDNA) via the extension of a second primer that was annealed to the single-stranded cDNA by reverse transcriptase. The promoter sequence present in the dsDNA product will function to initiate the synthesis of negative-sense RNA molecules (complementary to the original RNA molecules) by the DNA-dependent RNA polymerase (e.g., T7 DNA polymerase). The negative-sense RNA products will then serve as templates for another round of reverse transcription resulting in the generation of more RNA:DNA heteroduplexes leading to a cyclic reaction of the aforementioned whole process enabling an exponential amplification of RNA molecules complementary to the initial target RNA (Compton 1991; Zanoli and Spoto 2013; Zhao et al. 2015). Although NASBA can be conducted at 41 °C where a billion copies of the target molecule can be synthesized in about 2 h (Compton 1991; Deiman et al. 2002; Zanoli and Spoto 2013), it is recommended to conduct initial denaturation to facilitate RNA-primer annealing for reverse transcription (Blais et al. 1997; Simpkins et al. 2000; Abd El Galil et al.

2005; Zhao et al. 2015). In case that dsDNA is a starting material for NASBA, the denaturation step should be incorporated for primer annealing to the target DNA molecule followed by extension by reverse transcriptase (Compton 1991). The detection of NASBA single-stranded RNA products can be achieved in several ways including real-time detection using molecular beacon probe (Deiman et al. 2002; Polstra et al. 2002; Abd El Galil et al. 2005; Zanoli and Spoto 2013; Zhao et al. 2015), electrochemiluminescence (Simpkins et al. 2000; Deiman et al. 2002; Zanoli and Spoto 2013; Zhao et al. 2015), and colorimetric analysis (Gill et al. 2006; Zhao et al. 2015). Since its first report in 1991, NASBA was quickly adopted for pathogen detection and diagnostics, especially for the detection of RNA viruses including hepatitis C virus and human immunodeficiency virus (HIV) (Van Der Vliet et al. 1993; Vandamme et al. 1995; Hollingsworth et al. 1996; Craw and Balachandran 2012). In addition, the integration of NASBA into a lab-on-a-chip system after its miniaturization without compromising the detection sensitivity has been reported for novel pathogen detection (Gulliksen et al. 2004, 2005; Dimov et al. 2008; Craw and Balachandran 2012).

4.2.3.3 Strand Displacement Amplification (SDA)

While NASBA exploited the retrovirus replication mechanism, the SDA technique adopted the DNA replication process that takes place at a constant temperature (Walker et al. 1992a, b; Zhao et al. 2015; Zeng et al. 2019). Since the development of SDA technique in the early 1990s (Walker et al. 1992a, b), various modifications have been added to the original SDA to improve its efficiency and the specificity of DNA nick by endonucleases (Walker et al. 1992a; Mehrpouyan et al. 1997; Fang et al. 2014; Zhang et al. 2016a, b; Wu et al. 2018; Zeng et al. 2019). SDA requires two sets of primers, one set designed for both DNA nick and primer extension and the other for primer extension which targets outside of the first primer-binding sites. After heat denaturation of the template DNA, the first primer set that has an endonuclease-recognition site at its 5′ portion for DNA nick binds to the target DNA and primes the synthesis of a new strand, producing double-stranded DNAs. Then, the synthesized dsDNAs that have endonuclease recognition sites are subjected to the cycles of DNA nick followed by DNA extension accompanied by strand displacement of downstream DNA. The second primer set flanking the binding sites of the first primer set produces new DNA strands of which dsDNA product will then serve as a target for the first primer set to anneal, which will initiate another round of the cycle of DNA nick and amplification of new DNA strands (Walker et al. 1992a, b). The cycles of nicking DNA strand, primer extension, and strand displacement enable the exponential amplification of single-stranded DNA molecules complementary to the original target (Zhao et al. 2015). It has been shown that SDA can be used for the detection of microRNAs for the purpose of disease diagnosis (Shi et al. 2014).

4.2.3.4 Rolling Circle Amplification (RCA)

RCA technique utilizes DNA polymerases (e.g., Phi 29 DNA polymerase) that are capable of continuous amplification and strand displacement of circular DNA

templates, resulting in the production of long single-stranded DNA molecules with tandem repeats of the circular template (Nilsson et al. 1994; Walter and Strunk 1994; Banér et al. 1998; Zanoli and Spoto 2013; Ali et al. 2014; Zhao et al. 2015). While the linear amplification of nucleic acid by RCA is achieved by using a single primer (Fire and Xu 1995; Liu et al. 1996; Zanoli and Spoto 2013), exponential RCA method is done by including the second primer in the reaction which will hybridize at multiple locations to the newly synthesized single-stranded DNA product with tandem repeats of the circular target, initiating hyper-branched rolling circle DNA amplification (Mothershed and Whitney 2006; Zanoli and Spoto 2013; Zhao et al. 2015). Since RCA requires small single-stranded DNA molecules as a template, long dsDNA molecules, routinely obtained from biological samples, need to be converted into small circular single-stranded DNAs for RCA either by padlock probes or by primer-generation RCA (Nilsson et al. 1994; Banér et al. 1998; Murakami et al. 2009; Zanoli and Spoto 2013; Zhao et al. 2015). The padlock probe is composed of two sequences, each of which is complementary to one-half of a single target region, separated by a linker sequence (Nilsson et al. 1994; Banér et al. 1998). The hybridization between the padlock probe and the target sequence followed by DNA ligation circularizes the padlock probe forming a single-stranded circular DNA which will be used as a template for RCA (Lizardi et al. 1998; Zanoli and Spoto 2013; Zhao et al. 2015). The RCA mediated by the padlock probe is highly specific to the target sequence, which is suited for SNP analysis (Lizardi et al. 1998; Zanoli and Spoto 2013). Unlike padlock probe-mediated RCA, primer-generation RCA only requires a single-stranded circularized probe that has a nucleotide sequence complementary to the target and an endonuclease recognition site for a DNA nick (Murakami et al. 2009). Once the circularized probe with a nick site hybridizes to the single-stranded target, the linear RCA takes place producing a long single-stranded DNA with tandem repeats of a sequence complementary to the circular probe (Murakami et al. 2009). The single-stranded DNA product of linear RCA will serve as a template molecule for the hybridization of multiple circular probes and nicking reaction, resulting in the generation of primers for further RCA (Murakami et al. 2009; Zanoli and Spoto 2013; Zhao et al. 2015). Primer-generation RCA has been successfully used for a diagnostic purpose for the detection of bacterial pathogens (Murakami et al. 2009).

4.2.3.5 Multiple Displacement Amplification (MDA)

Unlike SDA and other isothermal amplification methods that amplify specific target nucleic acids, MDA and other whole genome amplification methods use random primers enabling efficient amplification of template DNA for next-generation sequencing (Dean et al. 2002; Zhao et al. 2015). Due to the nature of short random primers (e.g., random hexamer), new DNA strand synthesis and strand displacement are initiated at multiple locations on the template DNA which is mediated by Phi 29 DNA polymerase that has a strong strand displacement activity (Dean et al. 2002; Zhao et al. 2015). It has shown that MDA can synthesize ~10 kb-long DNA fragments in the amount of 20~30 μg from a very limited amount of starting materials (Zhao et al. 2015). Although MDA can be directly used for various

types of biological samples including lysed cells without purification step, since the amplification of DNA by MDA is nonspecific, any contaminated DNA in the sample will be amplified, causing that only small fraction of the amplified MDA products were derived from specific target molecules (Raghunathan et al. 2005; Zanoli and Spoto 2013). This kind of amplification bias of MDA can be reduced by reducing the reaction volume to the nanoliter level in microfluidic devices (Zhang and Xing 2010; Zanoli and Spoto 2013).

4.2.3.6 Loop-Mediated Isothermal Amplification (LAMP)

LAMP, developed by Notomi et al. (2000), uses DNA polymerases with strand displacement activity and two to three different primer sets (outer and inner primer sets and/or loop primers), each of which targets specific nucleotide sequence present on the sample in a single reaction, providing greatly improved specificity compared to other isothermal amplification methods (Notomi et al. 2000; Zanoli and Spoto 2013; Zhao et al. 2015). The two inner primers, forward inner primer (FIP) and backward inner primer (BIP), are comprised of two different sequences complementary to sense and antisense strands of target DNA segments, of which LAMP products will form a loop structure at its each end that will provide annealing sites for both inner primers during LAMP reaction (Notomi et al. 2000). The outer primers, F3 (Forward) and B3 (Backward) flanking the two inner primer-binding sites, synthesize a new DNA strand by strand displacement activity of the DNA polymerase following the first LAMP reaction initiated by two inner primers (Notomi et al. 2000). The new strands synthesized by strand displacement from outer primers are then served as templates for the LAMP reaction with two inner primers which will produce new single-stranded DNA molecules with loop structures that subsequently enters the cycling LAMP reaction mediated by two inner primers, resulting in geometrically and continuously branched out LAMP products from each single-stranded LAMP product, which can accumulate 10^9 copies of target molecule within an hour (Notomi et al. 2000; Zanoli and Spoto 2013; Zhao et al. 2015). The final LAMP products can be detected in real-time and by end-point assays (Xie et al. 2014; Yi et al. 2014; Zhang et al. 2014a, b). LAMP has been widely adopted for the diagnostic purpose for the detection of pathogens as well as for SNP analysis in various fields either with or without reverse transcription step depending on the biochemical status of target molecules (Thi et al. 2004; Ohtsuka et al. 2005; Misawa et al. 2007; Curtis et al. 2008; Mori and Notomi 2009; Zanoli and Spoto 2013; Keremane et al. 2015; Choi et al. 2018).

4.2.3.7 Helicase-Dependent Amplification (HDA)

HDA exploited DNA helicase-mediated DNA replication process together with other single-strand-binding proteins and DNA polymerases in the reaction, which can amplify long target DNA molecules (Vincent et al. 2004; Zanoli and Spoto 2013; Zhao et al. 2015). The original HDA, developed with *E. coli* UvrD helicase, was replaced with thermostable UvrD helicase derived from thermophilic bacteria to improve the specificity and efficiency of HDA and to amplify longer DNA targets (Mechanic et al. 2000; Vincent et al. 2004; An et al. 2005; Matson and Robertson

2006; Zanoli and Spoto 2013). The addition of helicase in the target DNA fraction mediates unwinding of dsDNA that generates two single-stranded DNA molecules which are accessible for two sequence-specific primers for new strand synthesis by DNA polymerase resulting in dsDNA formation. The newly synthesized dsDNA serves as substrates for DNA helicases in the following HDA reactions, resulting in an exponential amplification of the target DNA molecules (Vincent et al. 2004; Zanoli and Spoto 2013). HDA has been successfully used for the detection of bacterial and viral pathogens as well as SNP detection (An et al. 2005; Motré et al. 2008; Li et al. 2011a, b; Zanoli and Spoto 2013; Chen et al. 2015; Zhao et al. 2015).

4.2.3.8 Recombinase Polymerase Amplification (RPA)

RPA uses recombinase that interacts with primers forming a nucleoprotein complex which will be directed to the target sequences on the dsDNA molecules for primer-template DNA hybridization leading to a new strand synthesis accompanied by a strand displacement (Piepenburg et al. 2006; Zanoli and Spoto 2013; Zhao et al. 2015). In RPA, the nucleoprotein complex formed by primer and recombinase scans the dsDNA to locate the primer target sequence, then displaces the non-template strand which will be stabilized by single-strand-binding proteins while the primer extension takes place by DNA polymerase (Piepenburg et al. 2006; Zanoli and Spoto 2013). By incorporating two primers (forward and reverse primers), the primer extension events of each primer will generate a complete target amplicon which will be used as a template for continuous RPA enabling an exponential amplification of the target (Piepenburg et al. 2006; Zanoli and Spoto 2013; Zhao et al. 2015). TwistDx (Cambridge, UK) commercialized RPA kits for the detection of various pathogens (Shen et al. 2011; Crannell et al. 2014; Rohrman and Richards-Kortum 2015; Zhao et al. 2015).

4.2.4 Real-Time Polymerase Chain Reaction (RT-PCR)

The discovery of a thermostable DNA polymerase, Taq DNA polymerase I originating from a thermophilic bacteria, *Thermus aquaticus*, followed by its adoption in PCR made the PCR a major tool for the amplification of target nucleic acid molecules from various source materials (Chien et al. 1976; Saiki et al. 1988; Kralik and Ricchi 2017). Shortly after the utilization of a thermostable DNA polymerase for PCR, the concept of "real-time" monitoring of DNA amplification was probed using either a hydrolysis probe paired with 5′ nuclease activity of Taq DNA polymerase or an intercalating DNA-binding dye (Holland et al. 1991; Higuchi et al. 1992; Navarro et al. 2015; Kralik and Ricchi 2017). The commercialization of real-time PCR instruments from various vendors, which has a dual function of thermocycling and fluorescence detection after each PCR cycle, made the real-time PCR technique quickly widespread and then resulted in the development of various real-time PCR protocols for diagnostic purposes, quantification of gene expression, and mutation detection (Navarro et al. 2015; Kralik and Ricchi 2017). The real-time PCR techniques can be categorized into two major groups based on the detection

chemistry of amplicons (Navarro et al. 2015). The first group utilizes intercalating fluorescent dyes and the second group uses fluorophore-labeled oligonucleotides (Navarro et al. 2015). Unlike the intercalating dye-based methods, the latter group adopted various types or structures of fluorophore-labeled oligos for the detection of amplicons with improved specificity (Navarro et al. 2015).

4.2.4.1 Real-Time PCR with Intercalating Fluorescent Dyes

The applicability of DNA-binding dyes for real-time detection of the amplicon was first proved by Higuchi et al. (1992) using ethidium bromide. Among those DNA-binding dyes used for real-time PCR (e.g., SYBR Green I, SYTO9, BEBO, etc.), SYBR Green I is the most widely used fluorescent dye, which is often included in a commercial master mix for real-time PCR assays (Ririe et al. 1997; Wittwer et al. 1997). These dyes emit fluorescence when they bind to the minor groove of double-stranded DNA molecules, which is then measured during the course of the PCR. As the DNA-binding dyes preferentially bind to double-stranded DNA molecules, both specific and nonspecific amplicons as well as, if any, primer-dimers can be detected in the real-time PCR assay. To verify the specificity of the real-time PCR assay with DNA-binding dyes, a melt-curve analysis must be conducted at the end of the PCR (Navarro et al. 2015). Since the melting temperature of PCR products in the reaction is determined by their length and nucleotide composition, the specificity of the real-time PCR assay can be easily evaluated by melt-curve analysis (Ririe et al. 1997). Although SYBR Green I was proven to work for real-time PCR, it also has some drawbacks such as dye instability, PCR inhibition at high dye concentration and dye-redistribution, which made SYBR Green I unsuitable for high-resolution melt curve analysis (HRM) (Wittwer et al. 1997; Nath et al. 2000; Monis et al. 2005; Varga and James 2006; Mao et al. 2007). EvaGreen developed by Biotium Inc. (Hayward, CA, USA) has some advantage over SYBR Green I in terms of dye stability and less PCR inhibitory effect compared to SYBR Green I which made EvaGreen suitable for real-time PCR as well as HRM (Ihrig et al. 2006; Wang et al. 2006; Mao et al. 2007). Despite the drawbacks of SYBR Green I, various real-time PCR assays using SYBR Green I as well as EvaGreen were successfully used for the detection of pathogen and genetic variability (e.g., SNP) (Buh Gašparič et al. 2010; Li et al. 2010; Carrasco et al. 2013; He et al. 2014; Navarro et al. 2015).

4.2.4.2 Real-Time PCR with Fluorophore-Labeled Oligonucleotides

Fluorescent molecules, fluorophores, absorb energy from the light of a certain wavelength and emit the energy (donor fluorophore) as a form of light of a longer wavelength that can excite or be quenched by another fluorophore (acceptor fluorophore) nearby via fluorescence resonance energy transfer (FRET) (Cardullo et al. 1988; Mergny et al. 1994; Howell 2006). Real-time PCR adopts these two different FRET mechanisms for the detection of amplicons in the reaction mixture: (i) FRET-quenching by acceptor (e.g., TaqMan, Scorpion, Molecular Beacon, etc.) or (ii) FRET leading to fluorescence emission from acceptor (e.g., Hybprobes, Angler®, etc.) (Mergny et al. 1994; Marras 2006; Navarro et al. 2015).

I. *FRET-quenching Oligos*

(i) *Hydrolysis Probe (TaqMan Probe)*

Hydrolysis probes are modified, non-extendible oligonucleotides which are designed to hybridize to a specific sequence within the real-time PCR amplicon (Holland et al. 1991; Gibson et al. 1996; Heid et al. 1996). The hydrolysis probe has donor and acceptor fluorophores at the 5′ and 3′ ends, respectively. The fluorescence of the donor fluorophore in the probe is quenched by the acceptor fluorophore (quencher) due to the physical proximity of both fluorophores (Holland et al. 1991; Gibson et al. 1996; Navarro et al. 2015). The binding of the hydrolysis probe to the target region during real-time PCR leads to the release of donor fluorophore from 5′ end of the probe due to the 5′ exonuclease activity of Taq DNA polymerase, which results in the increase of the fluorescence that will be monitored after each PCR cycle (Gibson et al. 1996; Heid et al. 1996). Real-time PCR using hydrolysis probes can be multiplexed by using fluorophores with different excitation and emission wavelengths and has been routinely applied in diverse applications such as pathogen detection, quantification of gene expression, and SNP detection (Navarro et al. 2015).

(ii) *Scorpion Probe*

Scorpion probe has a stem-loop (hairpin) structure that functions as a PCR primer as well as a probe (Whitcombe et al. 1999). The scorpion probe is composed of three major functional units: (i) fluorophores (a donor fluorophore at the 5′ end and an internal quencher), (ii) a loop working as a probe, which is flanked with a fluorophore and a quencher as well as complementary sequences leading to the formation of a stem structure followed by (iii) an oligonucleotide sequence at its 3′ region which will act as a PCR primer (Whitcombe et al. 1999). Like the hydrolysis probe, the physical proximity between the fluorophore and the quencher results in the suppression of the fluorescence in the scorpion probe. During real-time PCR, the primer sequence present at the 3′ region of the scorpion probe initiates a DNA synthesis complementary to the target DNA, of which product will have a copy of the nucleotide sequence of the scorpion probe at the 5′ end. The denaturation step of the following PCR cycle will denature the scorpion probe sequence and allows the probe sequence residing on the loop portion to bind to the target sequence of the same newly synthesized product resulting in the increase of fluorescence due to a lack of quenching mechanism (Whitcombe et al. 1999; Navarro et al. 2015). Due to the structural feature (a hairpin) of the scorpion probe, nonspecific amplification and the formation of primer-dimers can be minimized during PCR cycles (Nazarenko et al. 2002; Navarro et al. 2015). Scorpion probes can be multiplexed and are used for the detection of pathogens and genetic variability (e.g., SNPs, etc.) (Solinas et al. 2001; Naserpour Farivar et al. 2014; Zhang et al. 2014a, b; Navarro et al. 2015).

(iii) *Molecular Beacon Probe*

Molecular beacon probe has the feature of both hydrolysis probe and scorpion probe in terms of the structure and mode of action. It has a hairpin structure where a fluorophore and a quencher are located at the 5′ and 3′ end of the probe, respectively, and like a hydrolysis probe, it contains a probe sequence on the loop (Tyagi and Kramer 1996). The native hairpin structure of the molecular beacon probe, placing a fluorophore and a quencher in close proximity, suppresses the fluorescence from the molecular beacon probe (Tyagi and Kramer 1996). The hybridization of the probe to the target DNA region takes place at the annealing step of PCR resulting in the increase of fluorescence (Tyagi and Kramer 1996). The molecular beacon probe has higher specificity due to its structural specificity than other hybridization probes and can discriminate target sequences with single nucleotide variation (Bonnet et al. 1999; Navarro et al. 2015).

II. *FRET-mediated Fluorescence Probe*

(i) *FRET Probe (Hybprobe)*

FRET probe is consisted of two oligonucleotides, which are specifically designed to bind two adjacent target sequences in the amplicon (Heller and Morrison 1985; Navarro et al. 2015). The first oligo has a donor fluorophore at its 3′ end, and the second oligo carries a second fluorophore at its 5′ end and a phosphate group at its 3′ end preventing the primer extension (Cardullo et al. 1988; Morrison et al. 1989; Navarro et al. 2015). These two oligos hybridize to the adjacent two target sequences on the amplicon at the annealing step during PCR, placing the two fluorophores next to each other. The second fluorophore emits the fluorescence by the FRET mechanism from the first fluorophore (Bernard and Wittwer 2000; Navarro et al. 2015). Real-time PCR with FRET probe can be multiplexed and was used for the detection of pathogen and genotyping (Liew et al. 2006; Lim et al. 2008; Navarro et al. 2015).

(ii) *Angler® and ResonSense® Probes*

Angler®probe is composed of two nucleotide sequences, a reverse primer and a probe sequence identical to the target region adjacent to the reverse primer-binding site, which were connected by a linker (Lee et al. 2002; Navarro et al. 2015). The real-time PCR with Angler®probe uses SYBR Gold as a donor fluorophore. The 5′ end of Angler®probe has a fluorophore which emits its fluorescence when the reverse primer portion of Angler®probe initiates a synthesis of a complementary strand of the target DNA region. During the denaturation step of the following PCR cycle, the probe sequence of Angler®probe binds to the complementary sequence of the same strand that leads to the formation of double-stranded DNA to which the intercalating fluorescent dye, SYBR Gold included in the reaction mix, will bind and act as a donor fluorophore for the acceptor fluorophore residing at the 5′ end of Angler®probe which will emit fluorescence via FRET derived from SYBR Gold

(Lee et al. 2002). The real-time PCR with Angler®probe together with an intercalating dye can distinguish nonspecific amplicon (SYBR Gold) and specific amplicon (Angler®probe) without melt-curve analysis (Lee et al. 2002; Naserpour Farivar et al. 2014).

ResonSense® probe functions as a primer that has an acceptor fluorophore at its 5′ end, but unlike Angler® probe, ResonSense® probe itself acts as a primer and a probe. The hybridization of ResonSense® probe to the target sequence will form a double-strand DNA where SYBR Gold will bind and act as a donor fluorophore for FRET leading to the emission of fluorescence from ResonSense® probe. Both Angler® and ResonSense® probes were successfully used for the detection of pathogen, mutation, and gene expression (Lee et al. 2002; Punia et al. 2004; Sanchez et al. 2006; Naserpour Farivar et al. 2014; Navarro et al. 2015).

In addition to the real-time PCR methods described above, there are other amplicon detection methods available for real-time PCR (Navarro et al. 2015). The review paper published by Navarro et al. (2015) and the papers cited in the review described details of these methods, some of which methods share a similar molecular structure or add a molecular ligand for improved specificity (e.g., hairpin structure in Scorpion and molecular beacon probes; MGB (minor groove-binding ligand)) for the amplicon detection or adopt a new approach shown in Cyclicon, Amplifluor®, Yin-Yang and Snake assays (Tyagi and Kramer 1996; Whitcombe et al. 1999; Kandimalla and Agrawal 2000; Kutyavin et al. 2000; Li et al. 2002; Nazarenko et al. 2002; Lukhtanov et al. 2007; Kutyavin 2010; Navarro et al. 2015).

4.3 Methods Under Active Development

4.3.1 Programmable Nucleic Acid-Binding Proteins

Advances in molecular biology and interest in the fundamental organization of eukaryotic chromosomes led to the identification of nucleic acid-binding proteins that bind to specific nucleotide sequences (Miller et al. 1985). Biochemical characterization of these proteins led to the conclusion that alterations in amino acid sequence could be used to alter their affinity for unique nucleotide sequences (Berg 1988; Choo and Klug 1994a, b) and subsequent targeting of genetic differences (Choo et al. 1994). Further development led to the use of zinc finger (ZnF) proteins in diagnostics while exploration, in other fields, led to the discovery of transcription activator-like effector (TALE) proteins and clustered regularly interspaced palindromic repeat (CRISPR)-associated (CAS) proteins. These proteins have all been adapted for use in diagnostics and, in the case of CRISPR-Cas in particular, represent active areas of research. The diagnostic approaches proposed for each of these protein classes are diverse and, below, we present a comprehensive summary of each method organized by protein class (Table 4.1). Each approach shows substantial promise, but their use, to date, has been limited.

Table 4.1 Characteristics of diagnostics systems based on programmable nucleic acid binding proteins

Type	System name	Effector	Target	Signal amplification	Readout	Sensitivity	References
ZFP	SEER	Fused ZFP-GFP	DNA	–	Fluorescence	4 μM	Stains et al. (2005)
	SEER-LAC	Fused ZFP-β-lactamase	DNA	–	Colorimetric	5 nM	Ooi et al. (2006) and Kim et al. (2011)
	–	ZFP-GST/anti-GST antibody	DNA	PCR	Chemiluminescence/ ELISA	10 copies	Osawa et al. (2008, 2009)
	–	ZFP/ZFP-luciferase	DNA	PCR	Bioluminescence	10 copies; 62 pM	Abe et al. (2012), Shi et al. (2017), and Takano et al. (2017)
	–	ZFP/ZFP-biotin	DNA	–	Chemiluminescence	0.5 nM	Kim and Kim (2016)
	–	ZFP/ZFP-glucose dehydrogenase	DNA	PCR	Electrochemistry	10 copies	Lee et al. (2017)
TALE	–	TALE/ TALE-β-lactamasee	DNA	PCR	Chemiluminescence	1.66 mM	Honarmand et al. (2014)
CRISPR	NASBACC	Cas9	RNA	NASBA	Colorimetric	1 fM	Pardee et al. (2016)
	PC reporter	dCas9-luciferase	DNA	PCR	Bioluminescence	1 copy/ 500 μL	Zhang et al. (2017)
	–	dCas9	DNA	–	Fluorescence	1 CFU/ mL	Guk et al. (2017)
	ctPCR	Cas9	DNA	PCR	Electrophoresis/ Fluorescence	5 ng of amplicon	Wang et al. (2018)
	CAS-EXPAR	Cas9	DNA/ RNA	EXPAR	Fluorescence	aM	Huang et al. (2018)
	RCH	dCas9	microRNA	RCA	Colorimetric	35 aM	Qiu et al. (2018)
	CARP	Cas9	DNA	PCR	Electrophoresis/ Fluorescence	2 pg of amplicon	Zhang et al. (2018)
	SHERLOCK	Cas13a	DNA/ RNA	RPA	Fluorescence	2 aM	Gootenberg et al. (2017)

	SHERLOCK V2	CcaCas13b, PsmCas13b, LwaCas13a	DNA/ RNA	RPA	Fluorescence; Colorimetric	8 zM	Gootenberg et al. (2018)
	DETECTR	Cas12a	DNA	RPA	Fluorescence	aM	Chen et al. (2018)
	HOLMES	Cas12a	DNA/ RNA	PCR/RT-PCR	Fluorescence	1–10 aM	Li et al. (2018)
	HOLMES V2	Cas12b	DNA/ RNA	LAMP; RT-LAMP; Asymmetric PCR	Fluorescence	10 aM	Li et al. (2019)
	Cas14-DETECTR	Cas14	ssDNA	RPA	Fluorescence	aM	Aquino-Jarquin (2019)

4.3.1.1 Zinc Finger Proteins

Comprising a highly diverse protein family, zinc finger (ZnF) proteins share a motif incorporating at least one zinc ion, which stabilizes their secondary structure. The domains formed typically involve molecular interactions such as binding DNA, RNA, proteins, or small molecules (Laity et al. 2001). Indeed, this specificity in binding particular DNA sequences first propelled these proteins to prominence in biotechnology. ZnFs were the first proteins engineered for gene editing, resulting in a diverse set of proteins capable of targeting desired genomic regions (Urnov et al. 2010; Perez-Pinera et al. 2012).

Stains et al. (2005) first took advantage of the sequence-specific binding of ZnFs for diagnostics. Building upon the availability of ZnFs to target desired sequences of DNA, they developed the sequence-enabled reassembly of proteins (SEER) approach. In order to prepare a fluorescent reporter for a desired DNA sequence, they split the green fluorescent protein (GFP), thereby removing its chromophore function, and fused each half to one of two ZnFs designed to specifically bind to unique DNA sequences. In the presence of the target DNA sequence, each ZnF binds to its respective nucleic acid sequence, the GFP halves are brought into proximity allowing reassembly, which catalyzes the chromophore formation producing a detectable fluorescent signal.

Ooi et al. (2006) subsequently improved the sensitivity of the SEER system by assessing the impact of distance (in nucleotides) between ZnF-binding sites. Similarly, they improved specificity by altering the length of the amino acid linker between the ZnFs and the signal domains. Finally, they converted the system to a colorimetric reporter by exchanging GFP for β-lactamase.

Subsequent work expanded on these methods by introducing new signaling systems, improving sensitivity, and removing the need for DNA amplification (Table 4.1).

4.3.1.2 Transcription Activator-Like Effector Proteins

Transcription activator-like effector (TALE) proteins are a class of DNA-binding proteins secreted by *Xanthamonas* sp. bacteria to manipulate host plant gene expression to aid colonization (Boch and Bonas 2010; Bogdanove et al. 2010). Unlike ZnFs, which target three nucleotide base arrays for binding, TALEs target single nucleotides providing a much more modular system (Sanjana et al. 2012; Sun and Zhao 2013). As a result, TALEs can theoretically target any sequence and show reduced off-target DNA-binding capabilities (Zischewski et al. 2017; Nerys-Junior et al. 2018).

Similar to ZnFs, TALE domains can be fused with other protein domains to confer novel function (Li et al. 2011a, b). This approach was taken by Honarmand et al. (2014) in the development of the first TALE-based detection system. By constructing two TALE arrays to bind uniquely to the target DNA, one could be immobilized on a nitrocellulose substrate, while the second was fused with a reporter protein. Upon addition of the test DNA, the nitrocellulose-bound TALE captures those DNA strands that present the appropriate sequence. If the target DNA carries the second TALE recognition sequence, the TALE-reporter construct binds. The

presence of both TALE recognition sequences immobilizes the TALE-reporter construct with the target DNA acting as a linker. Immobilization allows visualization and detection.

TALE approaches differ from ZnFs and CRISPR systems (see below) in several ways that benefit diagnosticians. TALE proteins are more stable than ZnF proteins, smaller than Cas proteins, do not require neighboring protospacer adjacent motif (PAM) sites for sequence recognition, and can target RNA-DNA hybrids (Yin et al. 2012; Pattanayak et al. 2013). While these advantages of TALE-based diagnostic systems warrant further attention, no other TALE-based diagnostic systems have been developed. These useful proteins apparently have lost the attention of researchers drawn to the newer CRISPR systems (Batista and Pacheco 2018).

4.3.1.3 Clustered Regularly Interspaced Short Palindromic Repeat-Associated Proteins

Clustered regularly interspaced short palindromic repeats (CRISPR) are genomic elements that retain a genetic memory with Lamarckian-style inheritance for many lineages of bacteria and archea. Together with CRISPR-associated (Cas) proteins, this genetic system forms an adaptive immune system that acquires sequences from foreign genomes and recalls this information for sequence-specific recognition and cleavage by Cas endonucleases (Mojica et al. 2005; Barrangou et al. 2007). With the development of engineered Cas9 endonucleases for genome editing, these systems have experienced intense research interest (Barrangou and Doudna 2016). CRISPR-associated proteins (Cas) and single-guide RNAs (sgRNA) have been adapted to function in sequence-specific genomic and epigenetic editing (Wang et al. 2016; Kungulovski and Jeltsch 2016), transcriptional regulation (Didovyk et al. 2016), loss-of-function screening (Shalem et al. 2015; Wade 2015), characterization of enhancer and regulatory sequences (Rajagopal et al. 2016; Lopes et al. 2016), visualization of DNA (Ma et al. 2016), creation of gene drives (Champer et al. 2016), antimicrobial and antiviral development (Fagen et al. 2017), suppression of the uptake of antibiotic resistance genes (Garneau et al. 2010), and in data storage (Shipman et al. 2016).

The excitement over CRISPR-Cas systems likely stems from the ease of designing and producing sgRNAs to target-specific sequences. This excitement has led to the exploration of CRISPR systems across taxa which has revealed substantial diversity (Luo et al. 2016; Koonin et al. 2017) only beginning to be exploited to advance diagnostics. To date, four Cas endonucleases have been utilized for diagnostics: Cas9, Cas13 (formerly known as C2c2), Cas12 (formerly known as Cpf1), and Cas14.

I. *Cas9-based Systems*

Pardee et al. (2016) pioneered the use of RNA-guided endonucleases for diagnostics. Using the nucleic acid sequence-based amplification method (NASBA; Compton 1991) to initially amplify the target RNA, they employed CRISPR-Cas9 as a sequence-specific nuclease to identify strain-specific sequences

and toehold switches to control signal expression in a cell-free, paper-based system to detect the RNA genome of Zika virus and distinguish between strains of the virus.

Specifically, they reverse transcribed the RNA target to DNA using a sequence-specific reverse primer to produce an RNA/DNA duplex. They degraded the RNA strand allowing a forward primer containing a T7 promoter to bind and initiate synthesis of second-strand DNA. The T7 promoter within the newly created double-stranded DNA allowed T7 RNA polymerase to amplify the RNA template (Deiman et al. 2002; Zhao et al. 2015). Due to NASBA primer design, the RNA product contained the trigger sequence of a toehold switch.

Toehold switches are synthetic riboregulator RNAs that contain hairpin structures that block a ribosome-binding site until the switch is bound to a complementary trigger RNA. In this system, complementary binding of the trigger RNA to the toehold switch drives conformational changes that reveal a ribosomal-binding site and initiate translation of a reporter gene. Pardee et al. utilized CRISPR-Cas9 to discriminate between strains of Zika virus, at the single nucleotide level, by identifying a target region in one strain containing a PAM sequence required for Cas9 to initiate cleavage. In the presence of the appropriate PAM, Cas9 destroys the trigger sequence which can no longer cause conformational changes in the toehold switch so reporter gene expression remains suppressed. In the absence of the appropriate PAM, the full trigger sequence is produced and results in reporter gene expression. Careful choice of target region and design of NASBA reverse primers allowed Pardee et al. to control signal production and distinguish strains of Zika virus.

Others have used CRISPR-Cas9 to recognize and generate the amplified signal. Huang et al. (2018) used a sequence-specific sgRNA along with an antisense PAM-presenting oligonucleotide (PAMmer) to nick single-stranded target DNA and generate the fragment for amplification and detection. The fragment, paired with an oligonucleotide designed for exponential amplification reaction (EXPAR; Van Ness et al. 2003), primes DNA polymerase to synthesize the remaining EXPAR template (designed as a copy of the target fragment). Nicking allows this fragment to be displaced by DNA polymerase and bind with a new EXPAR template initiating isothermal amplification (Zhang et al. 2016a, b). SYBR Green is used to detect this amplified double-stranded DNA product.

Zhang et al. (2018) similarly developed a Cas9/sgRNAs-associated reverse PCR (CARP) diagnostic tool using two sgRNAs to cleave genomic DNA into a target fragment. However, they employed T4 DNA ligase to concatenate or circularize the resulting fragment. Adding reverse primers designed to initiate exponential amplification of the target fragment in its concatenated or circularized form rather than its original orientation allows the production of double-stranded DNA capable of being visualized on agarose gels or with SYBR Green.

Wang et al. (2018) developed CRISPR-typing PCR (ctPCR) to detect and identify human papillomavirus (HPV) subtypes. The first step relies on traditional PCR with universal primers to amplify a target region associated with the virus. Amplicons with sequences matching a pair of specific sgRNAs are cleaved by Cas9, incubated with Taq polymerase to induce A-tailing, and ligated to a T adaptor. A second round

of PCR using primers matching the T adaptor produced specific fragments for visualization.

Other approaches take advantage of dead Cas9 (dCas9), a mutated form of the Cas9 protein that lacks the characteristic endonuclease activity of the native version. Zhang et al. (2017) utilized dCas9 as a protein reassembly reporter system similar to those used with Znfs and TALE. Designing sgRNAs to target neighboring sequences, they reassembled split halves of the bioluminescent enzyme, luciferase, bound to dCas9 proteins in a system they called PC reporter (Zhang et al. 2014a, b). A similar approach was later used by Qiu et al. (2018) to detect microRNAs amplified by rolling circle amplification and signaled by reassembly of split halves of horseradish peroxidase bound to dCas9 proteins. A different approach using dCas9 is based on fluorescent in situ hybridization (FISH; Guk et al. 2017). Using magnetic bead-labeled dCas9 and a sequence-specific sgRNA, Guk et al. captured and physically separated target DNA for visualization using SYBR Green without the need for amplification.

II. *Cas13-based Systems*

Unlike the well-known Cas9 RNA-guided deoxyribonuclease (DNase), Cas13a (previously called C2c2; Shmakov et al. 2015) is an RNA-guided ribonuclease (Abudayyeh et al. 2016). In addition to RNA-guided cleavage, binding with the target RNA also activates a nonspecific ribonuclease activity (Abudayyeh et al. 2016). This "collateral" cleavage was used by Gootenberg et al. (2017) to develop the specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) diagnostic system. This system accepts test material in the form of double-stranded DNA or RNA for amplification by recombinase polymerase amplification (RPA) or reverse transcriptase-RPA (RT-RPA), respectively (Piepenburg et al. 2006). The DNA product is then transcribed to RNA using T7 RNA polymerase and exposed to Cas13a. If the test sample contains the target site, Cas13a binds and initiates nonspecific "collateral" cleavage of single-stranded RNAs. By providing reporter RNAs labeled with a fluorophore at one end and a quencher at the other, the nonspecific "collateral" ribonuclease activity of Cas13a releases the fluorophore from its quencher only in the presence of the target RNA.

Two updates to the SHERLOCK method have been published. One, heating unextracted diagnostic samples to obliterate nucleases (HUDSON), focused on preparing samples for analysis to facilitate SHERLOCK deployment in the field (Myhrvold et al. 2018). The second extended the functionality and specificity of SHERLOCK. Aiming to extend their diagnostic method to simultaneously detect multiple targets, Gootenberg et al. (2018) characterized the cleavage preferences of Cas13a and Cas13b orthologs. These orthologs, paired with Cas12a (see below; Chen et al. 2018), allowed Gootenberg et al. to detect four targets in a single reaction. By altering the RPA primer concentrations and reaction volumes, they achieved the detection of as little as 8 zM target concentrations. By incorporating Csm6, a CRISPR type III nuclease that is activated by Cas13 collateral cleavage products, and a unique reporter molecule, they were able to amplify the signal strength for

reporting. Finally, they developed a lateral flow readout system by replacing the fluorophore quencher with biotin. Samples added to the test strip first acquire a nanoparticle-bound anti-fluorophore antibody, then a streptavidin band captures the biotin bound reporters and only those cleaved products accumulate at the antibody capture line. Combined with HUDSON, this method offers a sensitive, multiplexed, field-deployable diagnostic system.

III. *Cas12-based Systems*

While Cas13 systems can be converted to work with DNA target molecules through reverse transcription, Cas12 is a DNase with collateral activity that does not require an additional step for DNA targets. As such, SHERLOCK-like diagnostic systems have emerged since the characterization of Cas12 proteins. One-hour low-cost multipurpose highly efficient system (HOLMES; Li et al. 2018) and DNA endonuclease-targeted CRISPR trans reporter (DETECTR; Chen et al. 2018) work on a similar framework to SHERLOCK. With target amplification, by PCR or RPA (HOLMES and DETECTR, respectively), each uses collateral cleavage of single-stranded DNA reporters capped with fluorophores and quenchers.

These approaches were expanded upon by Wang et al. 2019 and Li et al. 2019 to incorporate amplification and signal production in a single tube to improve ease of use and avoid cross-contamination. The Cas12a-based visual detection (Cas12aVDet) utilizes RPA, a portable heater, and a blue light for visualization (Wang et al. 2019). HOLMES version 2 (Li et al. 2019) used loop-mediated isothermal amplification (LAMP; Notomi et al. 2000) for single tube testing and expanded the method for single nucleotide polymorphism (SNP) and RNA detection as well as quantitating DNA methylation.

IV. *Cas14-based Systems*

Through the use of metagenomic sequencing of uncultivated archea, Harrington et al. (2018) discovered a new Cas endonuclease, Cas14. Substantially smaller than other Cas endonucleases, this protein is an RNA-guided DNA endonuclease that, unlike other DNA-targeting endonucleases, selectively targets single-stranded DNA. Like Cas13 and Cas12, nonspecific collateral cleavage is activated in Cas14 by binding with the target DNA. However, unlike other Cas endonucleases, Cas14 does not require a PAM site.

Harrington et al. (2018) proposed that Cas14a could be used to modify the DETECTR platform. As proof of principle, they used Cas14-DETECTR to detect an SNP in the human HERC2 gene which is responsible for eye color. This diagnostic system will be useful to detect single nucleotide polymorphisms (SNPs) and single-stranded DNA viruses.

4.3.2 Optical Biosensors

Optical biosensor technologies for plant disease detection focus on identifying changes in the host plant properties due to biotic or abiotic stresses, by determining unique spectral profiles which correlate with these conditions (Martinelli et al. 2015). Generally speaking, plant diseases will modify the physiological state of plant leaves, and any modification to a leaf constituent will correspondingly modify the spectral properties of the leaves by affecting its light scattering and absorption characteristics (Mahlein 2016). Optical technologies therefore rely on light interrogation of a diseased plant to measure and then translate the resulting reflected light into optical spectra, which can be analyzed for unique spectral biomarkers associated with a particular disease state.

In this way, optical techniques for disease detection can be classified as indirect, in comparison to direct molecular or serological identifiers of pathogens such as PCR and ELISA based approaches (Fang and Ramasamy 2015; Martinelli et al. 2015). Other indirect technologies exist, such as chemical analyzers that detect the release of new gaseous plant volatiles (Schmelz et al. 2003; McCartney et al. 2016) or nuclear magnetic resonance spectroscopy-based detection of changes to plant metabolite profiles (López-Gresa et al. 2010; Freitas et al. 2015). By correlating host response to pathogen presence, indirect approaches are not necessarily reliant on the level of pathogen in a collected sample to determine whether the plant is diseased. Therefore, they provide the capability to overcome sampling issues associated with direct approaches due to uneven, low-level pathogen distribution in the host and can provide a more efficient means for determining disease state, especially in the early stages of development.

Optical technologies offer some additional advantages for plant disease detection. They can noninvasively interrogate plants (Behmann et al. 2015), which in many cases completely alleviates any requirement for sample collection. Further, spectral imaging-based solutions including hyperspectral remote sensing-based approaches provide a rapid assessment of large-area vegetation or crops, improving the odds for identifying disease in a timely manner. However, the sensitivity of spectral-imaging systems varies based on the desired spatial resolution. Aerial or drone-mounted hyperspectral systems provide very large area coverage, but have limited spatial resolution often resulting in entire plant or canopy averaging, limiting the sensitivity for detection of small-scale, leaf-level physiological changes.

Optical techniques utilize several spectroscopy approaches to identify the spectral biomarkers associated with plant disease. Primarily, these include visible and near-infrared (VIS-NIR) spectroscopy, fluorescence spectroscopy, and Raman spectroscopy techniques. VIS-NIR spectroscopy technologies focus on measuring the reflection of light from a leaf, which is affected by absorption due to leaf pigments and scattering due to heterogeneous cellular and intercellular structures. The visible spectrum (~ 400–700 nm) is dominated by pigment absorption, and in most plant leaves chlorophyll is the main absorber, including chlorophyll a and b, which absorb significantly in the blue and red spectral regions (Peñuelas and Filella 1998). There

are other pigments that also contribute to the visible absorption and include xanthophyll, carotene, and anthocyanin (Christensen 2004).

Fluorescence detection is also used to measure the photosynthetic activity of chlorophyll, both through natural light stimulus (Berdugo et al. 2014) and laser-induced approaches (Belasque et al. 2008). Since chlorophyll function presents as an easily accessible optical biomarker to analyze for a disease state, it is utilized extensively in optical plant disease detection (Mahlein 2016). However, chlorophyll function is also affected by other plant stressors such as nutrient deficiency, and often needs to be measured in conjunction with other biomarkers to distinguish between other plant stress states (Lowe et al. 2017; Zarco-Tejada et al. 2018).

In the near-infrared spectrum, the absorption of pigments is not significant, and leads to relatively flat reflectance from ~ 700 to 1200 nm. The near-infrared is mainly dominated by scattering due to inhomogeneous refractive index distribution arising from cellular and intercellular structures such as the cellulose to air interfaces (Mahlein 2016; Kuska et al. 2015). Near-infrared spectral signatures can therefore be correlated with certain structural changes associated with disease progression. Raman spectroscopy, on the other hand, can utilize infrared plant interrogation in a different manner. Unlike VIS-NIR, Raman spectroscopy focuses on utilizing near-infrared laser systems to probe for chemically specific information, and therefore can determine localized changes in leaf chemical composition (e.g., carbohydrates, proteins, lipids) and associate those changes with the disease (Pérez et al. 2016).

By determining unique spectral biomarkers for disease states, optical techniques have been shown to be sensitive to many disease conditions (e.g., fungal, viral, bacterial) across various plant species. Bravo et al. (2003) investigated *Pucciniastriiformis* (yellow rust) infection in wheat plants using a ground-mounted spectral imaging system. Rumpf et al. (2010) analyzed hyperspectral data collected on sugar beet experiencing Cercospora leaf spot, leaf rust and powdery mildew, and leveraged machine learning analytics to perform early disease differentiation. More recent demonstrations elsewhere have also harnessed recent developments in machine learning and other variants of artificial intelligence (AI) analytics to improve accuracy for disease detection. Some examples include Huanglongbing detection in citrus trees (Li et al. 2013), *Tobacco mosaic virus* in tobacco plants (Zhu et al. 2017), *Xylella fastidiosa* in olive trees (Zarco-Tejada et al. 2018), *Phytophthora infestans* in potato plants (Franceschini et al. 2019); reviews presented elsewhere provide comprehensive lists of other demonstrations and applications for plant disease detection (e.g., Mahlein 2016; Lowe et al. 2017; Sankaran et al. 2010; Sylvain and Cecile 2018).

Cellular Analysis and Notification of Antigen Risks and Yields (CANARY®) is a biosensor that utilizes genetically engineered B lymphocytes expressing bioluminescent protein and pathogen-specific antibodies on the membrane surface for specific and sensitive pathogen detection (Rider et al. 2003). CANARY® possesses an optimal combination of the sensitivity of PCR and speed of lateral flow devices and is under development and evaluation for several pathogens including *Ralstonia solanacearum*, *Phytophthora* spp., and potyviruses (Nargi 2006).

To summarize, the development of optical biomarker-based technologies for plant disease detection has progressed steadily over the last couple of decades. Recent advances in the mobilization of optical technologies coupled with emerging AI analytics are rapidly improving the availability and effectiveness of such solutions. Optical technologies offer some key advantages including indirect detection, noninvasive interrogation with no sample preparation, and rapid, large-area assessment for plant disease. In certain cases where sensitivity is an issue, the high-throughput nature of optical detection can be used to guide other detection technologies for follow-up or confirmation on disease state. Ultimately, large-scale detection methods are needed to identify and monitor for diseases in order to prevent epidemics, and optical technologies are showing some promise toward providing these capabilities.

Traditional methods of fungal identification by visual observation for disease symptoms, incubation methods, fungal isolations, colony and spore morphology, and microscopy are tedious, time-consuming, and may lead to wrong identifications. Fungal diagnosis using PCR-based methods has evolved rapidly and became the gold standard. Several diagnostic markers such as ribosomal genes, particularly the internal transcribed spacer (ITS) region, have been successfully used in fungal identification up to the species level (White et al. 1990). Analysis of more than one DNA locus is beneficial in phylogenetic interpretation and species identification of new isolates especially in certain genera such as *Mycosphaerella, Colletotrichum,* and *Fusarium*. Next-generation sequencing (NGS) is a versatile tool being used not only for diagnostics but also to learn the whole genome or transcriptome of the fungal isolates. Another technology that gained popularity for rapid and reliable diagnostics is matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) fingerprinting. A combination of techniques, multilocus PCR, and electrospray ionization –mass spectrometry (ESI-MS) can rapidly detect multiple fungal pathogens and provide genetic information in a single assay.

Microscopy, serological techniques such as ELISA including plant-trapped antigen ELISA, and double antibody sandwich ELISA and PCR-based technologies, and isothermal amplification such as LAMP, rolling circle amplification (RCA), and nucleic acid sequence-based amplification (NASBA) are routinely employed in the detection of phytopathogens. Recent diagnostic assays are all based on the nucleotide sequences of pathogen RNA or DNA, but even these can be combined with earlier assays such as immuno-capture PCR (Mulholland 2009). Modern diagnostic techniques such as CANARY® can accurately determine the minute amounts of the pathogen in the infected plant tissue very rapidly and is easy to use. Prokaryotic CRISPR-Cas immune systems have contributed to the advancement of the biotechnology field with an ability to precisely edit the genomes, gene disruption, and gene repression (Hsu et al. 2014). Several diagnostic methods based on CRISPR/Cas9 to detect nucleic acid of the pathogen were recently developed.

Despite the advances in molecular diagnosis, biological indicators still have their place. They are still required for newly discovered viruses to complete Koch's postulates, and they are also essential for indexing diseases for which no pathogen

has been identified. Even for viruses which are well characterized, the plant is still the only indicator for symptoms.

4.4 Conclusions

Critical to all efforts to fight plant disease, detection, and diagnosis of pathogens is the first step toward any treatment, suppression, or containment of the pathogen. While the variety of pathogens is innumerable, so too is the ingenuity of diagnosticians. Here we have provided an overview of and an entrance into the various methods that are being used or being developed to detect and diagnose plant pathogens. The variety of well-established methods should allow for the development of a diagnostic strategy for any known plant pathogen. The methods under development offer exciting promises for quicker, cheaper, and easier deployment of more sensitive and more accurate diagnostic methods. Much work is needed yet for these methods, but we encourage their exploration and implementation where appropriate.

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5 Plant Virus Diagnostics: Traditional to Recent and Emerging Advances

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Abstract

Viral diseases cause huge economic losses in agriculture systems globally and their management is a big challenge to growers as well as researchers. For any successful management of viral disease, detection and identification of plant virus associated with the plant disease is of foremost importance. Specific, robust and precise diagnostics of plant viruses is also essential to prevent the introduction of viruses in a new region as free trade agreement in absence of virus diagnostics can lead to the introduction of new viruses through transfer and exchange of planting material. The advancements in molecular biology have led to major breakthroughs in the form of newer, sensitive and efficient diagnostic techniques. Several detection techniques have been developed in the last three decades, which are broadly based on serological and molecular approaches for the detection of viruses. Next-generation sequencing has been used to detect unknown or new viruses in several crops. Several specific, simple, fast, farmer-friendly and sensitive technologies as point-of-care diagnosis have also been developed. Plant virus diagnostics, after integrating with portable devices, has a promising future for on-site field diagnosis. Here, we review various methods of plant virus detection including those methods that have been successfully used in field conditions.

Keywords

Plant viruses · Detection · Robust · Serological · Molecular

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5.1 Introduction

Viruses are catastrophic, tiny entities, which are visible only under a transmission electron microscope. The genetic material of viruses comprises of either DNA or RNA, encapsidated in coat protein. Being obligate in nature, viruses exclusively utilize the metabolic machinery inside the host cell for its multiplication. Plant viruses are ubiquitous and rank second among economically vital plant pathogens (Vidaver and Lambrecht 2004). It is difficult to estimate crop loss due to plant viral diseases as it is highly variable and depends on virus strain, region, time of infection and cultivar (Sharma et al. 2014). However, it has been reported that economically significant viral diseases cause economic losses more than several billion dollars annually throughout the world (Hull 2002; Joo et al. 2014; Islam et al. 2017). Viruses intervene by the allocation of resources produced through photosynthesis inside plant cells and alter physiology, which results in symptoms like mosaic, necrosis, mottling, curling and puckering. However, sometimes the phenotypic expression of symptoms may not be visible due to latent infection of plant viruses (Van der Want and Dijkstra 2006; Nabi et al. 2018). Additionally, plants can also express symptoms similar to viral diseases in response to nutritional disorders, harsh climatic conditions and other abiotic agents (Vander Want and Dijkstra 2006). Thus, symptom-based diagnostics of virus disease is complex in comparison to other pathogens (Lievens et al. 2005). The diagnostic methods as well as their application are influenced by several factors (Islam et al. 2017). Many virus species from closely related families show high mutation rates and exchange of genetic components, which can result in recombination events between these related species, thus enhancing the chances of genetic variability and aggressiveness in viral strains (García-Arenal et al. 2001). Similarly, the mixed infection of more than two viruses infecting a single plant results in either synergistic or antagonistic effects among the viruses (Syller 2012). Also the increased globalization of trade as well as the climate change has considerably lead to a rise in the plant virus movement. Consequently, viral disease prevention and economic damage warrant the robust, accurate and specific methods for detection. Several methods, including new high emergent throughput diagnostic techniques, have been developed to detect plant viruses, viz., biological, physical, morphological, immunological, molecular methods and point-of-care techniques (Lopez et al. 2008; Yadav and Khurana 2016; Rani et al. 2019).

5.2 Detection and Diagnostic Methods

Management of any plant disease depends on proper identification of disease and its causal agent. Therefore, detection and diagnosis is the most important aspect for managing plant viruses, as the viral infection remains systemic throughout the life cycle of the crop. Detection and identification of viruses is based on biological properties, morphological properties and intrinsic properties of the viruses. An overview of various methods suitable for plant virus detection is described in the following sections.

5.2.1 Detection Based on Biological Properties

Biological indexing was an essential tool for the detection of a particular plant virus followed by characterization. Biological detection is based on the symptom expression on the natural hosts or on susceptible herbaceous or woody indicator plants mechanically inoculated by sap or grafting or budding. In the present era, the labour-intensive and time-consuming biological indexing has little application and not preferred for routine virus detection.

5.2.2 Detection Based on Morphological Properties

5.2.2.1 Electron Microscopy (EM)

Morphological properties like shape, size and other surface features of the virus particle are a vital requirement for virus detection (Bernd and Gunther 2009). In electron microscopy, a negative staining leaf-dip preparation technique is used universally to detect differential filamentous and rod-shaped viruses rather than isometric and other viruses. The EM aids in rapid and precise results, and, in the majority of cases it is sufficient to determine the shape and size of virus particles (Harris 2007; Wild 2008).

5.2.3 Detection Based on Viral Proteins

Methods are generally called as serological or immunoassays which use viral proteins, mostly coat protein (CP) for virus detection. Numerous serological or immunological tests have been developed and used for plant virus detection. These approaches are based on the interaction between a protein or proteins (antigen) in the virus particle with antibodies raised against them in vertebrates. The various immunological tests are as under.

5.2.3.1 Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA is the plate-based immunoassay and most versatile due to its specificity, simplicity and robustness. The test is primarily based on the specificity of the antibody to interact with the antigen (coat protein of virus). The ELISA microtitre plate is coated with primary antibody (DAS-ELISA) followed by infected plant sap, which is further detected with the help of secondary antibody conjugated with the reporter molecule (an enzyme). If the target virus is present in the sap, it will interact first with the primary antibody, which is consequently detected by conjugate antibody in the presence of substrate. The plant virus detection is confirmed through the production of the visible colour. The quantity of the colour product is estimated through ELISA-Reader, which gives a quantitative estimation of viral load indirectly. Similarly sometimes microtitre plate is precoated with plant sap (DAC-ELISA), which is further detected by primary and secondary antibodies (Clark and Adams 1977). It has been widely used for the detection of many

important viruses including *Apple stem grooving virus* (ASGV), *Citrus tristeza virus* (CTV), *Potato virus X* (PVX), *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) (Sun et al. 2001; El-Araby et al. 2009; Nabi et al. 2018). Polyclonal antibodies have been produced against a large number of plant viruses using their recombinant proteins and have been successfully used for specific detection *of Grapevine leafroll virus*-3, *Grapevine leafroll virus*-4, *Garlic common latent virus, Leek yellow stripe virus and Banana streak Mysore virus* in India (Pramesh et al. 2012; Kumar et al. 2015, 2018; Rai et al. 2018). A more detailed information on serological detection of plant viruses in India has been reviewed by Bhat and Maheshwari (2017).

5.2.3.2 Tissue Immunobinding Assay (TIBA)

The TIBA is similar to ELISA, in which an impression is made onto the nylon or nitrocellulose by pressing plant tissue over it, which is further used to detect the virus following similar procedures as in ELISA (Webster et al. 2004). Several plant viruses have been detected using TIBA, which includes *Alfalfa mosaic virus* (AMV), *Capsicum chlorosis virus* (CaCV), *Bean leafroll virus* (BLRV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Soybean dwarf virus* (SbDV) and *Watermelon bud necrosis virus* (WBNV) (Kumari et al. 2008).

5.2.3.3 Immunosorbent Electron Microscopy (ISEM)

It is a combination of electron microscopy with serological specificity for morphological observation of virus particles (Derrick 1973) and has been used for the detection of a broad range of plant viruses (Rod and polyhedral) (Naidu and Hughes 2001). From samples, the virus particles are selectively entrapped on copper grids coated with antiserum specific to the virus (Lima and Purcifull 1980). The grid is treated with infected plant sap for 3–4 h at 25 °C after washing off the excess of antibody. Further, the grid is washed thrice and stained with 1.0% uranyl acetate in 50% ethanol and dried. The grid is finally observed under a transmission electron microscope for the detection of virus particles (Lima and Purcifull 1980). Because of its higher sensitivity and rapidity, it finds wide applicability in the detection of viruses present in low titre in plants (Khurana 1990).

5.2.4 Detection Based on Viral Nucleic Acid

Molecular detection methods involving amplification of nucleic acids (DNA or RNA) have been developed and revitalized day by day for the detection of most important plant viruses. A broad range of molecular diagnostic techniques such as polymerase chain reaction (PCR), reverse transcriptase-PCR, multiplex PCR and quantitative PCR proved to be the most important approaches. These methods are more advantageous over biological or serodiagnostic techniques, as any viral genomic region can be targeted to develop the detection tools. As many of the viruses are poor immunogens, nucleic acid-based detection method would be a more promising approach for detecting such viruses. These methods are highly efficient, robust,

specific and sensitive in comparison to immunological assays and are suitable for plant virus detection on routine basis accurately (Chen and Adams 2001; Zheng et al. 2010).

5.2.4.1 PCR and RT-PCR

Most important milestone in molecular biology was the invention of PCR (Mullis and Faloona 1987). Based on the sequence information, PCR has been effectively used for the detection and characterization of plant viruses (Yadav and Khan 2009). It is a highly accurate and sensitive detection protocol, as it relies mostly on the presence of distinctive sequences in the viral genome. In RNA viruses, the first cDNA strand is synthesized with the help of reverse transcriptase (RT) enzyme, which is further used as a template for amplification. The amplicons obtained in PCR from genomic regions of the virus need to be identified by cloning and sequencing. It has been used to detect several viruses such as Citrus yellow mosaic virus Papaya ringspot virus (PRSV), Potato virus X (PVX), Cucumber mosaic virus (CMV), Potato leafroll virus (PLRV), Apple stem grooving virus (ASGV), Prunus necrotic ringspot virus (PNRSV), Apple mosaic virus (ApMV), Leek yellow stripe virus (LYSV), Apple necrotic mosaic virus (ApNMV), and Bhendi yellow vein mosaic virus (BYVMV) (Baranwal et al. 2003; Jain et al. 2004; Rana et al. 2011; Gupta et al. 2013; Roy et al. 2015; Nabi et al. 2020). The PCR or RT-PCR has been optimized for a number of plant viruses in India (Sharma et al. 2017).

5.2.4.2 Multiplex PCR/RT-PCR

It is a procedure of simultaneous detection of two or more viruses/viroids in a single reaction tube (Nassuth et al. 2000). This technique is helpful for simultaneous detection of viruses in mixed infection in the same host. Many sets of primers are used in the single reaction mixture, which target different regions in the genome of multiple viruses. The oligonucleotides (primers) are designed in such a manner that these lack complementarities and have the same annealing temperature for amplification. It is advantageous by saving time and reagent costs against individual PCR (Deb and Anderson 2008; Roy et al. 2005, Bertolini et al. 2003). Several viruses such as *Apple mosaic virus* (ApMV), *Apple chlorotic leafspot virus* (ACLSV), *Prunus necrotic ringspot virus* (PNRSV) and *Plum pox virus* (PPV) were detected simultaneously in apple trees via multiplex-PCR (Menzel et al. 2002). Selvarajan et al. 2011 developed a multiplex-reverse transcription-PCR for the detection of BSMYV and *Banana bunchy top virus* (BBTV) simultaneously in banana samples. Multiplex RT-PCR for 4 viruses in garlic and for 4 viruses and a fastidious greening bacterium in citrus has been optimized in India (Majumder and Baranwal 2014; Meena and Baranwal 2015).

5.2.4.3 Immunocapture PCR (IC-PCR)

This technique involves a combination of PCR and immunoassay to entrap virus particles by antibodies, which is further amplified using specific primers. The virus is first adsorbed on antibody-coated PCR tube which is further removed in the presence

of non-ionic surfactant Triton X-100 by heating, followed by amplification using PCR. Entrapment of virus particle by antibody helps to purify the virions from the plant sap, hence proves very successful for viruses which are present in low titre and extracts having certain inhibitors which can hamper PCR. The IC-PCR has been employed for the detection of several viruses such as *Pepper mild mosaic virus* (PMMV), *Cucumber mosaic virus* (CMV), *Citrus tristeza virus* (CTV), *Grapevine fanleaf virus* (GFLV) and *Tomato spotted wilt virus* (TSWV) (Narayanasamy 2011). A duplex-immunocapture PCR protocol developed and optimised for simultaneous detection of two viruses, namely SCSMV and SCMV (Subba Reddy et al. 2011). It has also been optimized for *Grapevine leafroll-associated virus*-3 (Kumar 2013).

5.2.4.4 Real-Time PCR (qPCR)

It is an advanced version of molecular detection, which is accurate, sensitive and specific over conventional PCR. The basic principle is the monitoring of accumulation of amplicon in real-time by the labelling of primers or amplicon with fluorescent dyes. Several dyes have been used which binds to any dsDNA. It is a simple and economic way to quantify amplification. It avoids the downstream process like agarose gel electrophoresis as a product at each cycle is detected by fluorogenic molecules. It has wide application in plant virus diagnostics and quantification (Schaad and Frederick 2002; Boonham et al. 2004). A dye-based one-step reverse transcription-quantitative PCR (RT-qPCR) assay was developed for the robust and easy detection of *Cardamom mosaic virus* (CdMV) and *Banana bract mosaic virus* (BBrMV) which infect cardamom (Siljo et al. 2014), Piper yellow mottle virus (PYMoV) and CMV infecting black pepper plants (Bhat and Siljo 2014).

5.2.4.5 Microarray

It is evolved from southern blotting, which instead of nitrocellulose membrane uses glass as a support (Maskos and Southern 1992). It can detect virus-specific serotypes with high accuracy using specific probes in a single assay (Nam et al. 2014). The 25 bp to 70 bp nucleotide single-stranded synthesized DNA probes are hybridized with the viruses extracted from plant samples. It is capable to identify plant virus at the genus level and can also differentiate related strains (Boonham et al. 2007). The important demerit is cost, as it requires dust-free room, sophisticated machine for spotting probes and reading reactions. This platform is efficient and specific for the detection of viruses along with satellites like CMV, *Tomato infectious chlorosis virus*, TSWV, *Tomato mosaic virus*, *Pepino mosaic virus*, PVX, PVA, PLRV, PVY, PVM and PVS (Bystricka et al. 2005; Lee et al. 2003). A microarray chip has been developed at the Advance Centre for Plant Virology, ICAR-IARI, New Delhi for parallel detection of more than 1100 viruses and 30 viroids whose genomic sequences are available in the GenBank (Unpublished).

5.2.4.6 Isothermal Amplification

These methods were a major breakthrough in molecular diagnostics, avoiding the need for costly equipments (PCR) to amplify DNA or RNA. It depends on the non-thermal separation of the dsDNA. In this assay, the sequence-specific primers

amplify the target DNA just like in conventional PCR (Boonham et al. 2014). Several methods, viz., loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA) and recombinase polymerase amplification (RPA) have significantly been used in plant virus diagnostics (Zhao et al. 2015).

Loop-Mediated Isothermal Amplification

The limitations of PCR-based molecular methods, such as costly thermocycler requirement and low specificity in some cases, consequently lead to the development of LAMP. It can amplify target DNA at very low copy number in a short span of time. In a LAMP reaction, specifically *Bst* DNA polymerase having high processivity and strand-displacement activity along with three primer pairs (internal, external and loop primers) complementary to six specific genomic regions in DNA template are used. The reaction is set at a temperature range of 60–65 °C for 30–60 min. The primers are designed from online software Primer Explorer V4 (https://primerexplorer.jp/elamp4.0.0/). It has been used to detect plant viruses such as *Plum pox virus, Tomato yellow leaf curl virus*, *Wheat yellow mosaic virus, Banana bunchy top virus* and *Zucchini yellow mosaic virus* (Kuan et al. 2014; Fukuta et al. 2003; Varga and James 2006; Zhang et al. 2011; Peng et al. 2012; Ahmadi et al. 2013).

Rolling Circle Amplification

The discovery of RCA has revolutionized the field of diagnostics especially in begomoviruses, where it was first time used for cloning of a single-stranded circular DNA (Haible et al. 2006; Inoue-Nagata et al. 2004). The procedure involves *phi* 29 polymerase at isothermal temperature for sequence-independent amplification. It uses exoresistant random hexamer primers instead of specific primers that provide an advantage of amplification of variants as well. The RCA-amplified product can be characterized by restriction digestion using restriction enzymes and by direct sequencing (Haible et al. 2006). It is highly sensitive as compared to PCR for the detection of integrated and episomal viral sequences of *Badnaviruses* (James et al. 2011; Baranwal et al. 2013; Sharma et al. 2015).

5.2.5 Recent and Emerging Advances

With the demand of producers for access to the high yielding plant varieties, to facilitate the movement of germplasm from country to country, while maintaining phytosanitary values, the efficient, rapid, robust, onsite and cheap methods of diagnostics are need of the hour (Varvara et al. 2018). With continued efforts of plant virologists across globe, novel and emergent approaches have been identified to detect the causal agent of new and unusual diseases. These new high-throughput diagnostic approaches are classified as lab- and point-of-care-based.

5.2.5.1 Lab-Based Methods

Next-Generation Sequencing (NGS)

From last two decades advancement in sequencing technologies had led to the development of new methods for the detection, identification and characterization of viruses. The availability of NGS has revolutionized the discovery and ease at which novel phytovirome have been reported from agricultural ecosystems during the last decade (Villamor et al. 2019). The new approach referred as NGS, or deep sequencing or high-throughput or in-parallel, is the sequencing of total nucleic acid content in symptomatic/asymptomatic samples for subsequent identification of pathogen(s) using bioinformatics tools (Qingfa et al. 2015). Millions to billions of nucleotides can be sequenced in parallel, minimizes cloning of large number of fragments, which are used in Sanger sequencing. It substantially yields more throughputs. The NGS being sequence and culture-independent approach, hence concurrently detects RNA/DNA viruses and viroids even if present in low titre. It is a revolutionary technology for easy identification of novel and unknown viruses as compared to traditional diagnostics which uniquely target a species/strain (Baranwal et al. 2015). The virome of various plants like citrus, apple, grapevine, etc. (Rott et al. 2017) has been explored using the NGS technology.

Droplet Digital PCR (ddPCR)

Droplet Digital PCR invented in 2011 is a recent technology based on water-oil emulsion droplet technology to increase the amplification efficiency of standard PCR. The ddPCR is accurate, robust, sensitive and precise, producing simple readouts such as 'YES' or 'NO'. It provides absolute quantification as it eliminates the need for standard curves and normalization. It is useful, where virus titre is low and has uneven distribution in infected plants (Selvaraj et al. 2018). Until now, it has been used for the detection of *Grapevine red blotch-associated virus* (GRBaV) (Voegel and Nelson 2018).

5.2.5.2 Point of Care (POC) Detection

These approaches are on-site detection procedures without the use of sophisticated equipments and do not require expert personnel. The POC devices are advantageous, due to their sensitivity portability, accuracy and robustness (Lau et al. 2016).

Lateral Flow Immunoassay (LFIA)

It is a rapid and widely applied immune-chromatographic technique for plant virus detection, especially in horticultural crops (Salomone et al. 2004). Due to the use of simple devices, it is easy to perform even by a non-expert person. It is quick and simple, hence allows on-field detection of plant viruses. The LFD is a paper-based platform spotted with the sap of infected plant tissue; the sample flows via it and commences antigen-antibody interaction, which finally leads to chemical reaction detectable within 5–25 min as chromogenic bar/line for test and control samples. Several kits have been developed for the detection of plant viruses. It has been used

in the detection of several plant viruses either individually or in combination such as TMV, PVM PVX, PVA, PVY, PVS and PVY (Drygin et al. 2011).

Recombinase Polymerase Amplification (RPA)

It is a recent isothermal approach based on enzymatic denaturation. It uses recombinase, which forms primer-recombinase complex for initiation of DNA amplification. It performs amplification at the temperature range of 37–42 °C, but sometimes give amplification also at room temperature. The reaction mixture comprises of primers of length 32–36 nucleotides, with other buffers and three enzymes (recombinase, single-stranded DNA binding protein (SSB) and strand displacing polymerase). The process begins by scanning of double-stranded DNA with recombinase enzyme to bind primers on cognate sites in presence of ATP molecule and open the double-helical structure which in turn is stabilized by the SSB protein. The disassembly of recombinase is facilitated by ATP hydrolysis followed by the addition of complementary nucleotides into the primer sequence via strand displacing polymerase to form a new strand of DNA. The technique has been used for the detection of several viruses like *Plum pox virus* (PPV), *Banana streak Mysore virus* (BSMYV) and CMV (Zhang et al. 2014: Kapoor et al. 2017; Nishant et al. 2019).

Antibody-Based Biosensors

The antibody-based biosensors mainly work on the ELISA principle for signal generation after target antigen (Ag) capturing using immobilized antibodies (Abs) placed on a solid surface. The complex formed by Ag-Ab can be divided using an immuno-magnetic separator (Cho and Irudayaraj 2013) and signal strength depends on several types of potent transducers (Pilolli et al. 2013). The bigger limitation of this method is cost, cross-reactivity and longer time for antibody synthesis (Lau and Botella 2017). These biosensors are generally applied in several diagnostic including plant viruses, e.g., *Citrus tristeza virus* (Shojaei et al. 2016).

Aptamer-Based Biosensors (Aptasensors)

Aptamers are defined as single-stranded nucleic acid consisting of 30–32 bp length or amino acid polymers which displays a high degree of affinity to the target molecule (Bahadir and Sezginturk 2016). An aptamer-based method has significant advantages over the antibodies use due to their cheapness and less production time (2–3 days). Moreover, these show high stability, flexibility and versatile binding abilities (Seok Kim et al. 2016). The aptasensors involve the selected aptamer immobilization on a solid surface to arrest the target molecule, which further help to convert the resultant signal into readout with the help of the transducer. To enhance the sensitivity and specificity of aptasensors, several nanomaterials have been used (Khedri et al. 2018). The first aptamer detection method was developed against *Apple stem pitting virus* (ASPV) using the coat protein of virus (Balogh et al. 2010). However, its use is still limited.

Lab-on-Chip (LOC) Devices and Paper-Based Devices

It is a miniaturized microfluidic device, which integrates several laboratory methods onto a single chip, which includes biochemical methods, DNA sequencing and finally detection of the pathogen. The various modules of a LOC device include processor (for sampling), amplifier (for signal amplification), transducer (to produce a measurable signal) and software (for data analysis) (Luka et al. 2015). The major challenge faced by LOC devices is the requisite for label-free assays and new stable polymers which hinders their on-site pathogen detection. However, the microfluidics paper-based analytical devices (μPADs) development with 2-dimensional and 3-dimensional potential have been able to overcome the demerits faced using standard LOC devices with a permeable porous cellulose paper (pore size 1–10 μm) which helps to reduce the costs further. These LOC devices are simple, cheap, disposable, portable and easy to handle. The main LOC body is designed with four parts: the sample pad (to load the sample), an indicator pad (for the test and control line), a conjugate pad (for sample binding and label) and an absorption pad (for the absorption of leftover fluids) (Mahato et al. 2017). Initially, μPADs produced mostly colourimetric readouts but with the advancement in wireless connectivity (Novarum, Android app, iPhone and iBG star) the target quantitation task can be done with the help of a cell phone-based detection system (Syedmoradi et al. 2017). These techniques are restricted with few limitations, for instance, light scattering, non-specific adsorption, variable sensitivity and non-uniform wicking. However, further research in μPADs could potentially help in the development of a 'POCKET SIZED' diagnostic techniques for pathogens detection in nurseries (Mahato et al. 2017). Although this method has been used for the detection of several plant pathogens, it has to be still used for viruses.

Cell Phone (CP)-Based Devices

These devices are based on the integration of sensor technologies with modern communication systems to capture images of the target, followed by comparison with images of diseased plants already stored. The data thus generated with these devices could be easily transmissible from one location to another. Similarly, some applications developed for crop plants aid in detection of pathogens based on colour intensity, texture, edge outline and number of spots of the infected leaves. The Google app, Plantix was developed by an AgTech startup which contains more than 55,000 images of plants and could able to successfully detect over 60 plant diseases (http://www.fao.org/e-agriculture/news/plantix-app-detect-and-cure-your-plantdiseases). These CP-based methods need further refinement and optimization prior to their acceptance in plant virus diagnostics to substitute molecular and serological-based techniques, particularly in terms of accuracy (Vashist et al. 2015).

5.3 Conclusion

Diagnostics is the basis for any disease management programme and plant virus diagnostics is gaining significance, due to rapid spread and identification of novel plant viruses and to facilitate enforcement of quarantine measures. The detection and diagnostic methods mostly include a number of immunological and molecular techniques based on intrinsic virus properties. Developments of isothermal amplification methods are gaining high acceptance owing for their portability and appropriateness for resource crunch laboratories. Additionally, they also show sensitivity, cost-effective, rapid and robust methodologies to support onsite detection of plant pathogens. Point of care detection techniques are comparatively simpler, can be performed by non-expert persons and need little handling. However, the major challenge for the development of onsite technologies remains cost-effectiveness and affordability.

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6 Epidemiology and Management of Potato Virus Y

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Abstract

Potato virus Y (PVY) is an economically important disease agent in potatoes with worldwide distribution. It is mainly transmitted vegetatively by tubers and between plants by an aphid vector. PVY management is primarily through reducing viral inoculum in seed tubers and other sources and controlling transmission by aphids. Seed certification programs are designed to comprehensively test and restrict levels of PVY within a region, enforcing low levels of PVY in marketed seed lots and allowing growers informed choice to plant low-PVY crops. Sanitation techniques on the farm can also reduce volunteer, weed, and neighboring field sources of PVY. Major techniques to reduce aphid-mediated PVY spread include mineral oil foliar sprays, especially combined with insecticides. Spray timing is important, starting early after first plants emerge, continuing weekly through season to vine-kill, with additional sprays during rapid growth and periods of aphid abundance. Other practices for PVY reduction include avoiding mechanical transmission by field equipment, planting crop borders or intercropping as a barrier or sink for PVY, breeding PVY-resistant potato varieties, and roguing symptomatic plants from fields. Major remaining challenges in combatting PVY are management complacency, proliferation of PVY strains circumventing resistance and roguing, and informal trade of untested potatoes.

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PVY · Foliar spray · Seed certification · Crop management · Crop protection · PVY strains · Mechanical transmission · Aphids

6.1 Introduction

Potato virus Y (PVY; genus *Potyvirus*, family Potyviridae) is a viral pathogen of potato crops that causes significant economic impact in all major potato-producing regions. Potato is the fourth largest staple food crop in the world, behind corn, wheat, and rice, and the largest non-grain crop. PVY is considered the most important virus of potatoes because of its effect on potato yield and quality and because of its worldwide distribution. Economic costs associated with PVY are not only from reductions in yield and tuber quality, however, but also through the substantial costs incurred from in-field management, potato seed certification, and breeding programs to develop tolerant and resistant varieties (Lacomme and Jacquot 2017). Though primarily a threat to potato crops, it can also impact other important crops such as tomato, tobacco, pepper, and other solanaceous species (Karasev and Gray 2013a).

PVY is a positive-sense single-stranded RNA virus with a genome of ca. 9700 nucleotides (Singh and Singh 1996), encoding ten multifunctional proteins. It causes a mosaic disease in potato, characterized by a mottled yellow-green coloration of the foliage and often accompanied by wrinkling and stunting of the leaves and reduced growth vigor of the whole plant. PVY is primarily spread by an aphid vector, which acquires and transmits the virus during feeding and flights between plants, and secondarily by residing dormant in overwintering tubers and emerging in subsequent plants grown from these tubers.

PVY is thought to have originated and co-evolved with wild potato species in the Andes region of South America and was transported around the world subsequent to European colonization and adoption of potato as a food crop (Bellstedt et al. 2017). Differences in symptomology, particularly evident in different host species, lead to the recognition of different strains of PVY in the early twentieth century. Today, PVY occurs as a complex of many strains originating from mutation and genetic recombination, which have diversified rapidly around the world in recent decades, continuing to this day (Karasev and Gray 2013b; Green et al. 2018). Currently, the main strains infecting crops in major potato-producing regions exhibit varying symptomology and yield effects, complicating management schemes designed to control PVY.

Despite considerable efforts to control its spread, PVY remains an ongoing and evolving threat needing vigilant, continuous management (Gray et al. 2010). Management practices also must evolve in step with the changing challenges associated with PVY, being reassessed on an ongoing basis to best cope with continuing changes in PVY strain evolution, varietal selection of producers, and changes in environment and aphid vectors through time (Davidson et al. 2013). This chapter represents a brief overview of the epidemiology of PVY, peculiarities of its biology

and recent evolution, which present challenges to potato producers, and reviews diverse management practices with examples of current research to minimize its spread in the field.

6.2 Epidemiology of PVY

6.2.1 Sources and Modes of PVY Transmission

6.2.1.1 Vertical Transmission and Sources of PVY in the Field

Generally, potato is a vegetatively propagated food plant, with the vast majority of potato crops being planted with seed tubers harvested from field-grown plants exposed to PVY in previous growing seasons. Relatively few potato plants are grown from true potato seed, tissue culture, or lab-grown tubers unexposed to PVY. PVY cannot be transmitted in pollen or through true potato seed, and phytosanitary procedures can eliminate it from tissue culture; thus in the laboratory and through breeding programs, potato plantlets and mini-tubers can be produced free from PVY (Lacomme et al. 2017). Vegetative production of potentially infected tubers dominates the seed trade, however, both to maintain varietal characteristics of the crop over the seasons and because of the required scale necessary to supply the seed requirements of industry (Frost et al. 2013).

PVY travels systemically in the plant following infection; thus tubers developing from infected plants can also harbor PVY and carry the virus into subsequently planted crops – termed "vertical PVY transmission". Not all tubers of an aphid- or mechanically infected plant (called "primary infection") necessarily develop PVY, though plants grown from infected tubers will necessarily carry that infection throughout the daughter plant (called "secondary infection") (Nolte et al. 2004). PVY burden of harvested seed lots destined for the seed trade can be readily quantified by molecular testing or winter grow-out assay of a sample of the harvest. As this seed is used to plant subsequent crops, it is the most direct source of PVY inoculum in the crop and of greatest interest to potato producers. Thus, management of vertical transmission of PVY in the seed trade is the focus of strict regulation in seed certification programs, which are discussed further in Sect. 6.3.1.2.

Seed tubers, however, are not the only source of PVY in the field. Volunteers, unintended potato plants growing from tubers not harvested or destroyed by cultivation or overwintering in the field from a previous crop, can be a significant source of PVY (Jones et al. 1996). Also, neighboring fields with different, more susceptible, or PVY-tolerant varieties, or ware crops not requiring low PVY for certification, can represent PVY inoculum that can be transported into more valuable low-PVY seed crops by flying aphid vectors (Dupuis et al. 2017).

PVY also has a broad enough host range to include several weed species frequently found in or around potato fields in Eurasia and the Americas, including solanaceous weeds such as several species of nightshade (*Solanum sarrachoides*, *S. nigrum,* and *S. dulcamara*) and *Physalis floridana,* and non-solanaceous weeds like lamb's quarters (*Chenopodium album*; Amaranthaceae), stork's bill and crane's

bill (*Erodium cicutarium*, *Geranium pusillum*; Geraniaciae), milk thistle (*Lactuca serriola*; Asteriaceae), and purple deadnettle (*Lamium purpureum*; Lamiaceae), among others (Kaliciak and Syller 2009; Cervantes and Alvarez 2011; Nanayakkara et al. 2012). Indeed, no fewer than 43 non-crop weed species have been identified as hosts for PVY (Dupuis et al. 2017). Combined with potential threats from neighboring PVY-susceptible crops such as tobacco, pepper, tomato, and other potatoes, these weed reservoirs may represent a source of PVY inoculum that could be transported into the field by aphid flights. The actual impact of weed reservoirs of PVY in the field, however, has not been conclusively established (Gray and Power 2018). PVY is likely eliminated yearly with the death of annual weeds (i.e., it is self-limiting) as it has not been observed to transmit vertically via seeds, and the efficiency of aphids to transmit PVY from weeds to potato in the field is unknown and limited to a few lab-based demonstrations (e.g., Cervantes and Alvarez 2011). If weeds are present within the field, preemergent herbicides can be applied to eliminate early weeds and volunteer potatoes before the emergence of the planted potato crop, and regular cultivation and later row closure by the crop would reduce the threat of PVY in later growing in-field weeds.

6.2.1.2 Plant-to-Plant Transmission of PVY by Aphids

PVY is primarily transmitted between plants by an aphid vector. Aphids acquire PVY during probing of the plant epidermis as part of its feeding activities. PVY is drawn into the stylet of the aphid and is retained through complex interactions between the coat protein and the helper-CP protein of the virus and proteins in the acrostyle near the tip of the aphid stylet (Valli et al. 2018). PVY transmission by aphids is considered "nonpersistent." Once acquired, viruliferous aphids only remain able to infect plants briefly (minutes to hours) and to only a few other plants, as the virus is quickly eliminated from the aphid stylet after leaving an infected plant (Pirone and Perry 2002).

This mechanism of acquisition and binding of the virus to the aphid stylet is not species specific, as viral particles can be acquired this way from a number of host plants of PVY and by a wide range of aphid species. Indeed, no fewer than 65 species of field-collected aphids, including both known potato-colonizing and non-colonizing aphids, have been found naturally able to carry PVY in their stylets (Pelletier et al. 2012).While many aphids are capable of transmitting PVY, they do so with measurably different efficiencies. The "type species" widely considered most efficient at PVY transmission is the potato-colonizing green peach or peach-potato aphid (*Myzus persicae*), which is also used to calculate the relative efficiency factor (REF) for PVY transmission of other aphid species. Many common aphid species have had REFs calculated for several common strains of PVY (Halbert et al. 2003; Verbeek et al. 2010), and these REFs can be used in combination with aphid monitoring programs to calculate near-real-time cumulative vector pressure information in a potato-producing region (Fenton et al. 2012).

Given the importance of aphids in the spread of PVY, several regional efforts have been undertaken to quantitatively link aphid flights to PVY in commercial potato fields. Aphid abundance was strongly correlated to local PVY spread when

counted weekly from pan traps placed in study fields (MacKenzie et al. 2014) or at more regional scales using aphid abundance data collected and published by government sources (MacKenzie et al. 2016). Similar modeling studies have been used as the basis for designing regional notification services in a number of countries. For example, the TuberPro models produced since the 1990s in Switzerland employed regional enumeration of a number of aphid species with known REFs regularly during the growing season and forecasted a PVY risk level communicated to growers in a weekly newsletter. This model, however, has been simplified considerably more recently, to produce more realistically reliable forecasts (Steinger et al. 2015). The ambitious European EXAMINE program spearheaded by the National Federation of Seed Potato Growers of France has also worked on forecasting PVY risk originally through aphid monitoring, but more recently with additional data inputs relating to climate and geography. While useful as an alert service to growers, it has been recognized that the ultimate goal of accurate prediction of post-harvest PVY would be very complex and require more specific data on the planted inoculum and field-scale knowledge of the cultural practices of the growers (Lacomme et al. 2017). There have been challenges maintaining the applicability of these notification services continuously over time, due to more fundamental biological causes as well as bureaucratic reasons of funding and personnel (Radcliffe et al. 2008). Changes over time in aphid species assemblages and expertise in identifying them, types or locations of traps used to capture aphids, new or revised REF values of aphid species, weather-related differences in activity levels across aphid species, or changes in populations of PVY strains (with their varying intrinsic rates of spread) can all affect model predictions of cumulative vector pressure or PVY spread based upon aphid abundance measurements. Many of these factors, and case studies of different aphid monitoring programs around the world, are reviewed in Lacomme et al. (2017).

6.2.1.3 Mechanical Transmission of PVY

Relatively little is known about the mechanical transmission of PVY in the field. "Mechanical transmission" generally includes any artificial means of transmitting PVY between plants through infected plant sap on equipment, such as plant-to-plant transmission by tractor or other traffic in the field or transmission between tubers during seed cutting or handling. PVY is known to remain viable in infected plant sap on plastic, metal, and rubber surfaces for up to 2 days and in fresh water at least 1 week, and artificial transmission through sap transfer or abrasion between plant vines occurs readily (Mehle et al. 2014; Coutts and Jones 2015; Fageria et al. 2015). Recent experimental results show a very large increase in PVY transmission in commercial potato fields under normal industry management in New Brunswick (NB), Canada (MacKenzie et al. 2018a). In this study, replicated over six fields in two crop seasons, known PVY-infected tubers of three major PVY strains (PVY^{O}, $PVY^{N:O}$, and PVY^{NTN}) were planted in sprayer track rows where plants were routinely crushed, abraded, or otherwise disturbed from repeated tractor traffic through the season, compared to nearby control rows without such traffic. Subsequent PVY spread from planted inoculum tubers was two to seven times

greater (majority being PVY^{NTN}) in the tractor traffic rows than in the control rows, and the spatial distribution – in terms of greater along-row distance from inoculum to new infection and a significant frequency of infection closely matching the circumference of the growers' tractor tires – strongly suggested that the tractor equipment was transporting PVY through the field. An earlier in-field study in the same potato-producing region did not show convincing evidence of heightened PVY transmission, however, but this was solely based on a lack of an expected spatial patterning of PVY-infected plants observed in fields with natural assemblages of PVY (Sturz et al. 2000).

Handling of infected seed tubers, particularly cutting tubers prior to planting, is a concern in the industry for multiplying PVY inoculum when planting the field. Several studies, however, show that cutting known-infected seed tubers did not transmit PVY to subsequently cut PVY-free tubers at any measurable rate (Sturz et al. 2000; Fageria et al. 2015). This includes when tubers were infected with novel recombinant PVY strains that show evidence of greater rates of plant-to-plant spread via aphids (Fageria et al. 2015). These several studies, however, only investigated the potential for transmission from tuber cutting in three potato varieties, and it remains possible that more PVY-susceptible varieties could show some significant rate of transmission through this little studied route.

6.2.2 Biotic and Abiotic Effects on Rate of PVY Spread

6.2.2.1 PVY Strains Spread at Different Rates

PVY exists as a complex of at least 36 strains distinguished by genome sequencing, including the nonrecombinant parental strains PVY^{O}, PVY^{C}, and PVY^{N} first differentiated about a century ago based on symptomological effects on host plants, and many recombinant strains containing genomes consisting of various mixed sections of the parental strains that have evolved more recently (Green et al. 2018). Many strains, including the parental PVY^{C} and PVY^{N}, are now only seen rarely in some localities, while the recombinant PVY^{NTN}, $PVY^{N\text{-}Wi}$, and $PVY^{N:O}$ strains and to a far lesser extent the nonrecombinant PVY^{O} are more common in most potato-growing regions currently. This diversification of PVY strains is of global importance to the potato industry, because these novel strains exhibit different – often cryptic – symptoms than more traditional strains like PVY^{O} with which industry has long experience. These new strains also have different capacity to spread, including evasion of traditional resistance factors in some varieties, and in particular PVY^{NTN} can produce damaging tuber necrosis (Karasev and Gray 2013b; Gray and Power 2018; MacKenzie et al. 2019). Strain populations vary with locality and change over time, though often several strains co-occur within defined potato-growing regions and even within the same crop field. Examples of recently changing populations of major strains in western and eastern Canada and the USA are shown in Fig. 6.1. There are several proposed mechanisms likely responsible for explaining the proliferation of these novel recombinant strains over the original nonrecombinant strains, including intrinsically greater rates of plant-to-plant transmission

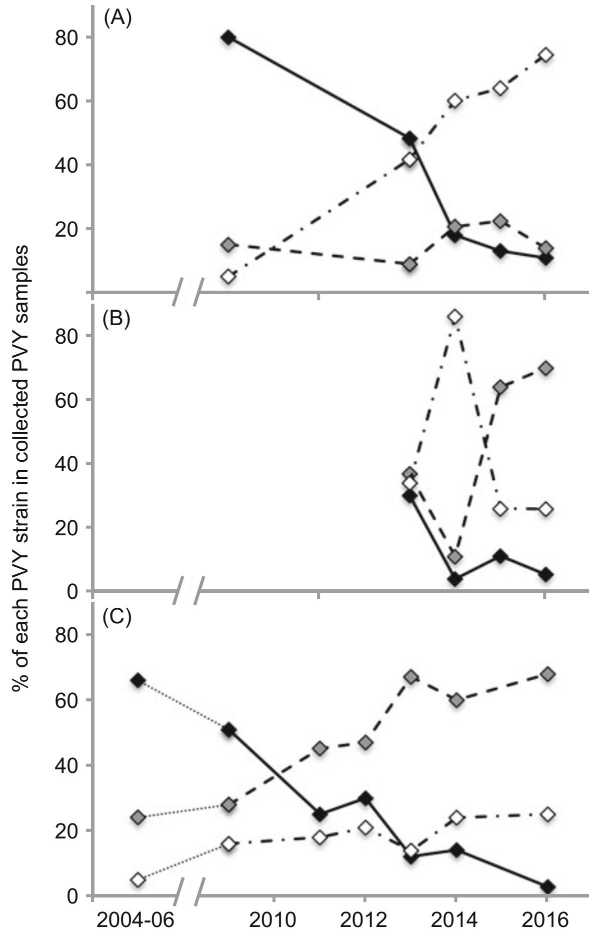

Fig. 6.1 Changing populations of PVY strains in (**a**) eastern Canada, (**b**) western Canada and (**c**) the USA (predominantly northwestern states). PVY strains are indicated as PVY^{O} (solid line, black symbol), $PVY^{N:O/N\text{-}Wi}$ (dashed line, gray symbol), and PVY^{NTN} (alternating line, white symbol) (Data in [a] and [b] adapted from MacKenzie et al. 2019 and Nanayakkara et al. 2012; [c] from Gray and Power 2018, with permission)

(MacKenzie et al. 2018b; Funke et al. 2017), failure to identify and rogue them from fields due to more cryptic symptoms in foliage (MacKenzie et al. 2019), and selective resistance of some potato varieties for older strains (Gray and Power 2018). These proposed mechanisms explaining their rise to prominence are also the primary issues complicating management of these novel PVY strains.

In a specific example comparing spread of several PVY strains, the authors conducted a series of experimental trials in NB and Manitoba, Canada, testing different management strategies for PVY, as well as simultaneously studying spread of three locally important PVY strains, the traditionally prevalent PVY^{O} and novel recombinant $PVY^{N:O}$ and PVY^{NTN} strains. In five large, replicated experimental trials, MacKenzie et al. (2018b) showed that on average the recombinant strains spread more effectively than PVY^{O}. These differences in virus spread, expressed as the spread of PVY strains to harvested tubers of initially virus-free plants in a field inoculated with a known number and strain identity of infected tubers, were variety

specific, however. The rate of spread of PVY$^{N:O}$ and PVY^{NTN} in cv. Goldrush trials averaged 2.5 and 7.7 times greater than PVY^{O}, though only 3.1 times greater in PVY^{NTN} and slightly less spread (0.8 times) of PVY$^{N:O}$ in cv. Russet Burbank crops. Varietal differences in relative spread of PVY strains may be due to differences in selective resistance to PVY infection, i.e., hypersensitive resistance (HR) in Goldrush against PVY^{O} infection.

Likelihood of mechanical transmission of PVY between plants also seems to be strain-dependent. In a field study of mechanical versus aphid transmission of PVY (MacKenzie et al. 2018a), the ratios of transmission of PVY^{O}, PVY$^{N:O}$, and PVY^{NTN} strains were consistent across six trial fields regardless of potato variety, and most importantly those ratios were nearly the same whether measured in high-transmission tractor traffic rows (mechanically dominated) or control rows far from tractor traffic (aphid-dominated). These results suggest the susceptibility of the recipient plant to infection is likely the selective agent for differential spread of the strains, not selection by the aphid vector or during injury or transport of the virus ex vivo on farm equipment.

6.2.2.2 PVY Resistance in Potato Varieties

A range of PVY-resistant potato varieties exist and can generally be categorized into three forms: extreme resistance (ER) which do not allow replication of PVY within the plant, hypersensitive resistance (HR) in which newly infected tissues experience rapid cell death to halt systemic spread of the virus, and PVY tolerance, in which plants show relatively little symptomological or yield-affecting impacts despite PVY infection and replication (Dupuis et al. 2017). Only the former two are truly resistant to PVY and can halt the replication of the virus in the field, whereas tolerant varieties allow continued proliferation of PVY despite it having little impact on the crop of that variety. Ideally for disease management, a critical density of truly resistant varieties could be planted in a region to reduce the available hosts for PVY over a number of years and ultimately eradicate it in a process similar to vaccination herd immunity and isolation in human viral diseases. However, the availability of resistant varieties with other commercially competitive characteristics is limited. Particularly in North America, breeding of commercially successful tolerant varieties like Russet Norkotah and Red LaSoda has allowed continued virus spread without consequence for growers of these varieties (Gray et al. 2010), or focus has been on intensive PVY management in fields of high-yielding but susceptible varieties like Russet Burbank. Thus far, market pressures and possibly grower familiarity seem to support continued use of commercially successful but nonresistant varieties, over the slow, costly development of potentially less economically competitive resistant varieties.

The genetics of HR- and ER-type resistance in potato varieties has been well studied, and its specificity for different PVY strains has also enjoyed considerable study. Many varieties show complex viral strain-specific PVY resistance, complicating their utility in industry. For example, the varieties Alturas, Umatilla Russet, and Ranger Russet show HR resistance slowing transmission of PVY^{O} in a greenhouse experiment, but the recombinant strains PVY$^{N\text{-}Wi}$ and PVY^{NTN} evaded

the HR response and were transmitted at two to three times greater rate (Funke et al. 2017). With current changes in PVY strain populations and availabilities of resistant potato varieties in different production regions, careful variety selection, crop management, and PVY testing are necessary to successfully exploit PVY resistance in the field. A deeper review of the genetics of PVY resistance and characteristics of resistant varieties is a complex subject outside the scope of this chapter, but is well reviewed in Valkonen et al. (2017).

PVY translocation within the potato plant generally slows with age across potato varieties, a phenomenon termed "mature plant resistance" (Beemster 1972). Most important to industry is the degree of PVY translocation to progeny tubers after primary infection of plants. PVY translocation through the phloem sap to roots and later to developing tubers is rapid in the first ~4 weeks after plant emergence and then progressively slows to rates insufficient to produce viral particles and carry them to tubers from new PVY infections after 8–10 weeks of further plant growth (Dupuis 2017). The expression of mature plant resistance also seems to be PVY-strain dependent, with nonrecombinant PVY^{N} and recombinant PVY^{NTN} and $PVY^{N\text{-}Wi}$ translocating to tubers more effectively than PVY^{O} after infection of older plants (Basky and Almási 2005; Dupuis 2017).

6.2.2.3 Abiotic Factors Affecting Spread

It is not uncommon that commercial growers contacted by this chapter's authors express opinions that PVY spread is largely under the control of weather and abundance of aphids. These weather and aphid factors are intimately related and not under control of the grower, and thus the pressure to expend the effort and costs of combatting PVY spread is lessened in a sense of resignation (M. Singh pers. obs.). In our own studies, however, weather is far less correlated with PVY spread than other factors directly under the control of the grower, such as planting low-PVY seed and managing in-field spread of PVY with foliar sprays (MacKenzie et al. 2014; MacKenzie et al. 2016). From these studies, the greatest climatological predictor of PVY spread was found to be the departure of previous winter temperatures from long-term average, with higher than normal winter temperatures associated with higher following-season PVY spread; temperature and precipitation values during the season, surprisingly, were less so or not correlated at all with PVY spread (MacKenzie et al. 2016). The link between warm winter temperatures and PVY is likely due to increased overwintering survival of aphids. Klueken et al. (2009) quantified specific temperature thresholds in spring conditions (e.g., 10–13 °C) under which particular aphid species would not fly from their winter host plants. Also, in studies of aphid flights in mountainous Switzerland, altitude and average wind speed of fields was strongly correlated with aphid abundance and could serve as a planning tool for growers deciding where to site valuable PVY-susceptible seed crops (Steinger et al. 2014). While not directly under the control of growers, however, information on previous winter conditions, prevailing temperature, and wind conditions or early forecasts nearing temperature thresholds for specific aphid activities would be known early enough to inform grower decisions on planting and in-field management of PVY at the beginning of the growing season.

6.3 Management of PVY

Many factors – biological, physical, and cultural – affect the spread of PVY in the potato crop, several of which described above that are under little control of the potato grower. There are, however, management practices available to effectively reduce the impact of PVY in the crop, which can generally be categorized into two approaches: (1) minimizing PVY inoculum planted within or from a neighboring field, and (2) reducing PVY spread during the growing season within the field.

6.3.1 PVY in Planted Seed and Seed Certification Programs

6.3.1.1 Efficacy of Reducing PVY in Planted Seed

The most effective source of PVY inoculum in the potato field is that which is planted into it with the potato seed. While there are several potential sources of PVY to infect a potato field, many of them can be managed and reduced. Infected volunteer potatoes and weeds can be reduced by tillage, herbicide application, and crop rotation, while impact of PVY from neighboring fields can be reduced by distance, vigilant scheduling of crops in nearby fields, and border crops. Strict testing and selection of low- to zero-PVY seed is the only way to reduce the amount of PVY imported to the field at planting time.

In a series of studies over the past decade observing commercial potato production fields and through conducting experimental plantings in NB, Canada, the strong and proportional influence of planted PVY on subsequent virus spread was clear (MacKenzie et al. 2014, 2016, 2017). Even when PVY was planted at relatively low and commercially acceptable levels (i.e., $<3\%$), the degree of resulting PVY spread through the field during the growing season was substantial and only partly mitigated by significant and costly application of foliar mineral oil and insecticide sprays after planting.

While a detailed cost-benefit analysis was not done as part of these studies, it was evident that the increased cost of sourcing lower PVY seed would be minor compared to the impact on the crop in terms of reducing PVY spread in seed production fields. These savings would not be primarily through marginal increases in yield with lower PVY in the field, but in the far greater reduction of risk of the field surpassing the strict regulatory caps on PVY to be rejected as a seed crop and reduced input costs of needed oil and insecticide sprays to manage PVY spread during the growing season. Processing and table stock fields, though, are not under the same pressure to maintain very low-PVY rates in the harvested crop, and the yield difference between, for example, 2% and 5% PVY at harvest may not justify the cost of sourcing very-low- to zero-PVY seed. Considerations other than minor yield reduction should be taken even in these crops, however, including the increased possibility of frequent tuber necrosis from the now-common PVY^{NTN} strain causing loss of the whole crop due to market rejection, or local buildup of PVY inoculum for growers or neighbors simultaneously growing processing and seed crops.

6.3.1.2 Seed Certification and PVY Distribution Across Seed Classes

Seed certification is a systematic program in regional potato industries to control aspects of the commercial seed trade, specifically variety purity, seed class, provenance, quality and labeling, and, of particular focus in this chapter, disease burden. Some aspects of organized potato seed certification have been in place in Europe and North America for about 200 years, and other potato-producing developed nations such as Australia and New Zealand for about a century (Dupuis et al. 2017). These programs are of great value to local potato producers and exporters, and the comprehensive third-party testing and monitoring of potato seed has allowed government-imposed and market-driven reductions and eradications of disease agents in many major production regions around the world. The economic return to growers participating in seed certification programs has also been assessed (Gildemacher et al. 2011) and has shown clearly that the output of high-yielding, high-quality, and low-disease potato crops from these programs is one of the most cost-effective strategies in seed potato management.

In terms of PVY management, seed certification traditionally relied on visual inspection in the field or from a winter "grow-out" of a portion of the harvested crop. More recently, however, molecular testing with ELISA or traditional or real-time RT-PCR has become standard. These molecular techniques have the advantage of allowing rapid and high volume testing, without the expense and time required to transport tubers and grow plants in distant warm regions during winter grow-out tests. Nor do they require expertise in recognizing symptomatic plants, especially with latent infections in tolerant potato varieties and cryptic infections with increasingly common recombinant PVY strains. Standardized, quantitative results from comprehensive PVY testing within a producing region thus allow identification and disposal of problem seed lots, predictable government regulation of disease limits, and market forces to reward production of clean potato seed.

A clear result of seed certification data is that PVY is not evenly distributed in local potato-producing regions, but instead it tends to be concentrated in a minority of problem seed lots, retained at higher levels in some varieties, and concentrated in later generation seed. This relatively small number of seed lots can thus be targeted for intensive management or elimination by ranking into identifiable categories such as seed class (generations grown in the field), potato variety, or growers' practices. Figure 6.2 shows the relative distribution of PVY incidences in seed lots from a 2008 to 2015 survey in a US seed certification program (from Gray and Power 2018). Quickly apparent is the stubborn stability of high PVY levels – though still within regulatory thresholds for planting – in a minority of seed lots (i.e., only 8% to 21% of tested seed lots contain PVY at >2% incidence). Calculated differently, however, the total impact of those relatively few high-PVY seed lots is more clear. Factoring together the number of seed lots and degree of PVY contamination, these relatively few poor seed lots carried about 50–70% of the annual burden of PVY industry-wide during these years. Identifying the underlying causes for these high-PVY seed lots and targeting them for special management attention would be a most cost-effective means to expend management effort at reducing PVY impact on a local industry scale.

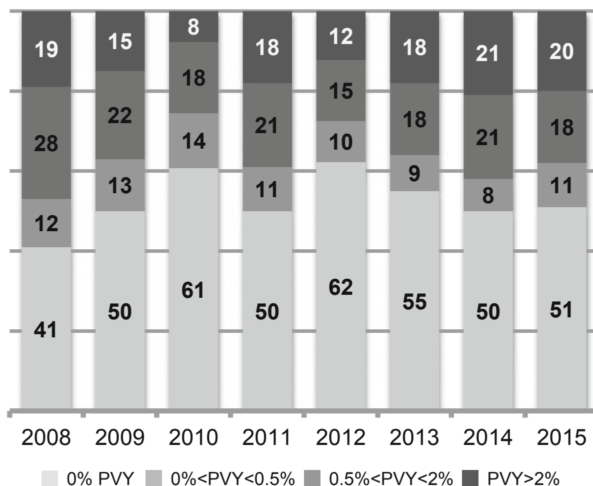

Fig. 6.2 Distribution of PVY in seed lots from a major potato-producing state of the USA. Values are percentages of lots tested in each indicated category of PVY contamination from all lots tested each year (Adapted from Gray and Power 2018, with permission)

The number of generations that a seed crop is grown in the field (seed class) determines the amount of exposure a crop has to PVY infection and spread. Though it varies with the background inoculum level, aphid pressure, and management of the crop, PVY incidence increases exponentially with seed class within a given region. In NB, Canada, despite a great overall reduction in PVY incidence averaged across the certified seed lots of the region over recent years, later generation seed still shows relatively high levels of PVY. As an example, 2018 seed certification in NB showed that E4 seed lots averaged 2.16% PVY compared to only 0.99% in all other seed classes (M. Singh, unpub.). Indeed, even though the number of lots and total acreage of this older-generation E4 seed is small, representing only 7.5% of 453 tested lots and 13.3% of seed acreage in 2018, the relatively high levels of PVY in those fields factored together with their acreage means that they contained 32% of the total PVY in the NB seed industry that year and are thus disproportionate contributing to the region's total PVY burden. Also, growers planting this seed class took on a significantly increased risk of failing certification, with nearly 14.7% of E4 seed lots surpassing the regulatory PVY cap to allow sale (4% PVY at harvest in 2018), compared to only 5.4% of lots in all other seed classes which failed. Though comprehensive PVY data do not exist for processing and table stock potato fields in the region, which represent about four times the acreage of the seed producers, the same buildup of PVY in the generations can be assumed to occur. Thus, especially if applied on an industry-wide scale, tighter grower marketing and acceptance or government-mandated restrictions on sale of later generation potato seed could remove a large component of the PVY from the industry at a minimum cost of lost potato production.

Lastly, results of seed certification can be used to identify particular growers within a region who produce atypically high-PVY seed. While singling out individual potato producers in a local industry could be controversial, the potential benefit to industry of focusing on such growers could be substantial. Two helpful approaches from such grower-focused data analyses would be (1) to identify specific

issues with their management practices and advise on practical ways of improving PVY management individually and (2) using a behavioral economics approach to "nudge" individual growers into more effective and competitive PVY management strategies. Identifying of PVY-problem fields, surveying management techniques, and tailoring industry-appropriate best management practices have proven very effective in the potato industry of NB, Canada (MacKenzie et al. 2016). Little information is available on practical behavioral economics approaches (i.e., "nudge theory," Thaler and Sunstein 2009) to disease management in the potato industry. However, these sort of "big data" approaches to identify and target individuals with specific messaging are increasingly and successfully being used in diverse fields, from affecting domestic energy usage (Sudarshan 2017), to increasing state tax compliance (Christian and Alm 2014), to reducing infectious and noncommunicable disease incidence in humans (Bond and Nolan 2011; Hansen et al. 2016). Such approaches could be easily and economically applied to managing PVY in a local industry with existing data from seed certification and marketing programs, such as informing growers of their relative performance or ranking in PVY control compared to anonymous neighboring competitors (social proof nudging) or personalized estimates of economic gain from better PVY control (economic messaging).

6.3.1.3 Informal Seed Trade as a PVY Source

Unregulated potato seed can also be a significant source of PVY inoculum and can originate from a wide range of sources described collectively as the informal seed trade. Examples of such are otherwise unmarketable seed crops planted in small personal gardens (i.e., "farm-saved" seed), small-batch seed sets sold at the retail level in the usually unregulated garden supply trade, or larger-scale unregulated trade more typical occurring in the developing world. In many developing countries, particularly in Africa, Asia, and South America, as much as 94% of potato seed is unregulated and of uncertain PVY status, because testing and seed certification infrastructure is not in place (Thomas-Sharma et al. 2016). Coupled with limited resources to manage PVY spread in these regions, PVY incidence and its consequences on yield and quality in the potato crop is often severe. However, because of the lack of certification programs in such regions, export of high-PVY seed tubers to low-PVY countries is generally blocked. Other than the obvious impacts on the local industry, one possible global concern of existence of such unregulated, high-PVY regions is that they may serve as incubators for rapid virus evolution and recombination, producing new PVY strains with novel characteristics that could increasingly challenge potato crop management worldwide.

On the smaller scale, farm-saved seed and the retail garden trade have been implicated in spread of PVY. A recent survey of potato seed available at garden centers in northwestern USA between 2016 and 2018 (Inglis et al. 2019) found widespread PVY contamination of retail-size sample batches (26% to 47% over the three annual surveys). PVY was present as PVY^{O}, $PVY^{N\text{-}Wi}$, and PVY^{NTN} strains or as mixed infections of multiple strains. Grown-out samples from these retail batches exhibited significant tuber quality deficits, particularly growth cracks highly

associated with $PVY^{N\text{-}Wi}$. While retail of potato seed at any commercial level could be regulated with the same strict seed certification standards as applied to large-scale industry, discouraging farm-saved seed or other informal noncommercial trade may need more creative approaches and local education efforts. As an example, in a high-value seed production area outside the main potato production region of NB, Canada, the local industrial producer freely provides small quantities of high-quality PVY-free seed potatoes to local residents with garden plots over a generous surrounding area (M. Singh pers. obs.). This service, at moderate cost, provides quality potatoes to residents and engenders local engagement, goodwill, and education while ensuring a substantial PVY-free buffer zone around the sensitive industrial production fields.

6.3.2 Management Factors Effective for Reducing In-Field PVY Spread

Once PVY inoculum is identified in or nearby the potato field, there are several management approaches to minimize virus spread in the crop during the growing season. Generally, these can be categorized into practices to directly combat transmission of PVY between plants by aphids, managing timing of crop planting, harvesting, and spraying to reduce unprotected exposure to aphid vectors, resistant variety selection, and other practices to reduce emergent PVY inoculum or spread.

6.3.2.1 Foliar Spraying of Mineral Oil and Insecticides Targeting Aphid Vectors of PVY

Regular foliar spraying of potato crops with agents to reduce plant-to-plant transmission of PVY during the growing season is a widely used practice to control in-field spread of the virus. Central to this strategy for the past half-century is the spraying of mineral oil-water emulsions onto growing plants to interfere with proper feeding or PVY acquisition by aphids (Bradley et al. 1966). Many studies have measured the effectiveness of mineral oils alone and in combination with other practices for reducing PVY spread (examples are Boiteau et al. 2009, Steinger et al. 2014, MacKenzie et al. 2016, 2017). The proposed mechanisms of action vary, including effects on retention of PVY in the aphid stylet, changes in aphid feeding behavior, priming of the natural defense mechanisms of the plant, and more. Many examples of the effect of mineral oils on PVY spread, and studies on these varied mechanisms of action, have been well reviewed elsewhere (Al-Mrabeh et al. 2010; Dupuis et al. 2017).

More controversial, the spraying of foliar contact insecticides targeting aphids has received considerable attention in recent years. This practice is questioned because of inconsistent early studies investigating the efficacy of foliar spraying of insecticides and the widespread opinion that insecticides act too slowly compared to the mere seconds required for an aphid to probe a susceptible plant with its stylet mouthparts and infect the plant (Al-Mrabeh et al. 2010).

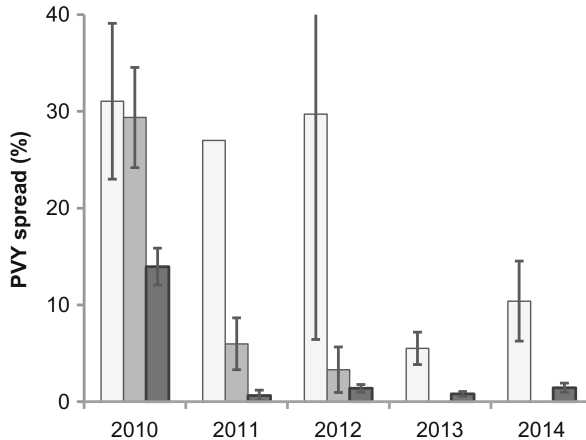

Fig. 6.3 In-field PVY spread grouped by foliar spray management strategy. Blank bars indicate fields with no foliar spraying of mineral oil or insecticides, light gray bars are fields with nine or fewer mineral oil and three or fewer insecticide sprays, and dark gray bars are fields with >9 mineral oil and >3insecticide sprays applied through the growing season. Values are means ± SEM from 56 study fields measured between 2010 and 2014 in NB, Canada (Adapted from MacKenzie et al. 2016, with permission)

Recent survey and observational studies and controlled experimental field trials by the authors' research group in NB, Canada, have produced substantial evidence supporting the use of mineral oils and specific insecticides in combination with mineral oil sprays. This Canadian province supports an intense, technically advanced local potato industry that produces the majority of agricultural revenues for the province; commercial growers here operate with significant government oversight, including a strict and comprehensive seed certification program and ongoing applied research to maintain and improve crop quality. From 2010 to 2014, an epidemiological study of PVY in commercial potato fields in the region was undertaken to elucidate the main factors associated with PVY spread and quantify the efficacy of management practices to control it locally. During the epidemiological study period, PVY spread was closely followed on 16 participating growers' farms, including 56 separate crop fields representing 13 locally important potato varieties (MacKenzie et al. 2016). The approach of this wide-ranging study was to select a range of experienced commercial growers and quantify on-farm PVY spread as a function of PVY inoculum level in the seed planted, aphid abundance and climate factors, and the effectiveness of in-field management practices, specifically the timing and number of foliar mineral oil and insecticide sprays. Through the years of this study, the growers of the region – including those participating in the study – were updated on the results of this and other local research and informed of developing best management practices. Over the course of the 5-year study, average PVY spread in the study fields dropped substantially, particularly in fields managed with the most intense foliar spraying programs; average PVY spread in these fields dropped nearly tenfold from 2010 to 2014 (Fig. 6.3).

The data gathered showed that not only the number of foliar spray applications but also timing and composition of sprays, as well as initial inoculum of PVY planted in the field, were correlated with in-field PVY spread (MacKenzie et al. 2016). Over the course of the study, growers who used approximately weekly simultaneous (tank-mixed) water emulsions of mineral oil and insecticide for most or all of their foliar spraying showed greatest reductions of in-field PVY spread. This degree of reduction in PVY spread was greater than with mineral oil-only sprays in other fields in the same season and increased over the years along with the industry-wide increase in use of simultaneous mineral oil-insecticide sprays. Later planting dates and shorter time intervals between planting and first spray application after crop emergence were also correlated with reduced PVY spread. Other factors, such as level of PVY inoculum in the planted seed, described in Sect. 6.3.1.1 above, and the abundance of aphids each year were unsurprisingly correlated with greater in-field spread of PVY.

6.3.2.2 Timing and Composition of Foliar Sprays to Maximize PVY Protection

Early application of foliar sprays to protect the emerging crop is critical, though in the local industry it has been difficult to convince growers of this necessity (M. Singh pers. obs.). Common opinion has been that prior to majority emergence of potato plants and significant plant growth, most sprayed mineral oil and insecticide is "wasted" as it lands on bare soil rather than plant leaves. Also, it is thought that if the potato crop is not substantially emerged, there is not a source of PVY for aphids to acquire and thus transmit the virus, and retrospective aphid counts from pan traps necessarily cannot alert growers to early aphid flights until they are counted (weekly starting in mid-June in this region). Given this information, potato crops are thus often left unprotected at a critical early stage, when aphid flights typically peak in this region, long before any mature plant resistance to PVY develops in the plant, and when winged aphids trapped in potato fields have been demonstrated to carry PVY in their stylets (Fig. 6.4, Pelletier et al. 2012). It is critical that this interval of time in which plants are unprotected from PVY-carrying aphids, from emergence to initiation of regular foliar spraying, is as short as possible – in our recommendations beginning at approximately 30% crop emergence.

Careful focus on the timing of crop-protecting sprays should keep both (1) periods of maximal aphid flight activity, and (2) changes in the susceptibility of the crop to infection, in mind. For example, in these NB, Canada, studies, aphid activity was maximum early in the growing season, with abundance in a 2-week period spanning late June to early July being best correlated with PVY outcome at harvest. Coupled with generally maximal susceptibility prior to development of mature plant resistance, a rapid growth of new unprotected leaves between sprays, intense and frequent foliar spraying of the crop was warranted. Many growers using this approach relaxed the frequency of insecticides somewhat later on, when plants mature and aphid numbers lessened, without apparent compromise on PVY spread. From this and other research, increased attention to early frequent spraying due to the dynamics of mineral oil coverage in rapidly growing crops has been recommended

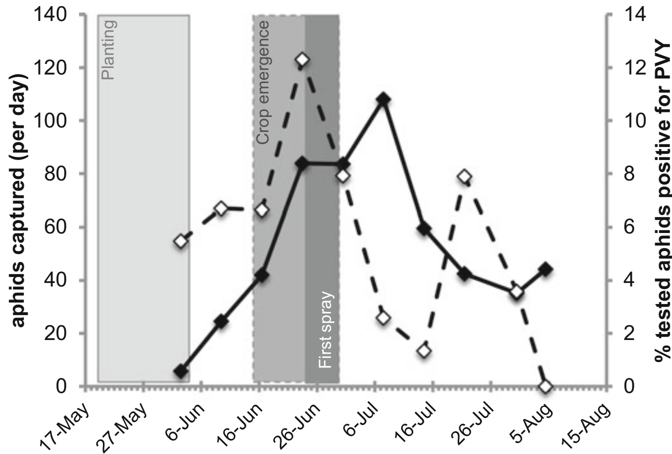

Fig. 6.4 Aphids captured from a network of yellow-bowl traps in the potato-growing region of NB, Canada, during the cropping season of 2010. Samples were collected approximately every 3 days, then averaged here into weekly values of total aphid abundance (filled symbols, solid line) and % of aphids tested by RT-PCR for presence of PVY in their mouthparts (open symbols, dashed line). Shaded regions indicate typical time periods locally for potato planting, range of time for emergence of the crop, and times of first foliar spraying of field; foliar spraying in this region usually continues at ~weekly intervals until late August/early September (Adapted from Pelletier et al. 2012, with permission)

and adopted in major potato-growing regions in North America (Fageria et al. 2014; MacKenzie et al. 2016) and Europe (Dupuis et al. 2017).

Late-season protection, however, also needs attention. In most years in NB, Canada, as an example, a second peak of aphid flights is measured in August well before vine-killing in potatoes (MacKenzie et al. 2016), which may be associated with local timing of grain harvesting. Also, while the most efficient known aphid vector of PVY, *M. persicae*, is relatively less common than other species in the region, it exclusively appears near season end and sometimes in very large numbers locally. Early vine-kill could avoid numerous, late, and effective vectors of PVY, as has been suggested elsewhere. Steinger et al. (2014) reported that under certain vector conditions at season end in their study fields, delaying vine-kill could increase likelihood of PVY infection by up to 3.5% *per day* of delay.

Changes with the types of insecticide chemistries used during the NB, Canada, studies were also associated with reduced PVY spread. Specifically, use of lambda-cyhalothrin (trade names Silencer®, Matador®) and flonicamid (Beleaf®) in sprays was strongly correlated with reduced PVY spread, while no significant correlation was found with many other insecticide chemistries. While controversy persists over the general efficacy of insecticides for reducing PVY spread, some particular classes of insecticides may show general utility as they have in our studies (MacKenzie et al. 2014, 2016, 2017). Synthetic pyrethroids as a group, including lambda-cyhalothrin,

have demonstrated a very rapid knockdown effect that has been shown elsewhere to slow aphid probing behavior (Collar et al. 1997) and reduce PVY spread by aphids (Perring et al. 1999; Boquel et al. 2015). Similarly, flonicamid has been demonstrated to produce a rapid anti-feedant behavior impact on aphids, which should slow acquisition of PVY from plants (Morita et al. 2007).

Another important consideration for using insecticides with specific and pharmacologically narrow mechanisms of action is the phenomenon of insecticide resistance in the aphid vector. Resistance mechanisms vary from genetic mutation of insecticide-targeted enzymes or their upregulation to overcome toxicity of the agent, increased metabolism of insecticide agents, or simple behavioral change to avoid exposure (Criniti et al. 2008). Surveying aphid DNA for known resistance mutations is the simplest approach to quantify heritable genetic insecticide resistance and has shown very high rates (up to beyond 75%) of genetically conferred resistance to pyrethroids in *M. persicae* and other aphid populations in limited surveys in North America (MacKenzie et al. 2018c) and the UK (Foster et al. 2014). Similar surveys have also found high population rates of mutations conferring resistance elsewhere around the world to this and other insecticides (e.g., Criniti et al. 2008; Slater et al. 2012). Other approaches to chemical control of aphids and PVY spread are through using synthetic analogues of pheromones to alter aphid behavior or elicitors which provoke natural defense responses in potato to reduce virus replication or translocation in the plant (Dupuis et al. 2017). The efficacy of these approaches has not been studied as well as more traditional chemical insecticides, though they may represent more environmentally benign treatments less likely to select for resistance in aphid populations than would specific insecticidal chemicals.

6.3.2.3 Modeling and Experimental Approaches to Elucidate Factors Associated with PVY Spread

Considering the multiple varying factors potentially affecting in-field PVY spread described above, several research groups including our own have attempted statistical modeling approaches to determine the significant factors associated with PVY spread, their relative effect size, and the efficacy of quantifiable management practices. Typically, a multiple logistic regression approach is used to model the likelihood of PVY infection during the season (or more simply the rate of PVY infection at harvest), a statistical approach commonly used in epidemiological studies (Hosmer and Lemeshow 2000) including in potato disease (e.g., Johnson et al. 1998; Martín-López et al. 2006). As part of the authors' research in the NB industry, a complex statistical modeling study was undertaken within our larger 5-year epidemiological study, to better quantify the interactions of various factors on PVY spread (MacKenzie et al. 2014). In-field PVY spread was very strongly related to PVY inoculum planted in the field, specifically to a compounded factor of planted PVY· Aphid abundance in the beginning of the growing season. Indeed, this PVY· Aphid interaction term representing the available virus inoculum together with early-season vector abundance was the single most significant factor determining in-field PVY spread in this modeling study. Clear from these studies was that the most effective, low-cost, and simple practice for reducing PVY spread in the NB potato

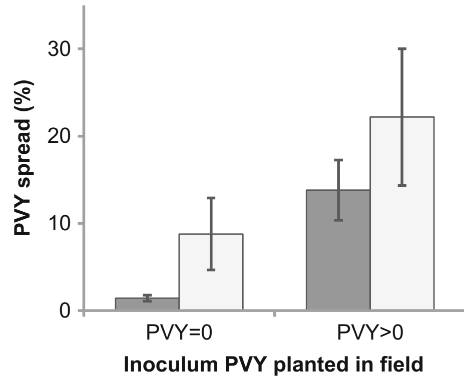

Fig. 6.5 In-field PVY spread as a function of initial PVY inoculum in the field and foliar spray management (fields with foliar spraying, gray columns; fields without spray, white columns). Values are means measured from 56 study fields in NB, Canada, 2010–2014 (Adapted from MacKenzie et al. 2016, with permission)

industry was to plant potato seed certified with low or zero PVY. While later labor-intensive and costly management of PVY spread was feasible, it was not as effective as simply planting low-PVY seed. Figure 6.5 shows this point clearly; over the 5-year study, fields with no detected PVY in seed averaged less PVY spread during the season *even without a foliar spray program*, than fields planted with >0% PVY in seed but managed with foliar sprays to reduce aphid-vectored PVY spread.

After accounting for planted PVY inoculum, the most statistically significant factor *reducing* PVY spread in the field was the number of combined mineral oil and insecticide sprays applied to the crop. Still significant, but of lower magnitude in effect, was the number of mineral oil-only (not simultaneous with insecticide) sprays. Interestingly, despite the wide range of mineral oil application rates, and opinions on it in the local industry, there was no statistically resolvable difference in PVY spread across the range of 2 to 5 L/hectare mineral oil application in each spray. Also worth consideration is that it may be safer to use more frequent, but lower areal rates of mineral oil spray rather than fewer more concentrated sprays to avoid potential phytotoxicity of the oils in the crop (Kirchner et al. 2014). As found more generally in the 5-year study, only insecticides of the lambda-cyahalothrin and flonicamid chemistries showed strong and significant reducing effect on PVY, while other chemistries (e.g., other older pyrethroids or organophosphates) did not. While the planting date and time from planting to first spraying significantly correlated with PVY spread over the larger and more varied data set of the 5-year study (MacKenzie et al. 2016), these could not be resolved into a statistically significant effect of quantifiable size in the smaller and more focused modeling study (MacKenzie et al. 2014).

From the early results of these observational epidemiological studies in cooperation with local industry partners, our research group also undertook large-scale experimental field trials. Among the more controversial findings of our studies within the operating industry (MacKenzie et al. 2014, 2016) was the apparent success of simultaneous mineral oil and insecticide foliar spray to reduce PVY spread. Thus our controlled and replicated field trials were primarily designed to rigorously test and compare the efficacy of regular foliar sprays of mineral oil-only,

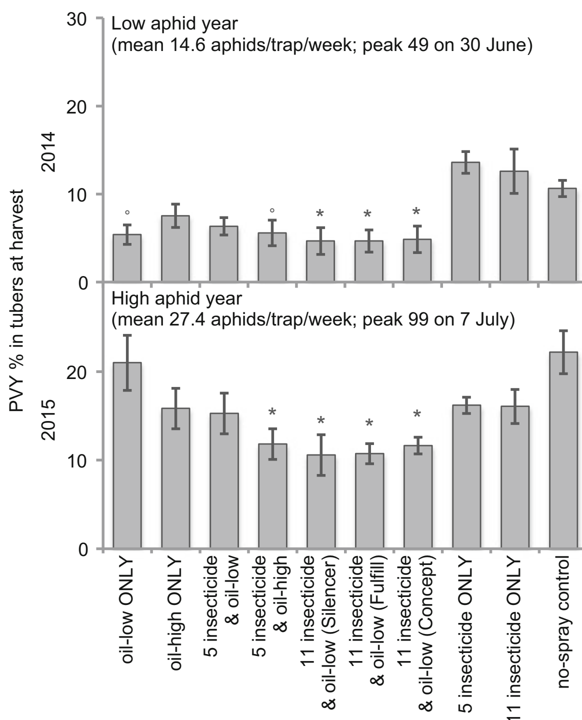

Fig. 6.6 PVY incidence in harvested tubers from field plots managed under ten different foliar spray treatments. Fields were treated identically, planted with PVY-free seed and hand-planted PVY inoculum tubers to 3% initial level, in relatively low- and high-aphid years (2014 and 2015, respectively) in NB, Canada. Values are means of PVY in 100 tubers from four replicate plots in each experiment; error is SEM, * indicates significant difference from no-spray control at $p < 0.05$, and ° indicates marginal significance at $0.05 < p < 0.1$ (Adapted from MacKenzie et al. 2017, with permission)

insecticide-only, and combined mineral oil and insecticide to reduce in-field PVY spread. These trials clearly concluded that frequent (weekly) mineral oil spraying can reduce PVY spread compared to unsprayed control plots and that combined mineral oil and insecticide sprays reduce PVY spread even further (Fig. 6.6). An additional important result to come from these trials was that insecticide-only sprays (without simultaneous application of mineral oil) had no significant effect on PVY spread in treatment plots. This result came to light only because the standard practice in the NB industry is to tank-mix mineral oil and insecticides to minimize labor in the field, and it may address some of the controversy in the literature about the effectiveness of insecticides in combatting PVY spread. One proposed mechanism to explain the synergistic effect of mineral oil and insecticide is that the oil-soluble insecticides may be carried into the leaf tissue after spraying, as these horticultural mineral oils have been shown to be rapidly absorbed into the interior of the leaf (Fageria et al. 2014), and thus be protected from loss due to washing by dew or rain or degradation from UV or air exposure. Another important result from these trials was that the degree of PVY protection by these foliar sprays was lower in a high-aphid than low-aphid pressure year (Fig. 6.6, comparing 2015, 2014); furthermore, when under higher aphid pressure, mineral oil-only spray treatments lost more of their protective function, which was retained in combined mineral oil and insecticide sprayed treatments. In a multiyear study in the UK, Dawson et al. (reported in Dupuis et al. 2017), however, found the opposite to be true, with mineral oil sprays

conferring more protection against PVY spread in relatively high vector pressure years; year-to-year changes in aphid species proportions were suspected to contribute to the variability in relative PVY reduction. With more study on aphid species-specific response to oil sprays, decisions on the intensity or frequency of such combined spray treatments will be better informed by timely information on relative abundance of aphid flights in the area, which are monitored and reported by local government agencies in many potato-producing regions.

6.3.2.4 Mid-Season PVY Testing as a Predictor of Crop Outcome

Testing individual potato crops in mid-season for measuring early PVY spread has a significant predictive power for estimating the ultimate outcome of PVY at harvest. As part of our survey (MacKenzie et al. 2016) and modeling studies (MacKenzie et al. 2014), a routine ELISA leaf test of PVY in ~100 plants was made from each study field in mid- to late July, about 1 month after full emergence. Increase in PVY from initial inoculum level to that mid-season test was strongly predictive of whether PVY spread would exceed the government regulatory threshold, which at that time was set at 5% PVY for sale of seed potatoes (Fig. 6.7). Across all fields, years, and varieties in this study, 86% of fields showing no increase from inoculum to mid-season (0% early PVY spread) remained at or below 5% spread at harvest and 63% of fields showing up to 1% early spread stayed below this level. Of fields showing higher early season spread, only 25% of fields showing 2% and no fields showing 3% or higher early PVY spread remained below 5% by the end of season (Fig.6.7c). This predictive capacity of a simple mid-season leaf test, available for <$200 and with data reporting within ~3 days of sampling, could be economical for growers to consider if producing a large and valuable seed lot.

Our modeling studies also showed that, in addition to knowledge of initial PVY level planted in the field and local aphid abundance, adding a mid-season PVY leaf test to models greatly increased the model's predictive power (correlation of predicted and actual PVY at harvest increased from $r^2 = 0.18$ to $r^2 = 0.86$ with addition of mid-season test, MacKenzie et al. 2014). Most commercially important, the rate of false negatives from these predictions, where the model predicted a field remaining below 5% PVY spread but it actually exceeded that regulatory threshold, fell from three of 19 modeled fields without the mid-season test to only a single false negative when mid-season PVY testing was added. Also important with this predictive modeling was that the impact of management, specifically the type and number of costly mineral oil and insecticide sprays, could be quantitatively assessed, allowing a powerful cost-benefit decision-making tool.

6.3.2.5 Roguing, Crop Borders, and Other Field Practices

Roguing is the inspection, identification, and removal of potato plants that are visually different from the majority of the crop to reduce disease inoculum in the field (Kerlan et al. 1987). Unusual looking plants may occur because they are a different variety or physiologically older (indicative of volunteers at greater disease risk) or show clear mosaic symptoms in leaves characteristic of PVY infection. Roguing, however, is very labor intensive, requiring walking rows and surveying all

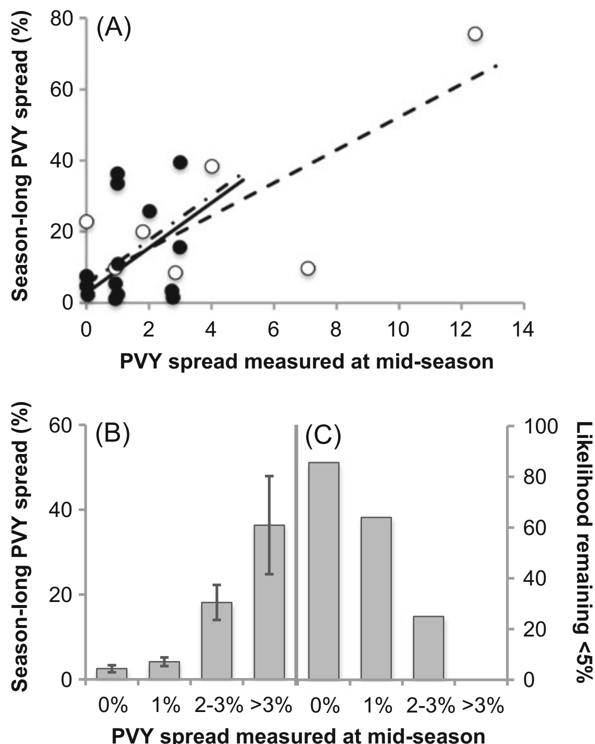

Fig. 6.7 In-field PVY spread predicted by mid-season ELISA leaf test of PVY in 56 study fields in NB, Canada, 2010–2014. (**a**) Correlations of season-long PVY spread with mid-season test in fields managed with foliar spray (filled symbols, solid line) and unsprayed fields (open symbols, dashed line). Alternating dashed line shows correlation of unsprayed fields matches that in sprayed fields almost precisely when two outlier fields >6% mid-season test are ignored. (**b**) Mean ± SEM season-long PVY spread as function of mid-season test in categories. (**c**) Likelihood that season-long PVY spread would remain below regulatory limit of 5% as a function of mid-season test result; values are % of all study fields in each category remaining within 5% PVY (Adapted from MacKenzie et al. 2016, with permission)

plants in the field, often multiple times per season, with personnel experienced in recognizing problem plants. In the field, different varieties show varying symptom expressions at different growth stages or time since PVY infection. As well, many varieties are cryptic when infected with some strains of PVY, particularly recombinant strains such as PVY^{NTN}, which are proliferating more rapidly in recent years in many potato-producing regions (MacKenzie et al. 2019; Karasev and Gray 2013b). Roguing may also cause disturbance of aphids during inspection of the crop, or high-contrast bare patches from removed plants serving to visually cue aphids, and thus may actually increase PVY spread in a crop (Dupuis et al. 2017). Thus, the efficacy and economics of roguing specifically for PVY management is increasingly

questionable and indeed may promote a false sense of security with some of the PVY-tolerant varieties or cryptic variety-strain combinations.

A number of other cultural practices have been investigated for reducing PVY spread into or within the potato crop, such as crop borders, mulching, or intercropping. Many of these approaches have been used in combination with other techniques to reduce PVY spread or have been employed to combat other disease organisms in addition to PVY (Dupuis et al. 2017). Crop borders use a narrow sacrificial crop surrounding a susceptible primary potato crop; it could be different crop such as a fast growing and tall grain or a tolerant or PVY-resistant potato variety that need not be harvested for marketing as seed. The border can serve as both a physical barrier and a virus sink, which can slow viruliferous aphids entering the field and physically clean viral particles from their mouthparts if it is a plant they would probe for feeding (Dupuis et al. 2017). Experimental studies have shown widely varying success rates at reducing PVY spread in bordered plots versus non-bordered ones. Relative reductions in PVY spread reached 27–60% with a very large (24 m wide) soybean border in a study by Difonzo et al. (1996). Boiteau et al. (2009), however, attained 20–60% reductions over 3 trial years with narrower (4 m) borders of grass or the resistant potato variety Kennebec, similar to the PVY-protective effect of regular mineral oil spraying without crop borders. Major considerations for using crop borders, which may limit their adoption by industry, are properly balancing the size and composition of a border to be sufficiently protective, yet not wasting valuable field area for regular production. Also important is the added management complexity of sourcing seed, maintaining and separate harvesting of both the primary and border crop, and care isolating tubers during harvest if an alternate potato variety is employed as a border.

Mulching, piling of dry plant materials such as straw from grain harvesting between potato rows, and intercropping, growing other crops between potato rows, have also been investigated for reducing PVY spread. Mulching is thought to reduce contrast and thus visual cues for aphids to land on potato plants and potentially infect them with PVY. In one multiyear study, relative reductions in PVY spread of 27–48% compared to non-mulched controls were realized (Saucke and Döring 2004). Similarly, intercropping may reduce visual cuing of aphids, but also may serve as a virus sink during aphid feeding, like crop borders. In a study comparing mineral oil spraying, straw mulching, intercropping with oats, and combined treatments (described in Dupuis et al. 2017), nearly 90% reduction in PVY was attained with combined mineral oil and intercropping treatments, about 70–80% reduction with all three combined or paired combined mineral oil and mulching, and somewhat less protection when each of the three were used alone. Several advantages of these techniques are that they are generally more environmentally friendly than more standard chemical foliar sprays, can help mitigate soil erosion, and add organic matter to field soil. However, the mulch may introduce other pathogenic organisms and requires a large source of grain straw or similar material early in the season and specialized equipment and labor to deploy. Intercropping also requires extra labor and equipment, cost of intercropping seed, and careful planning

of crop type, planting, and harvest of intercrop to minimize nutrient, light, or space competition with the potato crop.

6.4 Conclusions and Continuing Challenges

In recent decades, major advances have been made in understanding the evolutionary diversity, host interactions, and mechanisms of transmission of PVY, which have served the design of management practices to control PVY's impact in potato industries around the world. Despite this, PVY remains the most common virus in industrial potato production, causing a significant economic impact on these industries through crop yield and quality reduction and rejection of tubers from local and export markets.

Control of PVY has been presented with many new and continuing challenges, which remain important focal points for research and vigilant implementation in the field. These center on aspects of potato breeding, PVY detection and certification, in-field chemical control, continuous grower education and discipline, and the changing biology of PVY itself. Though several PVY-resistant varieties have been commercialized in recent decades, their often less-than-preferred performance, limited availability, or simple novelty in a sometimes conservative industry has limited their broad appeal in the market. Renewing efforts to produce high-yielding varieties resistant to all PVY strains while maintaining characteristics of locally important PVY-susceptible varieties should be prioritized, along with education and marketing efforts to encourage their uptake. Instituting seed certification programs in regions without them and more aggressive virus incidence targets under existing programs could rapidly force down PVY levels in the local seed supply. With the varying symptomologies of diverse PVY strains, molecular technologies are increasingly important for accurate assessment of PVY incidence in seed lots; many such tests (ELISA, RT-PCR) have been available for decades. A growing number of different molecular detection technologies, while some are relatively obscure and underutilized, may allow for faster, cheaper, and field-deployable testing to assist growers in rapid and informed decision-making during the growing season.

Particularly in Europe and North America, frequent insecticide limitations may restrict use of the few chemical controls which show some effectiveness at reducing aphid-mediated transmission. Also, mounting resistance in aphid populations to common insecticide chemistries like pyrethroids and neonicotinoids progressively reduces their effectiveness. Development and testing of novel, effective chemical controls should continue, particularly including biological agents that are more environmentally acceptable and less likely to generate resistance. Also, while many research groups like ours have developed locally relevant, economical, and effective best management practice recommendations to control PVY on-farm, these practices need to be validated in different regions, with different aphid species and abundances, potato varieties, climate, and labor and input cost structures. As well, in regions which have gained substantial control of PVY, continuous discipline in

monitoring, control, and engagement between growers, industry oversight bodies, and researchers is needed to prevent complacency and resurgence of the virus.

Finally, changes in the virus itself – namely, the rapid emergence and spread of novel recombinant PVY strains – present an extra layer of challenge and require all existing and future management practices to be reassessed in light of strain-specific responses of the virus. Vigilance surrounding the continued efficacy of management techniques, detection technologies and identification of PVY and its effects on the crop will be critical to coping with PVY at field, industry, and global scales into the future.

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7 Major Fungal French Bean Diseases: Epidemiology and Management

S. K. Gupta and Guervinder Singh

Abstract

French bean (*Phaseolus vulgaris* L.) is an important leguminous vegetable crop, grown throughout the world for its green pods as well as dry beans. During cultivation these crops are infected with several diseases of fungal, bacterial and viral nature which not only reduces the yield but also impair the quality of the produce. In this chapter, major fungal diseases like root rot and web blight (*Rhizoctonia solani*), angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*) and rust (*Uromyces appendiculatus*) infecting this crop are discussed in light of their epidemiology and management. The management practices include cultural, host resistance, biological and chemical control alone and their integration. It was also discussed how number of sprays of fungicides can be reduced by using epidemiological parameters.

Keywords

Phaseolus vulgaris · Fungal diseases · Epidemiology · Management

7.1 Introduction

French bean (*Phaseolus vulgaris* L.) is one of the most important leguminous vegetable crops grown throughout the world for its green pods as well as dry beans (rajmah). Various types of beans are grown throughout the world belonging

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to different genera of the family Leguminosae. The important members of broad group termed as beans are common bean or French bean (*Phaseolus vulgaris* L.), lima bean (*P. lunatus* L.), scarlet bean (*P. multiflorus* Wild.), tepary bean (*Mucuna* sp.), field bean or lablab (*Dolichos lablab*), sword bean (*Canavalia ensiformis* DC.) and cluster bean (*Cyamopsis tetragonoloba* (L.) DC.); these are grown as grain crops, as vegetables for green pods and as green manure crops for incorporating into the soil. It has gained increasing popularity due to its quality proteins and nutritional balance besides certain medicinal properties. The dry seeds are a rich source of proteins, calcium and iron and contain a large amount of Vitamin B1 (Stiebeling and Clark 1939). At present French bean is grown throughout the cooler tropics but not in the semi-arid or wet humid regions (Chatterjee and Bhattacharyya 1986).

French bean is a traditional crop of temperate region and it occupies an important position among various kharif vegetable crops and pulses grown in temperate hills of India at an altitude ranging from 900 to 2500 m above mean sea level (Sharma and Sohi 1989). In India, the crop is largely grown in Himachal Pradesh, Jammu and Kashmir, Uttarakhand, North Eastern Hills, Darjeeling, South Plateau, South Indian Hills (Nilgiri and Palni hills), Mahabaleshwar, Ratnagiri (Maharashtra) and Chickmangalore (Karnataka), having mild climate with humid environmental conditions. In India, it is cultivated in an area of 1,98,000 ha with a production of 2,012,000 MT (NHB 2017).

Successful cultivation of this crop is hindered due to the attack of various diseases of fungal, bacterial and viral in nature of which fungal diseases are very important. Under favourable environmental conditions, epiphytotics of these diseases have often reduced the yields considerably. Fungal diseases are either monocyclic or polycyclic in nature. Monocyclic diseases are mostly soil-borne diseases like wilts and root rots and they complete their one disease cycle in a year. In these diseases mostly disease incidence is recorded. In case of polycyclic diseases, the inoculum (s) is both soil- and air-borne and pathogen complete many cycles in a single season and can cause epidemic under favourable weather conditions. Examples are of leaf spots, rusts and anthracnose. In these diseases, disease severity is recorded in most of the cases.

The introduction of high yielding cultivars like pencil beans coupled with higher doses of fertilizers, assured irrigation and intensive agriculture has resulted in the higher susceptibility of the plants to diseases causing severe qualitative and quantitative losses thus making the produce less competitive in the national and international markets. These problems are further aggravated by the changing climatic conditions. A number of new diseases have appeared in different areas and many diseases which were of minor importance have become a major threat under this system. In favourable weather conditions, the cumulative losses have been estimated to 26–40% and even complete crop failure of the crop in certain cases which necessitate the role of plant protection. In this chapter, epidemiological parameters of major fungal diseases of French bean along with their management by including cultural, host resistance, biological and chemical methods alone and their integration have been discussed.

Epidemic is the "Change in disease intensity in a host population over time and space" whereas Epidemiology is the science of populations of pathogens in populations of host plants, and the diseases resulting there from under the influence of the environment and human interferences (Vander Plank 1963). Elements of epidemic are very well described by disease triangle.

Elements of epidemic are susceptible host, virulent pathogen and favourable environment. Interaction of these three components describes the disease development. Disease development is also affected by time and human activity. Each of these components affects the development of epidemic. Host affects the development of epidemic in many ways like genetic resistance or susceptibility, degree of genetic uniformity among host plants in a particular field, age of the host plants and types of crop (annual or perennial). Similarly, epidemics also depend on the virulence of the pathogen, type of reproduction like monocyclic, polycyclic or polyetic and also depend on the ecology and mode of spread of the pathogen. Environment plays an important role in the development of epidemic. Environmental factors like rain, dew, high humidity, temperature, wind velocity, etc. affect the diseases development and pathogen cycles. Time factors like season of the year, duration and frequency of favourable temperature and rain also affect the disease development. Human interferences also help in epidemic development in many ways like site selection and preparation, selection of the planting material, cultural practices followed and disease control measures used.

To study the epidemiology of a particular disease, one has to carry out two types of study: one is under control conditions, i.e. under glasshouse having controlled conditions and other under natural epiphytotic conditions. In glasshouse, the studies like the effect of temperature, humidity, leaf wetness and intermittent leaf wetness can be carried out. Under field conditions, mainly the role of environmental factors like temperature, relative humidity rainfall, wind speed, etc. are recorded under natural epiphytotic conditions. Disease severity of each interval is collected. Meteorological data is generally collected from the Meteorological sections of the University if the meteorological instruments have not been installed in the field where the experiment is laid out. Correlation and regression coefficients are calculated by following the procedures as described by Gomez and Gomez (1984) and the regression equation is developed. Besides this, infection rate (Vander Plank 1963) and area under disease progress curve (Shanner and Finney 1977) are also calculated.

Diurnal periodicity of spore release is generally conducted in the diseases like leaf spots and rusts where the inoculums are wind-borne. Studies on spores release in relation to climatic factors and diurnal periodicity of spore density in air are conducted in the experimental fields. For this purpose, the field air at the mid-height of crop canopy is periodically assessed for the presence of spores by a Burkard Air Sampler (Burkard Manufacturing Co. Ltd., Hertfordshire, England) which sucks in air at the rate of 10 l/min. The diurnal periodicity of the spore density in air is measured at every 4 h interval in the crop season for a week by counting the number of spores deposited on the slides. Simultaneously, the periodicity of

predominant weather parameters, *viz*., temperature, relative humidity and wind speed were also recorded and compared.

7.2 Epidemiology and Management of Major Fungal Diseases of French Bean

The information on epidemiology and management of important fungal diseases is given in this chapter.

7.2.1 Root Rot and Web Blight

This is an important disease of this crop world over. Root rot phase of this disease was first reported from New Jersey in the year 1924 (Cook 1924) while web blight was first described as a destructive disease of beans in Puerto Rico (Matz 1921). In India, root rot disease of French bean was recorded for the first time in 1980 from Bangalore (Sharma and Sohi 1981). Later, Paul and Sharma (1990) reported the occurrence of root rot from the Solan area of Himachal Pradesh while web blight was recorded during the 1994 crop season (Mathew and Gupta 1996) from the same locality. Rajnauth (1987) reported 25–100% yield losses in Trinidad due to this disease while whole bean crop has been destroyed within 48–72 h in Brazil (Sartorato 1988). In India, around 15% incidence of root rot in fields around Bangalore has been recorded (Sharma and Sohi 1981) while yield losses in green pods at different stages of plant growth varied from 8.4% to 64.6% (Sharma and Sohi 1980). The disease is caused by *Rhizoctonia solani* Kuhn. The basidial stage of the fungus (*Thanatephorus cucumeris*) also occurs but very rarely.

7.2.1.1 Symptoms

The disease occurs on roots, stem, leaves, pods and seed (Mathew and Gupta 1996). Sanchez and Cardenaz (1988) reported that root rot affects plant populations from emergence to the first 30 days of crop growth. The distinguishing symptoms of the disease occur in two phases and are described below:

Root Rot

Symptoms appear on roots and stem above and below the surface of the soil as reddish brown cankers. The lesions enlarge rapidly and engirdle the stem at the collar region, extending longitudinally downward to the roots. Roots are affected in advanced stages of infection leading to partial or complete rotting of the root system.

Web Blight

The symptoms on leaves appear as small, circular, water-soaked spots. These spots increase rapidly in size and cover extensive areas of the leaf blade. Initially, the spots are much lighter in colour than the surrounding unaffected area. At later stages, they become tan brown and surrounded by dark borders. The spots coalesce to form larger

areas on the leaf blade. Light tan hyphae develop on both surfaces of the infected leaf and at later stages are studded with minute sclerotia of the fungus. Defoliation occurs in advanced stages of infection. The pods are attacked at all stages of growth. On young pods, circular slimy water-soaked spots appear which are light tan in colour. On mature pods, the spots are dark brown, more or less circular, slightly zonate and definitely sunken. Spots coalesce to cover the entire pod. The distal end of the pods is the most affected. In favourable weather conditions, runner hyphae of the fungus are seen spreading from the point of infection to healthy parts, which later become studded with sclerotia. Tan brown to reddish brown discoloration is observed on infected seed, located immediately below the spots on the affected pods.

7.2.1.2 Epidemiology

The pathogen perennates as mycelium or sclerotia in the soil on plant debris. The germination of sclerotia gives rise to infective propagules, which are sufficient to cause infection. The fungus spreads with rain, irrigation or floodwater, tools and with infected or contaminated propagation material. The association of *R. solani* with bean seed has also been reported (Nitshe and Cafati 1985). Seed transmission up to 46% in cultivars like Kentucky Wonder and Contender (Gupta et al. 2000a) has been reported. Shama and Shama (1989) indicated that seed penetration of *R. solani* occurs directly through intact seed coat or through the hilum tracheids and then the pathogen is transferred from infected seed to the soil. The infected seeds after germination either rots in the soil itself or give rise to plants that later damped off. The soil-borne inoculum is splashed by rain to the foliage that causes web blight. On some plants or plant parts the pathogen can enter only through wounds or through openings in the epidermis while in others, it thrives in the soil or in mud splashed on to the plants until it can enter through healthy tissue. In favourable weather, runner hyphae of the pathogen are seen spreading from the point of infection to healthy parts, which later become studded with sclerotia.

Root rot is favoured by high soil temperature and soil moisture while web blight is more prevalent in the wet season. *Rhizoctonia* diseases of roots are most severe under moist soil conditions (Tu et al. 1992). Bruggen et al. (1986) reported a positive correlation between lesion size and soil moisture. If the moisture is sufficient enough or there is frequent drizzle, pronounced blight symptoms will appear. During humid periods fan-shaped whitish hyphae spread from the point of infection and produce the conspicuous and destructive phase of the disease. Diaz Plaza et al. (1991) reported that disease incidence and severity increased after rain. Continuous leaf wetness for at least 6 h was essential for disease initiation and increase in leaf wetness durations from 6 to 12 h showed a corresponding decrease in incubation period from 36 to 26 h whereas further increase in leaf wetness did not exert any effect on incubation period (Upmanyu and Gupta 2005). High rainfall and soil moisture coupled with high relative humidity and soil temperature (23–25 °C) were found to be favourable for web blight (Mathew and Gupta 1996) development. A high soil moisture (80%) and a temperature of 25 °C were the most favourable for root rot development, while web blight development was optimum at >85% relative humidity coupled with 25 °C temperature (Upmanyu and Gupta 2005).

7.2.1.3 Management

Since the causal fungus persists in the soil either as mycelium or sclerotia, therefore, an integrated approach of disease management combining the use of cultural, biological and chemical methods will be required to keep the disease in check.

Cultural Practices

Cultural practices, *viz.* planting disease-free seed, mulching, adjustment of planting date, medium (30–60 × 15 cm) plant spacing, crop rotation and soil solarization have been recommended to keep the disease under check (Sartarato et al. 1988; Tu 1992; Sharma and Gupta 2003). Deep tillage (30 cm sub-soiling) can reduce soil compaction and thereby, root rot severity in addition to increased yield (Tan and Tu 1995). Voland and Epstein (1994) reported the efficacy of fresh manure over composted manure in reducing bean hypocotyl lesions while soil amendment with cotton, mustard and neem cakes was found effective in reducing root rot incidence and increased yields (Upmanyu et al. 2002). The effectiveness of these amendments may be due to enhanced populations of non-parasitic microorganisms exhibiting antagonistic effects on the pathogen thereby inhibiting the mycelial growth and sclerotia production of the pathogen. Soil amendments with low soil C:N ratio increased incidence of *R. solani* whereas high C:N ratio amendment did not interfere with the incidence while the incidence was independent of soil moisture conditions (Fenille and Souzo 1999). Poultry manure, composted *Urtica* sp. and composted *Lantana camara* treatments were superior to other composts in suppressing Rhizoctonia root rot (Joshi et al. 2009).

Host Resistance

Prasad and Weigle (1976) observed that dark seeded PI accessions of French bean, *viz.* 109859, 163583, 174908, 226895 and 300665 were resistant to *R. solani* while most of the white seeded cultivars were susceptible to both seed infection and pre-emergence damping off. However, Sharma and Sohi (1981) reported all the three French bean cultivars, *viz.* “Contender”, “Premier” and “Giant Stringless” to be equally susceptible irrespective of their colour. Bush type cultivars/lines are more susceptible to the disease than pole type ones. The cultivars/lines, *viz.* Wisconsin RRR 46, BAT 477, BAT 332, BAT 1753, RIZ 30, EMP 81, A 300, ICA Pijao and Jackson Wonder have been reported to be highly resistant (Hagedorn and Rand 1980; Tu and Park 1993; Muyolo et al. 1993), while PLB-43, EC-18834, IC-18593, PI-249554, Phagu Chitra, Pole wall-I, Pole wall-II and K-13522 were found moderately susceptible to the disease (Gupta et al. 1997). Cvs./lines ET 8396 and IPR 96-4 were found resistant both under natural epiphytotic and in vitro conditions that can be used in breeding programmes as a source of resistance (Upmanyu et al. 2004; Khati and Hooda 2006).

Biological Control

Exploitation of the populations of bioagents may prove useful to combat the ravages of soil-borne diseases. Biological treatment of soil under glasshouse conditions with *Trichoderma viride* showed results similar to chemical control (Dumitras and Sesan

1990). Elad et al. (1980) found that in naturally infested soils, wheat bran preparations of *Trichoderma harzianum* significantly decreased the *Rhizoctonia* disease of beans. Tu and Vaartaja (1981) reported that *Gliocladium virens*, a hyperparasite of *R. solani*, inhibited production of sclerotia. Management of this disease by seed/soil treatment and foliar sprays with *T. harzianum, T. virens* and *T. viride* has also been reported (Mathew and Gupta 1998; Hazarika and Das 1998; Upmanyu et al. 2002). Inhibition of mycelial growth and sclerotial germination of this pathogen by *T. harzianum* and *T. viride* culture filtrate has also been observed (Hazarika and Das 1998). Roberti et al. (1993) indicated that *T. virde* produced non-volatile compounds while *T. harzianum* produced coils and hook pincer-shaped structures, which prevented the growth of *R. solani*. The efficacy of potential bacterial antagonists, *viz.* fluorescent pseudomonads and *Bacillus* spp. against root rot has also been reported (Sanchez et al. 1994; Wolk and Sarkar 1994, 1995). An endophytic *Pseudomonas florescens* carrying *Serratia marcescens* Chi A gene either on the plasmid or integrated into the chromosome controlled effectively the phytopathogenic fungus *Rhizoctonia solani* on bean seedlings under plant growth chamber conditions (Downing and Thomson 2000). Coinoculation of seed with *Bacillus subtilis* MB1600 (Epic) and *Rhizobium tropici* significantly reduced root rot severity and enhanced yield (Estevez-de-Jensen et al. 2002). Efficacy of seed treatment and soil application of *B. subtilis* in the reduction of pre- and post-emergence root rot have also been reported (Sharma and Gupta 2003). The water extracts obtained from green parts of potato, tomato and rape and *Trichoderma* PBG-1 showed marked antagonistic activity against *Rhizoctonia solani* (Smolinska and Kowalska 2006). Out of 60 isolates of *Trichoderma*, 7 isolates significantly reduced the incidence of root rot compared to the control. *T. harzianum* isolates Tr-34 and Tr-14 were the most effective, resulting in only 20.8% and 23.4% root-rot incidence, respectively (Joshi et al. 2008).

Chemical Control

Different fungicides have been reported to be effective for the management of this disease. Seed treatment with fungicides, *viz.* benomyl, captan, thiram, thiophanate-methyl, carbendazim, carboxin, terrachlor, pencycuron, iprodione + thiram (Raffat 1992; Maringoni et al. 1992) gave maximum protection against pre- and post-emergence mortality and also significantly increased the yield of green pods. However, seed treatment followed by foliar sprays with carbendazim or bitertanol gave best results against web blight (Thakur et al. 1991). Gupta et al. (1999) reported good efficacy of Bavistin (0.2%), Celest (0.1%), Topsin-M (0.2%) and Raxil (0.2%) against pre-emergence damping off of beans. Good control of web blight phase of this disease was achieved by seed treatment with Celest (0.2%) or Raxil (0.2%) or Bavistin (0.2%) followed by foliar sprays of Bavistin (0.05%) or tebuconazole (0.05%) (Mathew and Gupta 1996; Upmanyu et al. 2002).

Integrated Approach

Integration of seed treatment with bioagents and fungicides has been found to be more effective to manage soil-borne pathogens. Tu and Zheng (1993) studied the

compatibility of seed treatment with *Bacillus subtilis*, *Pseudomonas fluorescence* or *Gliocladium virens* and subsequently dressed with diazinon + captan + thiophanate-methyl (DCT). They observed that fungicide treated seed showed inhibition of bioagents, which was transient, and the growth and sporulation of the bioagents resumed as the effect of the fungicides diminished. They further suggested that bioagents such as *B. subtilis* and *P. fluorescence* are more mobile in the rhizosphere of seedlings grown from treated seed and hence, their incorporation together with fungicides can extend the effect of the chemical to give protection against root rot. Upmanyu et al. (2002) also reported the tolerance in *T. viride* to carboxin, tebuconazole and carbendazim. The combined seed treatment of French bean with Contaf (0.025%) and *Gliocladium virens* (0.1%) resulted in higher seed germination, lower incidence of the disease and higher grain yield (Mukherjee et al. 2001). Combined treatment of seed (soaking in *Trichoderma harzianum*, 1×10^7 conidia/ml) and soil (20 and 100 kg/calcium and micronized sulphur (40% micron sulphur), respectively) before sowing reduced the root rot incidence and increased green pod yield (Ziedan and Mahamoud 2002). Soil amendment with mustard cake along with carbendazim seed treatment and foliar sprays were found highly effective in reducing the pre-emergence and post-emergence root rot and web blight severity (Upmanyu et al. 2002). A combination treatment including soil solarization (SS) for 50 days + soil amendment (mustard cake) + combination of *Allium sativum* ethanol extract and *Bacillus subtilis* was found effective in reducing pre- and post-emergence damping-off while web blight was best contained by SS + soil amendment + *A. sativum* (ST + FS) and increased yields (Sharma and Gupta 2003).

7.2.2 Angular Leaf Spot

It is an important disease of French beans particularly in mid-hill conditions where moderate temperatures with high humidity conditions favour the development of this disease. The losses due to angular leaf spot are of two types. Firstly, there is reduction in photosynthetic capacity because of the heavy leaf spotting which drop down prematurely those results in subsequent poor pod setting and secondly, the green pod infection makes the pods unfit for market. Up to 50% yield losses under epiphytotic condition have been reported from the USA whereas in Mexico, it has been considered as a principal fungal disease where up to 90% incidence has been recorded causing significant losses (Campos and Fucikovsky 1981). In India, the total loss including damaged and unmarketable pods has been estimated to be about 40–70% due to this disease (Singh and Saini 1980) whereas up to 20–25% loss in grain yield every year has been recorded in Sikkam (Srivastava and Gupta 1994). The disease is caused by *Phaeoisariopsis griseola* (Sacc.) Ferr.

7.2.2.1 Symptoms

The pathogen infects all green parts of the plants, *viz.* leaves, petioles, stems, pods and seed, producing varied symptoms. On cotyledonary leaves, the symptoms appear as circular spots while on true leaves, 3–5 angled spots appear in between veins and veinlets (Fig. 7.1). The spots are dark greyish on the upper surface and light grey on the lower surface of the leaves but with the passage of time, change to reddish brown and finally attain dark brown colour. On the lower surface, light reddish discolouration appears on the veins and veinlets, which delimit such spots. Grey mouldy coating covers the spots on the lower surface of the leaves. On close observation, the spots reveal the presence of coremia bearing a large number of spores. Such severely infected leaves show upward curling and defoliate prematurely. Elongated dark brown spots appear both on petioles and stems. Under severe conditions, complete defoliation occurs resulting in premature death of the plants. Pods are infected at all the developmental stages. Lesions on the pods are smooth, usually circular and rarely elongated (Fig. 7.2). At first, these spots are superficial having reddish brown centre with well-defined ashy black borders but with the passage of time, deeper tissues are involved. In the central portion reddish brown, coremia and spores are produced. Severely infected pods either bear no seeds or produce only shrivelled seeds. In late infections, i.e. at maturity stage, the seeds underneath the pod lesions show yellowish brown discolouration which is more prominent on hilum region but can occur anywhere on the seed surface.

Fig. 7.1 Symptoms of angular leaf spot on leaves

Fig. 7.2 Symptoms of angular leaf spot on pods

7.2.2.2 Epidemiology

The pathogen (*P. griseola*) overwinters both on plant debris and in infected seed (Sohi and Sharma 1974; Sindhan and Bose 1980). The viability of conidia on plant debris is 6 and 8 months in laboratory and field, respectively (Sindhan and Bose 1980), thus it serves as a source of primary inoculum. Other source of primary inoculum is an infected seed. Though the infestation site has been identified in the hilum of seed but in some cultivars both hilum and seed coat seem to be infected (Saettler and Corea 1988). While disease can initially result from the planting of infected seed causing spots on cotyledonary leaves, the primary route in most bean growing areas involves the rain splashed conidia from plant debris or windblown conidia with soil particles attacking leaves, pods and stems. Infected leaves become inoculum source for secondary spread of the disease. However, the rate and degree to which secondary spread occurs is dependent on the interactions and influences of all factors involved with the disease and its spread such as climate, pathogen and the host. Moderate temperatures and moist conditions favour the arrival of viable inoculum and successful field infections. Optimum temperature for growth and sporulation, spore germination and development of the disease is 21–24 °C, 18–24 °C and 24 °C (Sindhan and Bose 1980; Sarotorato 1988), while optimum RH for sporulation, spore germination and development of symptoms has been reported as >90%.

Mathew et al. (1998) studied the effect of leaf wetness durations at optimum temperature and reported that leaf wetness of at least 12 h duration coupled with moderate temperature (25 °C) exhibited minimum incubation period of 9 days (Table 7.1).

The inoculum concentration from 1×10^4 to 5×10^4 conidia of *Phaeoisariopsis griseola*, leaf wetness durations from 3 to 9 h showed a progressive decline of the incubation period of angular leaf spot on French bean. Intermittent wetness up to 3 cycles (12 h dry and 12 h wet) was found effective to increase the number of

Table 7.1 Effect of different leaf wetness durations on number of spots per leaf and incubation period

Leaf Wetness (h)	Average spots/leaf (No.)	Incubation period (days)
3	9.22	12
6	24.25	12
9	33.25	9
12	38.33	9
18	40.50	9
24	49.25	9
48	46.25	9
LSD($\leq$ 0.05) 1.5		

Evaluated at temperature 24 ± 1 °C

lesions per leaf. Gupta et al. (2005) reported that an increase in relative humidity from 85.7% to 89.9% coupled with moderate temperatures of 25 °C resulted in a sharp increase in disease development. These workers also studied the role of environmental factors on disease development and reported the consistent effect of rainfall, soil moisture and relative humidity on the disease development. Simple correlation coefficients between disease severity and rainfall (0.7596), soil moisture (0.7070) and relative humidity (0.5253) were found to be highly significant and positive. But Chhetry et al. (1994) reported non-significant linear correlation and regression coefficients of disease incidence and severity with microclimatic factors.

7.2.2.3 Diurnal Periodicity of Conidial Release of *P. griseola*

The observations pertaining to conidial density were recorded at 4 h intervals starting from midnight and the release of conidia was continuous and that the air over the field was never free of conidia of *P. griseola*. It revealed a distinct rhythm at different times of the day with maximum conidial density being observed between midnight and early morning hours (Gupta et al. 2005). Conidial formation and release was favoured by temperature (23–24 °C), high relative humidity (>90%), prevalence of dew (20.00–8.00 h) and dark period.

7.2.2.4 Management

Cultural Practices

Various cultural practices, *viz.* use of disease-free seed and removal of diseased leaves and seedlings (Trutmann and Kayitare 1991); 3–4 year crop rotation (Correa-Victoria and Saetler 1987); destruction of diseased plant material; deep ploughing (Hocking 1967); wider spacing (Navarro et al. 1981; Gupta et al. 2000d) and altering of sowing dates (Bhardwaj et al. 1994; Gupta et al. 2000d) and staking of pole type varieties (Gupta et al. 2000d) have been recommended from time to time for the control of this disease.

Moreno (1977) studied the effect of different cropping systems (bean grown alone or in combination with maize, sweet potato or Cassava) on the severity of angular leaf spot and reported that severity was maximum when maize was included

and lowest with sweet potato and Cassava. However, during a multi-location trial in Tanzania, Kikoka et al. (1989) found intercropping with maize effective to reduce the severity of the disease. Boudreau (1993) and Gupta et al. (2000d) reported similar observations from Kenya and India, respectively. Poultry manure, composted *Urtica* sp. and composted *Lantana camara* treatments were superior to other composts in suppressing angular leaf spot (Joshi et al. 2009).

Host Resistance

French bean cultivars are known to possess a varying degree of resistance against the pathogen (Buruchara et al. 1988). Various cultivars/lines like Albama No.1, Café, California Small White, Case Knife, Epicure, Mexico blank, McKastan, Navy bean, Negro Costa Rica, Scotia Rojo Chico, Mexico 11, Mexico 12 and Cauca 27, Carpoata (Santos Filho et al. 1978); Negro Tacana (Lopez et al. 1997) and Manteiga Maravilha (Paula et al. 1998) are reported as resistant. Araiyo et al. (1989) reported that non-black cv. Ouro and black coloured cvs. Rio Tibagi and Milionario 1732 possess resistance to *P. griseola*.

In India, cvs./lines, *viz.* EC 38921, EC 44621, PLB 148, Kentucky Wonder, Canadian Round, Canadian Long Red, IC 47651-6, EP 146 (BAT-482), HPR-54, HPR-63, HPR-92, HPR-93, HPR-111, HPR-232, HPR-260, HPR-299, HPR-332, HPR-326, Hans, Nagar Local, SVM-1, JK-8, Him-12, IIHR 42-2, IIHR 901, EC 97830, EC 44758 and NIC 14402 were reported to be resistant against this disease (Singh and Sharma 1976; Bhardwaj et al. 1992; Srivastava et al. 1995; Mohan et al. 1995; Gupta et al. 2000c).

The effectiveness of supplementing local bean mixture with 25% or more resistant lines (BAT 76 and A 285) was evaluated and it resulted in a significant reduction of angular leaf spot severity in Africa (Pyndji and Trutmann 1992; Trutman and Pyndji 1994). Of the 68 French bean genotypes/lines screened for resistance to *Phaeoisariopsis griseola* during 2002 and 2003 at two locations namely, Shalimar and Wadoora in Kashmir, India, 9 genotypes/lines, *viz.* SL-4-PL-1, HIM-1, Bountiful, JKRO-3, L-27, L-28, L-8 and Local Big Beans, exhibited highly resistant reaction on pods. On leaves, 6 genotypes, *viz.* Uri-Red, EC-285559, Medur Tral, Local Red, Local Big Beans and SAW/GH/TA/41A at Wadoora location, and 4 genotypes, *viz.* Gurez Local, Big Bean Black, EC39855 and Local Big Beans at Shalimar location showed a highly resistant reaction, while the genotype Shalimar French Bean-1 gave a highly susceptible response on leaves at both locations (Bashir et al. 2006).

Chemical Control

Various fungicides have been reported to give good control of this disease in India and other countries. However, during continuous rains which are essential both for good growth of bean plants and rapid development and spread of the disease, routine application of fungicides is sometimes delayed which often results in a heavy buildup of the disease. Proper timing of initial sprays is of prime importance to achieve a desirable level of disease control.

Attempts have been made to control the disease by both seed treatment with mercurial and other fungicides and foliar sprays with various fungicides. Sprays of

fungicides like wettable sulphur, mancozeb, Bordeaux mixture, zineb, carbendazim, benomyl, triforine, triademorph (Fortugno 1977; Sindhan 1984; Issa et al. 1982; Hidalgo and Araya 1993; Singh et al. 1995), chlorothalonil, TPTH, TPTA (Castro et al. 1991; Vierra et al. 1998) and mancozeb (Issa et al. 1982; Castro et al. 1991; Vierra et al. 1998) have been reported effective to reduce disease intensity and increase yield. Oliveira et al. (1992) reported stannic triphenyl (fentin) acetate, bitertanol and tebuconazole as more effective when compared to Bavistin while, Srivastava and Gupta (1994) reported captan, thiophanate methyl, carbendazim, ziram and captafol to be approximately equally superior to mancozeb, copper oxychloride and tridemorph. Combi fungicide sprays like benomyl + captafol, chlorothalonil + copper oxychloride (Issa et al. 1982) and thiophanate methyl + thiram (Rodriques et al. 1987) have also been found effective. Combination treatments of carbendazim (seed treatment + foliar spray) and bitertanol (seed treatment + foliar spray) were also found effective in reducing the severity and increasing the green pod yields than seed treatment or foliar spray alone (Mathew et al. 1998).

Various EBI fungicides have longer post-infection and eradicant activity being systemic in nature. Mathew et al. (1998) studied the mode of action of EBIs like hexaconazole, penconazole and bitertanol along with conventional fungicides like mancozeb and carbendazim by providing artificial weather conditions like temperature, relative humidity and leaf wetness for infection recorded in epidemiological studies.

Protective Activity

Potted French bean plants sprayed with test fungicides and subsequently inoculated with the conidial suspension (5×10^4 spores/ml) after 24, 48, 72, 96, 120, 144, 168 and 192 h of fungicide spray. Inoculated plants were kept in saturated humidity for 12 h and then transferred to the net house. Humidity >75% and temperature 25 ± 1 °C 25 were maintained inside the net house for 9 days of incubation. Carbendazim provided 100% control of the disease up to 120 h while mancozeb up to 96 h EBIs exhibited poor protective activity only up to 72 h confirming the increased protective activity of mancozeb (protectant) and carbendazim (systemic).

Post-infection Activity

Spray inoculated potted French bean plants were kept in moist chambers for 24, 48, 72 and 96 h and subsequently sprayed with a dilute suspension of test fungicides. EBI fungicides demonstrated post-infection activity up to 72 h while it was only 24 h in case of carbendazim and mancozeb.

Pre-symptom Activity

Spray inoculated potted French bean plants were kept in the moist chamber at 20–25 °C for 12 h before being returned to the net house for normal maintenance. Fungicide suspension was sprayed on the seventh and eighth day of inoculation. Upon drying plants were maintained in nethouse and the data was recorded 14 days after treatment. Mancozeb when applied as pre-symptom spray it had little effect and produced sporulating spots while EBIs and carbendazim produced reddish brown non-sporulating spots when applied seventh and eighth day after inoculation (Fig. 7.2).

Post-symptom Activity
To evaluate anti-sporulant activity 15 naturally infected bean pods (5 pod/plant) having 5–10 spots each (one treatment) were tagged and subjected to 5 fungicide spray. After 3, 5 and 10 days of single spray, five tagged pods were removed from each treatment and one lesion (5 mm^2) was cut from each pod with the help of cork borer and conidial concentration was determined using a haemocytometer. Carbendazim, hexaconazole, flusilazole and bitertanol had high level of antisporulant activity and their residue persisted for more than 10 days. These properties of EBI fungicides can be utilized in planning different spray programmes for effective control under different situations.

Comparative Efficacy of Protective Versus Post-infection Spray Programme
A Thermohygrograph was placed in the field to record hours of RH, i.e. >80%. Continuous occurrence of RH >80% for 12 h was considered as one infection period. Short intervening periods of (up to 3 h) of RH >80% in between the periods of high humidity (>80% RH) was also considered while calculating infection period. Sprays were usually applied at various times depending on weather conditions but always within 72 h of initiation of infection period. No spray was repeated within 9 days even if additional infection period has occurred.

In all 25 infection periods occurred. Post-infection sprays (Table 7.2) of bitertanol and hexaconazole within 72 h of predicted infection period and protective sprays of carbendazim were equally to manage the disease. Two less number of sprays were required in post-infection spray programme in comparison to 7 sprays under protective spray programme.

Spray schedules involving the strobilurin (azoxystrobin) and triazoles (tebuconazole, propiconazole) alone and their combinations were also found effective in controlling angular leaf spot (Oliviera and Oliveira 2003).

Integrated Approach
Fungicidal treatments coupled with careful selection of varieties and sowing dates proved highly effective to reduce disease severity and increased the yield of the crop (Bhardwaj et al. 1994). Effect of two mulches (Pine needles and *Eucalyptus* leaves) alone and in combination with carbendazim (0.1%) sprays on mulch and foliage on

Table 7.2 Comparative efficacy of protective and post-infection (curative) spray programme against angular leaf spot of French bean

Treatment	Dose (μg/ml)	Disease severity (%)	Disease control
Protective spray programme[a]			
Carbendazim	500	6.00	90.21
Mancozeb	2500	11.73	80.87
Curative spray programme[b]			
Bitertanol	500	8.66	85.87
Hexaconazole	500	7.33	88.04

[a]No. of sprays = 7
[b]No. of sprays = 5

the disease severity and pod yields has also been studied by Mathew et al. (1998). Sprays of carbendazim on mulches and foliage were highly effective in reducing the disease severity and increasing green pod yields. Sprays of carbendazim on mulches alone were also found quite effective and reduced the severity and increased green pod yields significantly with an additional advantage of residue-free pods.

7.2.3 Anthracnose

Anthracnose is an important disease of French bean world over but it is more severe in tropical and subtropical regions (Pastor-Corrales et al. 1994). Scribner (1888) was the first to use the name anthracnose for this disease, which is still generally used. The term anthracnose is derived from the Greek word meaning ulcer and is appropriate because of the ulcer like depressed lesions appear on the pods. Though this disease occur world over including tropical and subtropical regions, it causes greater losses in the temperate zones than it does in tropics. In India, its occurrence was first noticed in Nilgiri hills during 1915 (Hutchinson and Ram Ayyar 1915). Since then the disease has been recorded in the entire bean growing areas of the country, which have cool and moist weather during the growing season. Losses from this disease can approach up to 100% when highly contaminated seed is planted under favourable weather conditions for disease development. Yield losses of 90–100% have been reported in many countries throughout tropical America and Africa (Pastor-Corrales and Tu 1989; Lenne 1992). In Himachal Pradesh, India, the incidence of this disease has been reported to range from 5.0% to 65.0% in different localities leading to considerable yield losses in certain years (Sharma et al. 1994; Padder and Sharma 2010). The fungus, *Colletotrichum lindemuthianum* (Sacc. and Magn.) Briosi and Cav. is responsible for this disease with *Glomerella lindemuthiana* Shear (Shear and Wood 1913), but now it has been renamed as *G. cingulata* (Kimati and Galli 1970) as its perfect stage.

7.2.3.1 Symptoms

The disease may appear on any above-ground plant part depending upon time of infection and source of inoculum but most striking symptoms appear on immature pods. Initial symptoms appear on the cotyledonary leaves as small, dark brown to black lesions. Conidia and hyphae then may be disseminated by rain or dew to the developing hypocotyl where rust coloured specks develop. The specks gradually enlarge lengthwise along and partially around the hypocotyl and young stems, forming a sunken lesion. On leaves, the infection may occur on both sides but an early sign of infection usually appear on the under leaf surface as blackened dead portions of the veins. These may extend to limited adjoining areas. Later, such spots also appear on the upper leaf surface. Lesions are also formed on petioles and stems. On pods, black sunken spots with lighter or grey central area are seen (Fig. 7.3). The central portion of the spots shows pinkish masses of spores of the fungus, especially in wet weather. Later, the sides of these spots appear raised. The seeds obtained from

Fig. 7.3 Symptoms of bean anthracnose on pods

heavily infected pods show brown to light chocolate coloured sunken cankers on the seed coats.

7.2.3.2 Epidemiology

The pathogen overwinters in infected seeds and plant debris. Ravi et al. (1999) reported that in seed the pathogen was mostly present in seed coats and cotyledons and rarely in embryonic axes. Conidia and/or dormant mycelia in the infected seeds and/or infected plant debris germinate and infect the young seedlings. It can remain viable in infected seed for several years (Tu 1983). When infected seed germinates, lesions appearing on cotyledons serve as the source of secondary inoculum. The spores are almost entirely water-borne. Primary leaves and the hypocotyl are foci of secondary infections. Borucka and Marcinkowska (2001) reported the importance of wet seasons at early growing period besides availability of a source of inoculum for the initiation and occurrence of this disease. The pathogen is very sensitive to changes in temperature and humidity. It develops most abundantly in cool, wet weather and largely disappears under hot and dry conditions. A relative humidity of 92% and above is essential for infection, the optimum being close to 100%. The fungus requires about 10 mm of rain to establish initial infection (Tu 1981). The optimum temperature for disease development ranges from 18 to 27 °C with maximum intensity at 21 °C (Sindhan 1983) and is markedly reduced at 13 °C. Heavy and frequent rains with moderate temperatures (19–25 °C) and high relative humidity (>70%) favoured the progress of the disease in terms of vertical and horizontal spread of the disease (Kumar et al. 1999). Moderate rainfalls at frequent intervals are also essential for the local dissemination of conidia present in water-soluble gelatinous matrix and the development of severe anthracnose epidemics. The movement of insects, animals and man may spread conidia particularly when foliage is moist.

7.2.3.3 Management

Various management strategies have been studied to keep this disease in check, which are based on use of fungicides, resistant cultivars, cultural practices and biocontrol of seed-borne infection. It is always better to integrate all available disease management methods to reduce the losses caused by this disease to bare minimum.

Cultural Practices

Use of healthy seed, clean cultivation and three-year crop rotation has been recommended for the management of this disease (Sharma and Sohi 1989). Disease-free seed can be produced either by surface irrigation in semi-arid regions where conditions of high temperature and low humidity prevent infection of this fungus, or under a pedigreed seed programme, in which seed plots were isolated and subjected to strict inspection for disease-free seed. Best sowing time of French bean in hill regions is in between mid-April to mid-May for maximum yield and minimum anthracnose incidence.

Host Resistance

The most promising strategy to control this disease is the use of resistant cultivars. However, the diversity and the high variability of the fungus with the continuous emergence of new races pose problems in breeding programme. Although cultivars with new resistance genes are resistant to majority of pathotypes, this resistance can be broken when such variety is planted widely (Lenne 1992). Anderson et al. (1963) reported that the resistance in cv. Charlevoix against alpha and beta races of *C. lindemuthianum* is conferred by a single independent dominant gene. A glycoprotein inhibitor in the cell wall of resistant varieties imparts resistance (Sharma et al. 1986). Due to the presence of much pathogenic variability in the pathogen, non-race-specific resistance has been used in breeding for anthracnose. Cultivar IAPAR-Rio Negro-8 is resistant to all races of the pathogen under field conditions (Alberini et al. 1987) while Menezes and Dianese (1988) have identified varieties (Aroana 80, Ayso, Carioca 80, Iguaco, Moruna 80, Riuo Piqiuri, Rio Vermelho, Mulatao, Olho-de-Pombo and Vermelhao) which were resistant to at least 7 of the 9 races of the pathogen and no race was virulent to all the 70 cvs. screened in Brazil. Chakrabarty and Shyam (1990) demonstrated resistance in cvs./lines VL 60, VL 63, Jawala, HPR 33, B 4 B 6 and P 48 against eight isolates of the pathogen. However, Jawala later exhibited highly susceptible reaction, which may be due to the development of new pathotypes. Kumar et al. (1997) screened 60 cultivars/lines of kidney bean against ten races, *viz.* beta, gamma, Ind I, Ind II, Ind II, Ind IV, Ind V, Ind VI (alpha-Brazil), Ind VII and Ind VIII of *C. lindemuthianum* under field and artificial epiphytotic conditions and found two accessions (AB-136 and G 2333) as highly resistant to all the above races prevalent in Himachal Pradesh, India, while cultivars Cornell 49-242, EC 43036, EC 57080, KRC 5, PI 207-262 and Widusa exhibited resistance to more than five races whereas rest were susceptible and these can be utilized in the breeding of race-specific resistant varieties. Later Gonalves-Vidigal et al. (2001) reported that a single dominant gene controls the resistance of

cv. AB 136 to both races 31 and 69 and the symbol Co-6 was assigned to the gene. In addition, linkage analysis using random amplified polymorphic DNA marker indicated that Co-6 also controls the resistance of this cultivar to other races of the pathogen, or that different genes are present in the same linkage block.

Few cvs./lines/land races, *viz.* Negro Otomi (Acosta-Gallegos et al. 2001), SRC 74, SRC 89A, SRC 90, SRC 95, SRC 201, PDR 14 (Sud and Sood 2002) and Morden 003 (Mundel et al. 2004) and HUR-5, HUR-137, IPR 96-4, VL-63, PDR-14, HUR-385 (Khati and Hooda 2006) have been reported resistant to this disease. Some exotic accessions like G 2333, Cornell 49242, PI 207262, Mexique 222, TO, Perry Marrow, Kaboon and Widusa were resistant to more than five Indian races, whereas two Indian accessions KRC-5 and Hans showed resistance to six and four races, respectively (Pathania et al. 2006).

Common bean germplasm lines comprising of 65 indigenous and 34 exotic were evaluated against four races, *viz.* 3, 515, 529 and 598 of *C. lindemuthianum* under laboratory conditions and four indigenous accessions namely 47, 34, 32 and 18 showed resistant reaction to race3, 515, 598 and 529, respectively, whereas in exotic accessions 16, 22, 18 and 11 exhibited resistance to these races (Sharma et al. 2012). However, accessions like IC-328537, IC-328538, IC-448888, IC313294, IC-27823, IC-339645, IC-341862 (Indigenous), EC-169813, EC-398530 and EC 500226 (Exotic) were found resistant to all races evaluated.

Biocontrol

Limited work on this aspect in this disease has been carried out like in vitro evaluation of the antagonists against this pathogen (Gupta et al. 1991) or the phenomenon of cross-protection by inoculating a less virulent strain of the fungus to reduce the severity from severe strains (Sutton 1979). Infected seed when soaked in culture filtrate (10%) or treated with talc formulation (0.4%) of *Trichoderma viride* recorded minimum seed infection and maximum seed germination (Ravi et al. 1999). Treatment with *P. chlororaphis* PCL1391 resulted in best biocontrol of anthracnose, while *P. fluorescens* WCS365 showed no significant difference compared to the positive control (Bardas et al. 2009). Padder et al. (2010) evaluated three bioagents (*Trichoderma viride, T. harzianum* and *Gliocladum virens*) and five biopesticides (Achook, Neemgold, Wannis, Spictaf and Neemazal) under in vitro and in vivo conditions against this disease. All the three antagonists caused significant mycelial growth inhibition, maximum being with *T. viride* (69.21%) followed by *T. harzianum* (64.20%). Among the biopesticides tested at four concentrations, Wanis applied at 1000μl/ml caused maximum inhibition of 82.12% followed by Spictaf (52.85%). *T. viride* and Wanis at 1000μl/ml were most effective in reducing the seed-borne infection.

Chemical Control

Various systemic and non-systemic fungicides such as captan, thiram, carbendazim, etc. have been used by various workers to reduce seed infections (Sindhan and Bose 1981; Trutman et al. 1992). Foliar sprays of carbendazim, benomyl, zineb, maneb and mancozeb have been recommended for its control (Chakrabarty and Shyam 1988). Benlate and Bavistin persisted in the plant up to 15–20 days after seed

treatment (Sindhan and Bose 1981). EBI fungicides like triadimefon and triforine were effective against the pathogen under in vitro conditions but were phytotoxic to *P. vulgaris* seedlings when used as a seed treatment (Chakrabarty and Shyam 1988). Carbendazim alone or in combination with thiram as seed treatment followed by two or three sprays of mancozeb at 45, 60–75 days after sowing effectively managed this malady (Sharma and Sugha 1995). A combination of seed treatment with tebuconazole (0.1%) and foliar sprays of tricyclazole (0.03%) reduced the disease severity both on leaves and pods while maximum seed yield was recorded in case of seed treatment and foliar sprays of carbendazim (Gupta et al. 2000b).

Continuous use of systemic fungicides has been shown to be associated with the development of resistant biotypes (Tu and McNaughton 1980). Maringoni et al. (2002) reported that all isolates of *C. lindemuthianum* collected from *P. vulgaris* fields located at Paranpanema Valley, Brazil were insensitive to benomyl, carbendazim and thiophanate methyl showing the occurrence of cross-resistance to different benzimidazole fungicides while all isolates were sensitive to chlorothalonil. Recently, Oliveira and Oliveira (2003) reported that strobilurin fungicides like azoxystrobin and trifloxystrobin efficiently controlled this disease while chemicals like carbendazim and fluquinconazole applied without rotation were least efficient treatments. However, a combination of systemic fungicides with non-systemics and rotation of fungicides can be an effective approach for management of this disease instead of constant use of one systemic fungicide.

Plant Origin Pesticides

Under greenhouse conditions, the extracts of *M. argyrophylla* and *O. vulgare* caused maximum reductions (41.82% and 37.65%, respectively) in disease severity when a local effect assay was carried out (Pinto et al. 2010). Antimicrobial activity of cascalote phenolics against phytopathogenic fungus *C. lindemuthianum* was carried out which indicated that cascalote phenolics have fungistatic activity against races R-0 and R-1472 of the pathogen under in vitro conditions. Under in vitro conditions, spore germination and cellulase and polygalacturonase activities in this fungus have been inhibited. In vivo assays with cascalote phenolics under greenhouse conditions using susceptible cv. PI 206272 of common bean resulted in a good protection against anthracnose severity especially as a preventive treatment (Garcia et al. 2010).

7.2.4 Rust

Rust is a polycyclic disease can cause significant losses under favourable environmental conditions by causing premature defoliation, decrease in number of pods/ plant and their reduced weight. The disease has been reported from the entire bean growing areas of the world and it was first described from Germany in 1795 (Persoon 1795). Since then it has been reported from many bean growing countries of the world (Gupta and Thind 2018). In India, the disease was reported as early as in 1918 (Butler 1918). Now the occurrence of this disease has been reported from Karnataka (Sharma 1989), Tamil Nadu, Uttarakhand (Sharma 1998), Meghalaya (Bhat et al.

1999) and Sikkim (Bhat 2002). Recently, the occurrence of this disease has been reported on pencil beans in Solan area of Himachal Pradesh (Gupta et al. 2008). Several workers have reported varying degree of losses due to this disease ranging from 25% to 100% (Grafton et al. 1985; Wagacha et al. 2007). In India, losses in green pod yield due to this disease ranged in between 4.7% and 69.0% (Sharma 1989; Devi et al. 2019). The disease is caused by *Uromyces phaseoli* var. *typica* Arth. and *U. appendiculatus.* Being autoecious rust, all its spore stages are produced on bean plant but pycnial and aecial stages are not commonly encountered in nature. The pathogen has limited host range. Besides French bean (*Phaseolus vulgaris*), it attacks *P. lunatus, Dolichos lablab, D. biflorus, Vigna chinensis,* etc.

7.2.4.1 Symptoms

Under favourable environmental conditions, all above-ground parts of the plant are infected but leaves and pods are the principal plant parts attacked by the disease. On leaves, the disease may appear on both the surfaces, but is more common on the abaxial surface. Pustules of the disease usually appear first as small and slightly raised spots that are almost white in colour. These pustules enlarge by forming reddish brown sori, up to 2 mm in diameter, containing the urediniospores (Fig. 7.4). After coalescing, they may occupy larger areas. A ring of secondary sori may develop around the original infection on susceptible cultivars. Minute elongated and raised spots also appear on petioles and stems when the severity of this disease is more on leaves. Reddish brown, elongated raised pustules also appear on pods. The affected leaves may turn yellow and dry or fall prematurely. Wherever telial stage appears, they are dark brown to black in colour and linear.

7.2.4.2 Epidemiology

Being autoceious rust, i.e. it has its life cycle confined to a single host. The aecia are rare in nature but have been observed in bean fields in Oregon (Eastman 1944). The Urediniospores are produced in great numbers in sori on the upper and lower

Fig. 7.4 Symptoms of bean rust

surfaces of the leaf. These urediniospores are air-borne and blown to long distances and cause severe epidemics. The urediniospores germinate as soon as they are mature. One or two germ tubes developed from germinated urediniospores (Xin et al. 2000). The penetration of the germtube occurs mainly through the stomata but occasionally penetrated directly through the epidermal cell. A well-defined appressorium is formed after penetration by the pathogen. The fungus forms a sub-stomatal vesicle when it penetrates through the stomata and a round inflated body usually occurs. One or two primary hyphae then develop from the inflated body. Once within the bean leaf, the fungus grows intercellularly and the penetration to the leaf cell occurs after a haustorium mother cell is formed at the hyphal tip in contact with a host cell. The branches emerge from behind the site of the primary hyphae where a haustorium mother cell develops. The secondary hyphae spread intercellularly.

Obligate parasitism and autoecious nature of rust pathogen helps it to complete the entire life cycle on bean plants. It is not seed-borne. Davison and Vaughan (1963) suggested that rust overwinters in or as urediniospores in trash and on trellis poles because 16% urediniospores of *U. phaseoli* race 33 germinated even after storage at –18 °C for >600 days and produced abundant infection on bean leaves. In cooler regions, survival is ensured through plant debris in the form of teliospores while in other areas through the continuous growing of crop including other hosts. Cloudy and humid days, allowing dew deposition on leaves for some time in the morning, favour germination of spores and subsequent infection. Optimum infection takes place at 17 °C (Harter et al. 1935; Mendes and Bergamin-Filho 1989) or 18–21 °C (Shands and Schein 1962) Singh and Gupta (2019) studied the effect of different temperature regimes on the disease development under glasshouse conditions (Fig. 7.5) and reported that maximum disease severity (82.99%) and number of pustules/cm^2 (69.40) were recorded at 20 °C followed by 25 °C where 72.3% disease severity and 63.20 pustules/cm^2 were recorded while least number of pustules/cm^2

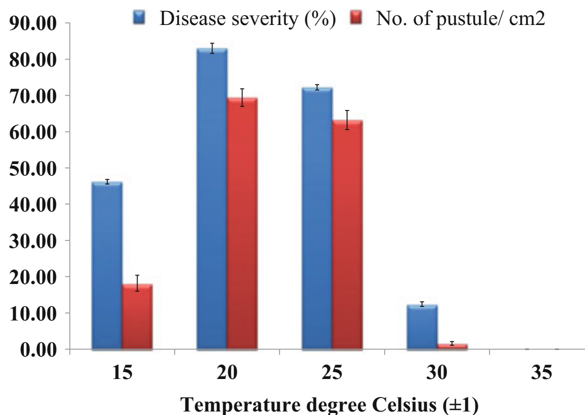

Fig. 7.5 Effect of temperature on bean rust development under glasshouse conditions

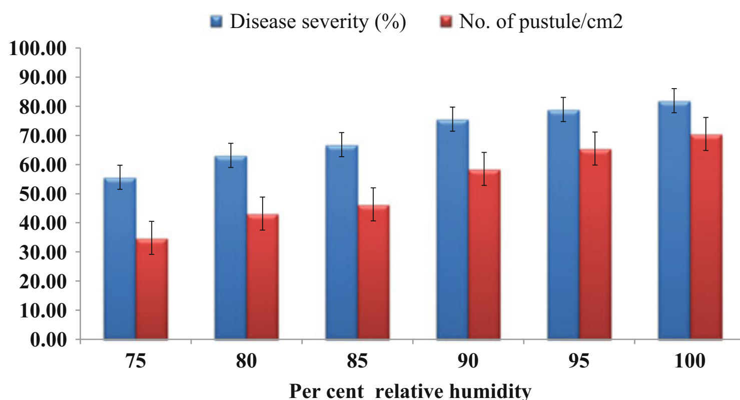

Fig. 7.6 Effect of different relative humidity levels on bean rust development under glasshouse conditions

(1.60) and disease severity (12.44%) was recorded at 30 °C, however, no disease developed at 35°°C.

Harter et al. (1935) obtained high level of rust infection when plants were exposed to 96% or more RH. Maximum percentage of bean rust infection was observed when plants were exposed to 11 h or more to 100% RH (Rey and Lozono 1961). Mendes and Bergamin-Filho (1989) attained maximum infection (Number of sporulating pustules/leaf) after 22 h of high RH. Singh and Gupta (2019) reported that there was a corresponding increase in the disease severity and number of pustules as humidity increased from 75% to 100% (Fig. 7.6) with maximum disease severity (81. 92%) and number of pustules/cm^2 (70.50) at 100% RH level followed by 95% and 90% RH levels on which 78.90% and 75.58% disease severity was recorded while it was minimum (55.63%) at 75% RH level. Sharma (1977) also studied the effect of number irrigations on the disease development and observed that irrigation given at 3 days intervals favoured disease development than with 18-day intervals.

Effect of duration of leaf wetness also has a significant effect on disease development under field conditions which is mostly provided by rain and dew deposition under field conditions. Singh and Gupta (2019) also carried out experiments to see the effect of leaf wetness (Fig. 7.7) and intermittent leaf wetness (Table 7.3) durations on disease development under glasshouse conditions and reported that no disease developed when plants were exposed to 3 h leaf wetness whereas continuous leaf wetness of 6 h was sufficient to initiate infection by rust pathogen. They further reported that when leaf wetness durations were increased from 6 to 24 h, there was a corresponding decrease in the incubation period from 243 to 168 h with the increase in the disease severity and number of pustules from 30.0% to 83.6% and 6.0 to 72.3/cm^2 of leaf, respectively. However, further increase in leaf wetness did not exert any effect on the incubation period.

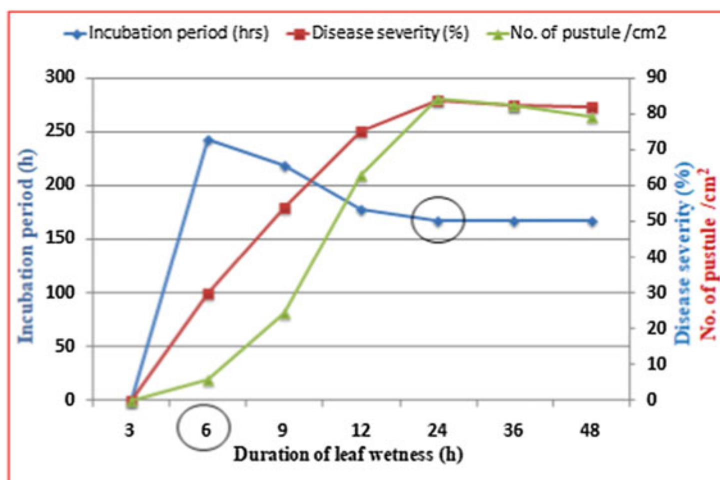

Fig. 7.7 Effect of duration of leaf wetness on initiation and development of bean rust under glasshouse conditions

Table 7.3 Effect of intermittent leaf wetness on development of the disease under glasshouse conditions

Total leaf wetness (h)	Wet/Dry sequence (hr)								Disease severity (%)	No. of Pustules/cm^2
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry		
12	12	12	–	–	–	–	–	–	68.28 (55.71)	57.33
24	12	12	12	12	–	–	–	–	82.49 (65.31)	72.50
36	12	12	12	12	12	12	–	–	78.90 (62.67)	72.17
48	12	12	12	12	12	12	12	12	76.75 (61.19)	69.83
$LSD_{0.05}$									(2.44)	8.28
SE(m)									(0.82)	2.79

Number of pustules and disease severity increased with the interruption of leaf wetness with dry periods up to two cycles (72.50 pustules/cm^2 and 82.49% disease severity) and further extension of wet and dry periods resulted in a non-significant decrease in number of pustules and disease severity (Table 7.3).

Sharma (1998) analysed the meteorological data for 8 years (1986–1993) and showed that excessive precipitation was deleterious for disease development, which explains the occurrence of severe infection during periods of low rainfall. Singh and Gupta (2019) studied the effect of different meteorological factors on disease

progress under natural epiphytotic conditions and observed that the disease was initiated in the second week of August and reached at peak in mid-September. Multiple correlation coefficients between disease severity and these environmental factors suggested that 92.36% rust severity was due to mean temperature, average relative humidity and cumulative rainfall while rest of the variation may be attributed to the factors not included in the investigations. The multiple linear regression equation was fitted to the data and the equation arrived for all the weather parameters was $Y = -52.4852 + 1.2535\ X1 + 0.8008\ X2 – 0.2862\ X3 + 0.4622\ X4 – 0.7755\ X5 + 0.2428\ X6$. With the step, down procedure, three variable, i.e. maximum temperature (X1), cumulative rainfall (X5) and wind speed (X6) were eliminated and final equation fitted to the data is $Y = -37.4111 + 1.0622\ X2 + 0.5299\ X3 – 0.2868\ X4$. Stepwise regression analysis of the data in relation to weather variables revealed that the R^2 value varied from 0.53 to 0.86. The regression equation clearly demonstrated that minimum temperature, morning RH and evening RH played a major role in the development of French bean rust, in addition to other independent variables.

Disease intensity is not influenced by varying levels of N, P and K. Farm implements, insects, humans and animals help in local dissemination of the rust fungus (Sumartini 1998).

7.2.4.3 Management

The disease can be managed by cultural, chemical, biological methods and host resistance.

Cultural Practices

Moreno and Mora (1984) have suggested minimum tillage or no tillage to prevent the spread of the disease and intercropping of bean with maize since severity and rate of infection was more in monoculture. Mixed cropping reduced rust incidence level by 51% and 25% with sole cropping and row intercropping, respectively (Fininsa 1996). Plant density and disease incidence are directly related (Flores Revilla et al. 1994). Mishra et al. (1998) evaluated seven dates of sowing under Orissa conditions and Arka Komal variety was sown in between 12 October and 23 November; 9th November was found as the optimum date of sowing because the rust incidence was minimum. Sharma and Sohi (1989) also recommended some cultural practices like long crop rotations, collection and destruction of plant debris, wider plant spacings and removal of weeds to permit aeration and drying which check rapid build-up of inoculum and spread of the disease under field conditions.

Host Resistance

More than 35 physiologic races of the rust fungus (*U. appendiculatus*) are present in Australia, Brazil, Hawaii and the USA. Extensive cultivation of the new cultivars may make them susceptible to new virulence. Golden Gate Wax and Brown Kentucky Wonder 298 have been used in breeding for resistance to four races (Dundas 1942). Cultivars/lines like Virginia 119, No.780 (Foster 1947), Hawaiian Wonder (Frazier and Hendrix 1949), Seminole (Anonymous 1953), Redlands

Greenleaf and Redlands Pioneer (Anonymous 1966), Jackson Wonder and Strikton (Makraw et al. 1973), Brazil K-12973 (Sokol et al. 1977), Catu and Carioca (Issa 1985) Serva Negra, Ag497 and Agrorrico (Azevedo and Kushalppa 1986), Gresham (Anonymous 1989), PV 136, PV 88, PV 89 and PV 3 (Prakasam and Thumburaj 1991), white seeded Enganador, Chevere and CC 25-9B, Negro Inifap (Villar-Sanchez and Lopez-Salinas 1993), Negro Cotaxtle 91 (Becerra Leor et al. 1994), IBRN-6, IBRN-11, Hawaiian Wonder, Bush Bean S-9, Bush Blue Lake and Contender (Ghimire et al. 1995), Ouro Negro (Faleiro et al. 1996), IIHR 220, stringless bean (Mohan et al. 1997), Mimoso Rasteiro (Paula et al. 1998), SVM-1, Hans (Sharma 1998) and IC 47643, PI 201489 and PI 247761 (Bhat et al. 1999) have been found to be resistant in different parts of the world. Cv. Ouro Negro showed immunity to 3 out of 4 races tested and PI 181395 was the best source of resistance to rust, as it was immune to all the four races of *U. appendiculatus* (Faleiro et al. 1998). Inheritance studies indicated that resistance to rust was controlled by a single, dominant gene (Aghora et al. 2007). Line “Arka Anoop” developed by the breeding programme is resistance to rust and has high pod yield and good pod quality. Germplasm lines/cvs. like Alapa Trey, Sing Tamey and Local Collection were found resistant to this disease under Solan, Himachal Pradesh conditions (Sharma et al. 2014).

Biological Control

Verticillium lecanii has been suggested to be a potential biological agent for the management of rust as it penetrates hyphae and invades urediniospores of the fungus (Allen 1982). Under field conditions *Verticillium lecanii* reduced the disease incidence and severity to 56% and 17% as compared to 98% and 85%, respectively, in agrochemical package while seed yield was slightly less in biocontrol than agrochemical package which may be due to the use of fertilizer in latter package (Carrion et al. 1999). Different isolates of *Bacillus subtilis* like APPL-1 (Baker et al. 1983, 1985) and FF-1, FF-5 and FF-6 (Mizubuti et al. 1995) have been selected as the potential biocontrol agents. In culture medium *B. subtilis* isolates released a thermostable substance(s) antagonistic to the rust fungus as pustule number was reduced by >95% (Centurion et al. 1994). Isolate B 206 showed the greatest efficacy, when cells of the antagonist were automized on to the plant.

Chemical Control

Different workers suggested various chemicals from time to time for keeping the disease under check. Straib (1943) suggested the disinfection of stakes with formaline (0.1%) before reuse. Systemic fungicides are more effective than the non-systemic owing to the difficulty of obtaining under leaf coverage with the latter. Efficacy of various systemic fungicides like oxycarboxin, carboxin, triforine, bitertanol, triadimefon, tridemorph, hexaconazole and HWG 1608 has been reported against this disease (Jaffer 1971; Rolim et al. 1981; Singh and Musymi 1979, 1984; Prakasam and Thamburaj 1991; Gonzalez and Garcia 1996a, b; Sumartini 1998) and possibly eradicated the already established infection of *U. phaseoli* in bean plants

after three sprays without any sign of phytotoxicity (Singh and Musymi 1979, 1984; Gonzalez and Garcia 1996b).

Seed treatment with the thiram and subsequent sprays with mancozeb was found best combination treatment for controlling rust (Mahanta and Dhal 2000). Three sprays of hexaconazole (0.1%) at 15 days interval were found best in keeping the disease under check (Bhat 2002). Contaf (0.1%) alone and in combination with mancozeb (0.2%) were highly effective and reduced the disease incidence from 86.6% to 24.1% (Singh and Bhat 2002). Sprays of propiconazole were found to be most effective in reducing the severity of rust to 10.93% as compared to control (41.20%) and gave about 91.54% disease control (Shukla and Sharma 2009). Sprays of Azoxystrobin (0.1%) were found significantly most effective in reducing the disease severity to 4.64% and increased green pod yield by >97% (Sharma et al. 2018). This increased yield may be due to the phytotonic effect exhibited by azoxystrobin sprays which delayed the crop senescence.

Systemic Resistance Inducer

Treatment of bean leaves with salicylic acid (SA) or 2, 6-dichloro-isonicotinic acid (DCINA) induces resistance against the rust fungus *U. fabae* resulting in reduced rust pustule density (Rauscher et al. 1999). Saccharin application in broad bean (*Vicia faba*) induced resistance to the rust fungus *Uromyces viciae-fabae* (Boyle and Walters 2005) and provided systemic protection to rust infection after 14 days of application. Bion was also used to induce resistance in bean cultivars like Borlotto Nano Lingua di Fuoco (BLF), Borlotto Taylor, Cannellino, Cannellino Montalbano, Saxa and Top Crop, against *U. appendiculatus*. A single 0.3 mm BTH spray 7 days before inoculation was sufficient to fully control the disease in all the examined cultivars (Iriti and Faoro 2003).

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8 Whitefly-Transmitted Plant Viruses and Their Management

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Abstract

Whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) are major agricultural pests that cause economic damage worldwide. These pests commonly referred to a group of tiny, soft-bodied, sap-sucking winged insects generally inhabiting on the underside of the leaf. Moreover, they are enormously polyphagous showing intercrop movement, high reproduction, resistances to insecticides and virus transmission. Since decades, genetic complexity of whiteflies is debatable. Despite the presence of several whitefly species, biotype and genetic species concepts also exist. Like most arthropods, whiteflies too harbour endosymbionts (both primary and secondary), essential for its survival and development. These endosymbionts are specific to species, host and geographic location, which enable easy differentiation among populations of the same species. Apart from causing losses through direct feeding, they also act as vector for various economically important plant viruses like *Begomovirus*, *Geminivirus*, etc., and transmit viral particles via persistent and semi-persistent mode of transmission. Management of whitefly populations, and, in particular, management of the viral plant diseases it transmits, is difficult. At present, the use of insecticides is the main approach employed to manage whiteflies. However, due to both environmental concern and resistance issue, this practice is greatly restricted. Hence, integrated

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pest management programme with all available tactics would help in reducing the pest population.

Keywords

Begomovirus · Biotype · Endosymbionts · *Geminivirus* · Vector management · Whiteflies

8.1 Introduction

Crop diversification with vegetables, flowers, medicinal plants and fruits provides a viable option to enhance farmers' income. Various biotic and abiotic stresses may hamper agricultural production. Among biotic stresses, insect pests and diseases are the major constraints resulting about 10–30% yield loss in various crops (Rai et al. 2014). Sucking pests like whiteflies cause incalculable loss globally as they pose serious threat to many host plants. Crops grown under protected conditions like greenhouse as well as in open fields are very prone to whitefly attack causing damage through direct feeding and virus transmission. Previously, sporadic infestation of this pest and the diseases they transmit was managed through a cocktail of insecticides; however, uncontrollable outbreaks at recent times have become serious cause for concern. Once established under favourable microclimate, pest density rises to outbreak levels where its control becomes difficult. Intervention to control pest should also be made when population reaches the economic threshold limit (ETL), while subsequent delay with initial control measures may prove expensive to farmers. In field crops like cotton, greenhouse crops like tomatoes and cucumbers and ornamental species like poinsettia and gerbera, whiteflies are of major concern. Many weed species act as alternate hosts to whiteflies and often serve as sources of infestations.

Whiteflies/snow flies are tiny, sap-sucking, soft-bodied winged insects commonly found in clusters on the undersurface of the leaves (Liu et al. 2007; Zhang et al. 2007; Wan et al. 2009). They are so named as their entire body including wings is covered with white waxy powder. Adults of these whiteflies resemble small moths, while the nymphs look more like a scale insect with a flattened oval shape. Both nymphs and adult stages of whitefly have piercing and sucking mouthparts and usually penetrate the phloem and suck the sap by inserting their stylets into the leaf. During this feeding process, they acquire plant viruses. Adult whiteflies may disperse and transmit the virus to new plants at subsequent feeding. They also secrete honeydew, a sticky substance on which saprophytic sooty mould develops and thereby hinders the photosynthetic efficiency of the plant. Irrespective of the instars, whiteflies at low densities are usually not damaging, and adults do not cause significant damage unless they transmit plant viruses. Excessive feeding affects plant growth by causing distortion, discoloration, yellowing or silvering of leaves. The role of whitefly is more important as a vector of various plant viruses than its direct damage as a sucking pest. The disease attains significance because the virus is

capable of attacking the crop in all the stages of its growth period (Brown 1994; Rajinimala et al. 2005). However, the combination of direct feeding loss and vector behaviour has promoted this pest to one of the most damaging pests in agricultural production (Perring et al. 1993).

During the past decades, whiteflies have already taken a heavy toll and risen as important sucking pests of most vegetables and horticultural and ornamental crops around the world. The escalating whitefly population has resulted in increased usage of insecticides, which is not only harmful to the environment but also resulted in resistance problems. Also, various whitefly species and biotypes morphologically look similar, yet have subtle physiological differences. Due to these differences, whiteflies exhibit differential behavioural response towards management strategies. Hence, proper identification of the whitefly (i.e. species, biotype) is critical for successful control measures.

8.2 Whitefly Taxonomy and Genetic Structure

Whiteflies are genetically complex and are economically most devastating pests. Substantial effort is made in investigating the taxonomy and systematics of whitefly for decades; still its species status is being a subject of debate. Despite their name, whiteflies are not true flies; these are small hemipterans which belong to Aleyrodidae, the only family in the superfamily Aleyrodoidea.

8.2.1 Systematic Position

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Hemiptera
Suborder:	Sternorrhyncha
Superfamily:	Aleyrodoidea
Family:	Aleyrodidae

Several species of whiteflies exist, of which *Bemisia tabaci* (tobacco whitefly) and *Trialeurodes vaporariorum* (greenhouse whitefly) are few belligerent pests that cause significant damage to a wide range of economically important crop plants. Apart from several species, biotype and genetic species concepts also exist. Biotype concept in whitefly became evident with *B. tabaci* variant, a totally different population from the native population of southern United States (De Barro et al. 2011). To identify these biotypes, molecular tools like various DNA markers such as mitochondrial cytochrome c oxidase subunit I (MtcoxI) or the ribosomal internal transcribed spacer (ITS) regions were used. Microsatellite markers are also effective for population genetic studies in insects (Dalmon et al. 2008; Boopathi et al. 2014). Recent studies with microsatellites have revealed six genetic populations with little

or no gene flow between *B. tabaci* from the Asian and Pacific region. Further, out of the six genetic populations, four subsequently split into two subpopulations. However, *B. tabaci* had 24 morphologically indistinguishable species which barely interbreed and form different phylogenetic clades, hence called as cryptic species complex exhibiting high variability in their biology and genetics (Dinsdale et al. 2010; De Barro et al. 2011).

Based on genetic differences in *B. tabaci*, 33 extant biotypes have been reported (Brown 2010; Gill and Brown 2010; Hadjistylli et al. 2010). Subsequent research suggested roles of *Wolbachia* and mating interference in *B. tabaci* revealed 11 well-defined genetic groups with at least 36 putative species (Dinsdale et al. 2010; De Barro et al. 2011; Boykin et al. 2012). These putative species are morphologically indistinguishable and genetically distinct and differ in their virus transmission capability, host range, fecundity and insecticide resistance (Dinsdale et al. 2010; Wang et al. 2010). Middle East Asia Minor 1 (MEAM1, known as B biotype) and Mediterranean (MED, known as Q biotype) (Hu et al. 2011; Skaljac et al. 2013) are two widespread putative species that caused extensive damage to numerous crops worldwide. Later on, three putative species (MEAM1, MED and JpL (*Lonicera japonica*)) were identified. MEAM1 and MED were first detected in 1998 and 2004 (Lee et al. 2000, 2005), respectively, whereas JpL was first recorded in 2014 (Lee et al. 2014). B biotype was first reported from Kolar district of Karnataka, India (Rekha et al. 2005). Whole genome-wide variants between Asia II 1 (indigenous to Indian subcontinent and South-East Asia) and MEAM1 (originated from Middle East that has spread globally in recent decades) contribute to resolving species delimitation of whitefly. MtcoxI sequence analysis helped in identifying cryptic species showing 3.5% pairwise divergence within *B. tabaci* species. About 42 distinct species have been reported: Africa, Asia I, Asia I-India, Asia II 1–12, Asia III, Asia IV, Asia V, Australia, Australia/Indonesia, China 1–5, Indian Ocean, Ru, Middle East Asia Minor I-II (MEAM), Mediterranean (MED), MEAM K, New World 1–2, Japan 1–2, Uganda, Italy 1 and Sub-Saharan Africa 1–5 (Firdaus et al. 2013; De Barro et al. 2011; Boykin et al. 2007; Hu et al. 2018; Roopa et al. 2015). Lately, in the identification of cryptic species within this species complex, 4% genetic divergence was found more realistic than 3.5% (Lee et al. 2013).

Like most arthropods, whiteflies too harbour endosymbionts (bacterium called *Candidatus Portiera aleyrodidarum*) confined to bacteriocyte cells (Thao et al. 2004; Sloan and Moran 2012). This bacterium is essential for host survival and development and has a long co-evolutionary history with all members of the subfamily Aleyrodidae (Thao et al. 2004; Moran and Telang 1998; Baumann 2005). Apart from primary endosymbiont, secondary endosymbionts, namely, *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Hemipteriphilus*, *Fritschea*, *Rickettsia* and *Wolbachia*, have been reported from *B. tabaci* populations around the world.

8.3 Whitefly: An Overview

Whiteflies consist of more than 1500 species in approximately 126 genera (Martin, 2004), but relatively few transmit plant viruses (not even 1%). Whiteflies in the *Bemisia* and *Trialeurodes* genera are vectors of various plant viruses. Reports suggest that the following five species of whitefly are identified as transmitting virus to plant: *Bemisia tabaci*, *Bemisia afer* (Priesner & Hosny), *Trialeurodes abutilonea* Haldeman (banded wing whitefly), *Trialeurodes vaporariorum* Westwood (greenhouse whitefly) and *Parabemisia myricae* Kuwana (bayberry whitefly) (Ng and Falk 2006; Gamarra et al. 2010; Navas-Castillo et al. 2011). Whitefly vector diversity is much lower than other vectors, yet considered as the second most important type of vector due to its capacity to transmit many plant viruses. Notably it transmits 90% of the viruses belonging to the genus *Begomovirus* which currently comprises around 200 members. Further, whitefly-transmitted viruses include ipomoviruses and criniviruses of the family Potyviridae and Closteroviridae, respectively.

8.4 Whitefly Distribution

Whitefly is a serious pest in agricultural, horticultural and ornamental crops; apart from direct losses by its feeding, the major concern lies with its role as a virus vector. Depending on the molecular phylogenetic data and genetic group of reciprocity, about 35 cryptic species complex are recognized worldwide in *B. tabaci* (De Barro et al. 2011). *B. tabaci* species complex has been reported in 83 countries globally in all six continents, namely, Asia, Africa, Australia, Europe, South America and Central America. In Indian, infestation of whitefly was first reported on cotton (Misra and Lambda 1929). Earlier *B. tabaci* was considered as a single species, but recent reports confirmed it as a complex species comprising Asia I, Asia I-India, Asia II 1, Asia II 5, Asia II 7, Asia II 8, Asia II 11, Asia II 13, MEAM K, China 3, MEAM1 types (Kanakala and Ghanim 2019). *B. tabaci* species complex has been reported in almost all Indian states starting from Jammu and Kashmir to Tamil Nadu and Arunachal Pradesh to Gujarat.

8.5 Whitefly Host Range

B. tabaci is polyphagous and has a broad host range which includes some of the common plants like cassava (Lal 1981), cinnamon (Koya et al. 1983), tomato (Bhardwaj and Kushwaha 1984; Qiu et al. 2007), eggplant (Balaji and Veeravel 1995), okra (Bhagabati and Goswami 1992), sunflower (Men and Kandalkar 1997; Qiu et al. 2007), black pepper (Ranjith et al. 1992), pulses (Patel and Srivastava 1998), cucumber, groundnut, cauliflower, cabbage and tobacco (Ellango et al. 2015) and several other host plants (Lisha et al. 2003).

8.6 Whitefly Life Cycle

Life cycle of all whiteflies is similar, staring from egg to adult phase through four nymphal instars. Both male and female adults are similar, except that the female is usually larger. Eggs are oval shaped and laid on the underside of the leaf in horseshoe or circular patterns. Subsequently, upon hatching from the eggs, crawlers/first instar nymphs (about 0.3 mm long) wander over the leaf surface. After a week's time, crawlers gradually settle down and remain sedentary. Later on, they pass through second, third and fourth nymphal instars. Both legs and antennae are lost after the first moult, and the subsequent nymphs remain fixed to the leaf surface. Generally, whitefly nymphs are flat and oval in shape and yellowish or black in colour and often resemble small-scale insects. Second and third instar nymphs are pale green. Fourth instar nymphal colour is acquired based on the host plant, upon which they feed. For example, yellowish individuals are associated with herbaceous plants while black on woody plants. Fourth instar nymphs feed initially and thereafter ceases feeding upon maturity (i.e., during gradual transformation into adult internally). From the fourth instar nymphs, winged adults emerge and hence often incorrectly called as pupa. After eclosion, adult whiteflies are often pale green or yellow in colour and thereafter secrete a white waxy coating. Irrespective of the instars, all stages of whitefly suck the plant sap and excrete the excess liquid as honeydew. Whitefly populations have several generations in a year, where warm weather generally flourishes the population with its peak at spring and autumn. The entire life cycle may be completed in 18 days at temperatures of 28 °C, depending on species. All growth stages can often be found on leaves at any one time (Fig. 8.1).

8.7 Whitefly and Virus Association and Disease Transmission

Geminiviruses are a large family of plant viruses with circular, single-stranded DNA genome (H). The genome organization and molecular properties have classified Geminiviruses into seven genera, namely *Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocuvirus* and *Turncurtovirus* according to International Committee on Taxonomy of Viruses. In tropical and semi-tropical region, *B. tabaci* majorly acts as a vector of *Begomovirus* and transmits virus to both monocots and dicots (Navas-Castillo et al. 2011; Brown et al. 2010). As far as whiteflies are concerned, it transmits viruses by two modes, namely, persistent and semi-persistent; their acquisition and retention time has been discussed in Fig. 8.2. Likewise, the number of viruses transmitted by different species of whitefly was given in Tables 8.1 and 8.2 (a, b).

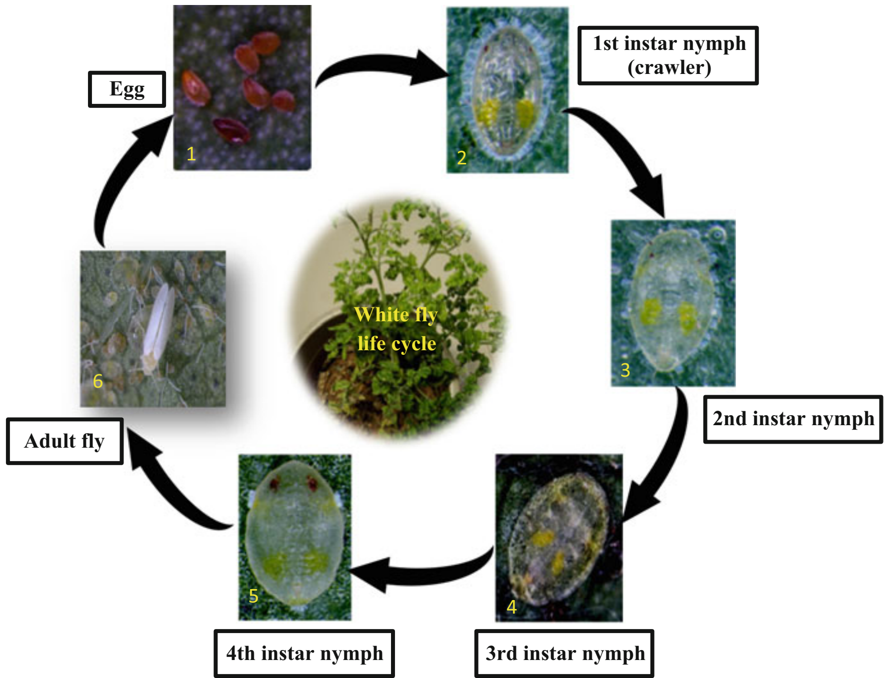

Fig. 8.1 Life stages of *Bemisia tabaci*. 1. Egg, 2. First instar nymph (Crawler), 3. Second Instar nymph, 4. Third instar nymph, 5. Fourth instar nymph and 6. Adult fly (Source: Naveen 2016)

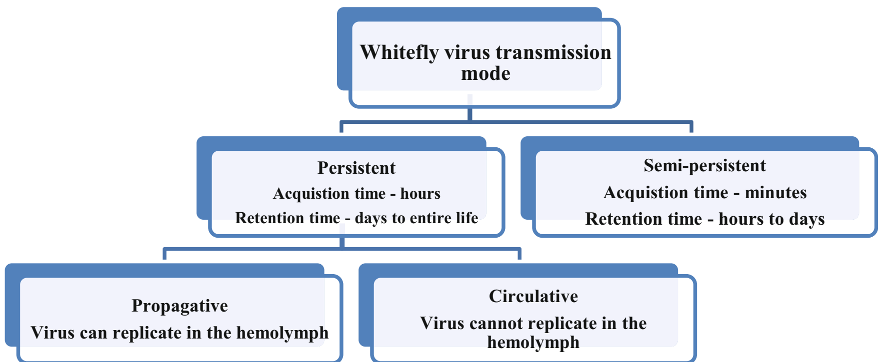

Fig. 8.2 Transmission mode of viruses by whitefly

Table 8.1 Summary of virus transmitted by whitefly species (Navas-Castillo et al. 2011)

Virus (genus; family)	Transmission mode	Whitefly species	No. of approved species[a]
Begomovirus; Geminiviridae	Circulative, persistent	*B. tabaci*	192
		T. ricini	1
Ipomovirus; Potyviridae	Semi-persistent	*B. tabaci*	4
Crinivirus; Closteroviridae	Semi-persistent	*B. tabaci*	4
		B. afer	1
		T. abutilonea	4
		T. vaporariorum	4
Carlavirus; Betaflexiviridae	Semi-persistent	*B. tabaci*	3[b]
Torradovirus; Secoviridae	Unknown	*B. tabaci*	2[c]
		T. vaporariorum	1

[a]According to King et al. (2011) except as noted
[b]Includes Cucumber vein-clearing virus (Menzel et al. 2011)
[c]Includes Tomato necrotic dwarf virus (Wintermantel and Hladky 2013)

8.8 Whitefly Management

Management of whitefly populations and in particular the viral disease it transmits is difficult. Polyphagous nature, high reproductive rate, short generation time, biotypes, rapid evolution of resistance to insecticides and the relatively protected location of the individuals on the underside of the leaves contribute to its survival and dominance in the agroecosystem. Invariably different management strategies have been employed vector management worldwide. Hence, durable and cost-effective pest-control strategies that include good agricultural practices like routine monitoring fields, breaking the pest continuum, conserving natural enemies and using pesticides only when necessary are required. Whiteflies can be controlled effectively when crops are grown under protected environment like greenhouses or polyhouses. If farmers practice open cultivation, they will have to adopt some preventive practices to avoid complete crop loss:

- Management of virus and vector reservoirs
- Host plant resistance
- Use of mulching
- Virus-free seed/planting material
- Trap crop
- Application of insecticides

Table 8.2 Whiteflies transmitting viral diseases with global significance

2a) *B. tabaci* transmitting viral diseases						
Crop	Disease	Other host	Affected area	Affected region	Monetary loss (US$) and yield loss	References
Tomato	Tomato yellow leaf curl disease	Eggplants, potatoes, tobacco, beans and peppers	7 million ha	Middle East Asia; Mediterranean Basin; North America, Australia	Early stage – 100% 30 Days after planting – 80%	CABI (2019)
Cassava	Cassava mosaic disease	Soybean, *Jatropha*, castor bean, Ceara rubber	2.6 million ha	Africa Indian subcontinent	1.9–2.7 billion; Early stage – 90% 30 Days after planting – 80%	Legg et al. (2006)
Bean	Bean golden yellow mosaic virus	*Phaseolus lunatus*, *P. acutifolius and P. coccineus*, *Vigna luteola* *Macroptilium lathyroides*, *Malvastrum coromandelianum*	4 million ha	North America, South America	Early stage – 75%	EPPO (2019)
Cucurbit	Cucurbit yellow stunting disorder virus	Alfalfa, lettuce, snap bean	1 million ha greenhouse/ polyhouse	Africa, Asia, Europe, North America	40–60%	Abrahamian and Abou-Jawdah (2014) and CABI (2019)
Cucurbit	Cucurbit chlorotic yellows virus	Lettuce, spinach, sugar beet	2 million ha	Asia, Africa, North America	60%	Abrahamian and Abou-Jawdah (2014)
Sweet potato	Sweet potato mild mottle virus	Beets, sowbane, devil's snare, tomato, tobacco, petunia and zinnia	4–5 million ha	Africa, South America	90%	Data from FAO and regions calculated from this study

(continued)

Table 8.2 (continued)

2b) ***Trialeurodes*** **spp. transmitting viral diseases** (Jones 2003; CABI, 2019)				
Crop	Disease	Other host	Affected region	Monetary loss (US$) and yield loss
Tomato	Tomato infectious chlorosis virus	*Callistephus chinensis*, *Cynara cardunculus*, *C. scolymus*, *Lactuca sativa*, *Lycopersicon esculentum*, *Nicotiana glauca*, *Petunia hybrida*, *Physalis ixocarpa*, *Picris echioides*, *Ranunculus* spp.	Asia, Europe, North America	Greenhouse – 80–100% Field condition – 50%
Tomato	Tomato chlorosis virus	*Datura stramonium*, *Lycopersicon esculentum*, *Solanum nigrum*	Europe, North America	Greenhouse – 80–100% Field condition – 50%
Potato	Potato yellow vein virus	*Catharanthus roseus*, *Lycopersicon* spp., *Polygonum* spp., *Rumex obtusifolius*, *Solanum nigrum*, *S. tuberosum*, *Tagetes* spp.	South America	Up to 50%
Beetroot	Beet pseudo-yellow virus	*Beta vulgaris*, *Lactuca sativa*, *Cichorium endivia*, *Cucumis melo*, *C. sativus*, *Capsella bursa-pastoris*, *Taraxacum officinalis*, *Conium maculatum*	Australia, Europe, North America	Early stage – 50%
Sweet potato	Sweet potato chlorotic stunt virus	*Ipomoea batatas*	Asia, Africa, North America	50% or more

8.8.1 Management of Virus and Vector Reservoirs

Control of weeds and alternate hosts near to the main crop could reduce the off-season survival of vector and virus load effectively (Adkins et al. 2011). Virus-infected fields are often the most important source of inoculums. For example, virus-infected tomato fields are often the most important source of the tomato yellow leaf curl virus (TYLCV) and its vector (Polston and Lapidot 2007). Hence, removal of virus-infected plants can contain the spread of the disease to healthy plants. Not every time visible symptoms appear. Symptomless infection of pepper can also provide a reservoir of TYLCV for tomato (Morilla et al. 2005; Polston et al. 2006). Hence, to reduce the initial TYLCV inoculum levels, pest continuum needs to be disrupted through creating host/crop-free period of tomato and pepper (Polston et al. 2009). This will reduce the spread of virus as without the susceptible host plant it cannot spread and also it breaks the phonological synchrony.

8.8.2 Host Plant Resistance

Potential source of resistance have been identified in wild germplasm that could reduce the virus spread. Screening is done for both the vector (white fly) as well as the virus in wild germplasm; if the screened material was found resistant against any one, it shall be considered as a good donor to develop resistant genoptypes (Table 8.3).

8.8.3 Nutrient Management

Like in most crops, nutrient management is an essential component in pest management. Excessive use of nitrogenous fertilizer promotes luxuriant plant growth, thereby increasing the susceptibility to whitefly infestation. Also phosphorus and magnesium levels of the plants need to be monitored, as deficiencies in these nutrients are believed to contribute to whitefly infestations.

8.8.4 Use of Mulching

Under open-field situation, plastic/polyethylene soil covers (mulch) are a popular strategy for protection against whiteflies and the viruses they transmit (Table 8.4). Apart from reducing the pest inoculum, it also inhibits germination and growth of weeds.

8.8.5 Virus-Free Seed/Planting Material

To avoid the infestation of any pest and disease at the field level, healthy and virus-free planting material like seeds or vegetatively propagated planting material is best. Vegetatively propagated crops like cassava, banana and sweet potato are particularly

Table 8.3 Summary of virus resistant sources

Crop	Virus	Resistance source	References
Tomato	TYLCV	*Solanum pimpinellifolium*, *S. peruvianum* (PI 126926, PI 126930, PI 390681), *S. chilense* (LA1969, LA2779, LA1932) and *S. habrochaites* (B6013), *S. arcanum* (LA0441)	Lapidot et al. (2014)
Tomato	Tomato infectious chlorosis virus (TICV)	Wild *Solanum* species (acylsugars derived gene)	Lapidot et al. (2014)
Watermelon	Squash vein yellowing virus (SqVYV)	Wild citron - PI 500354 (*Citrullus lanatus* var. *citroides*) PI 386024 (*C. colocynthis*) PI 459074 and PI 392291 (*C. lanatus* var. *lanatus*)	Kousik et al. (2009)
Watermelon	Zucchini yellow mosaic virus and PRSV-W	Virus-tolerant rootstocks	Wang et al. (2002)
Watermelon	Cucurbit yellow stunting disorder virus (CYSDV)	PI 313970 and TGR-1551	Lapidot et al. (2014)
Bean	TYLCV	Breeding line - GG12	Monci et al. (2005)
Cassava	Cassava mosaic virus (CMD)	African landraces (CMD2 gene)	Akano et al. (2002)

Table 8.4 Types of mulching used for controlling whiteflies

Particulars	Coloured (yellow) mulching	Silver (aluminium) coated plastic mulching
Mode of action	Attractant whiteflies attract to the mulch instead of to the host	Deterrent Strongly reflects light, which acts as a deterrent to the invading whiteflies
Crop period	Early crop	Early crop
Successful field application	Tomato field against TYLCV in Israel	*Tomato mottle virus* in Florida; Cucurbit leaf crumple virus (CuLCrV) in Zucchini squash (*Cucurbita pepo* L.)

vulnerable to virus infection. Hence, tissue culture technique is successfully employed to produce virus-free seed/planting material for vegetatively propagated crops (Clark et al. 2012). In cassava, tissue culture and virus indexing with thermotherapy has been routinely used for many years to ensure virus-free germplasm exchange between continents.

8.8.6 Trap Crops/Barrier Crops

Trap crops are those crops which are grown along with the main crop to attract the pest towards them, so that the main crop suffers no or little damage. Similarly, as the name suggests, barrier crops are crops grown along the borders of the main field as barrier to the movement of the pest from nearby fields. Both trap crops and barrier crops have shown to be effective in reducing the whitefly populations, consequently reducing the level of virus infection (Table 8.5).

8.8.7 Sticky Traps

Generally, yellow sticky traps or yellow pan traps are used to attract the whiteflies. It helps in both monitoring and mass trapping of the pest. These traps (25/acre) are hanged close to the crop canopy or at 30 cm from the ground.

8.8.8 Biological Control

In general, biological control is more expensive than chemical control and does not eliminate the pest completely from the agroecosystem. Biological control is often successfully used to suppress whitefly populations in greenhouses in Europe but is less widespread in the United States. Natural enemies include entomopathogens, predators and parasitoids. The only disease-causing organisms known to attack whiteflies are fungi. Currently, commercially available entomopathogenic fungi are Mycotal® (*Verticillium lecanii*), BotaniGard® (*Beauveria bassiana*) and

Table 8.5 List of trap/barrier crops used against whiteflies/viruses

Trap crop		
Main crop	Trap crop	Vector/virus to be trapped
Tomato	Squash	*Bemisia tabaci* (TYLCV)
Tomato	Cucumber	*B. tabaci* (TYLCV)
Cotton	Cantaloupes	*B. tabaci*
Cotton	Sunflower	*B. tabaci*
Cassava	Soybean	*Aleurotrachelus socialis*, *Trialeurodes variabilis*
Barrier crop/intercropping		
Tomato	Sorghum	*B. tabaci*
Tomato	Maize	*B. tabaci* (TYLCV)
Cassava	Maize, cowpea	CMD (Cassava mosaic disease)
Cotton	Maize	*B. tabaci*
Cow pea	Pearl millet	Virus transmission
Cucumber	Maize	CVYV
Squash	Maize	Squash leaf curl virus
Zucchini	Okra, sun hemp	*B. tabaci*

PreFeRal® (*Paecilomyces fumosoroseus*). However, various predators and parasitoids are also found effectively reducing the whitefly population. Habitat management through border cropping with perennial plants provides year-round refuge for these predators. General predators like the coccinellid (both adult and grub) beetles feed on whiteflies. Another predatory beetle, *Delphastus catalinae*, consumes whitefly eggs and nymphs and attacks all species of whitefly. It avoids eating whitefly nymphs that have parasitoids developing within them, which means that it can be released together with a parasitoid without interfering with parasitism. Apart from predators, a nymphal parasitoid, *Encarsia formosa*, is smaller than the whiteflies it attacks and oviposits on whitefly nymphs; subsequent parasitoid larva develops within the host body by consuming it from the inside over a period of 1–2 weeks. This parasitoid could parasitize three whitefly species, namely, banded winged whitefly (*Trialeurodes abutiloneus*), greenhouse whitefly (*T. vaporariorum*) and sweet potato whitefly (*B. tabaci*), but provides the best control against greenhouse whitefly, especially at cooler temperatures. Likewise, *Eretmocerus eremicus* is another parasitoid that is commercially available, provides better control against *B. tabaci* than *Encarsia inaron* and performs better at higher temperatures.

8.8.9 Chemical Control

Whiteflies are mainly controlled through multiple applications of insecticides with different modes of action. This method delays the development of insecticide resistance and gives better results at the field level and limits the spread of whitefly-transmitted viruses. The list of insecticides used in India against whiteflies with respect to crop has been given in detail (Table 8.6). In India, only 5 different modes of action insecticides are available for whitefly management, whereas the United States had registered 11 different group of synthetic chemical along with 6 different groups of mixer and biopesticides (Table 8.7). Comprehensive control of whiteflies with conventional insecticides is difficult to achieve. Therefore, novel insecticides with relative target pest specificity are recommended for the effective management of whiteflies, which prove to be less harmful to natural enemies and the environment. Accordingly, they are also more suitable for integrative combination with other methods.

8.8.9.1 Insect Growth Regulators (IGRs)

Insect growth regulators like buprofezin and novaluron are chitin synthesis inhibitors effective in managing the pest. Chitin is the integral part of the insect exocuticle, due to action of chitin synthesis inhibitor; chitin is not produced as the result procuticle of the whitefly nymph loses its elasticity and the insect is unable to moult. While pyriproxyfen is a juvenile hormone (JH) mimic that affects the hormonal balance in insects, suppresses embryogenesis, metamorphosis and adult formation.

Table 8.6 List of insecticides recommended against whitefly in India (CIBRC 2020)

Insecticide name	Crop	Mode of action		Application rate	
		Site of action	Code	a.i (g/ha)	Formulation (g/ml)
Acetamiprid 20% SP	Cotton	Nicotinic acetylcholine receptor (nAChR) agonists	4A	10–20	50–100
Diafenthiuron 50% WP	Cotton	Inhibitors of mitochondrial ATP synthase	12A	300	600
Flonicamid 50% WG	Cotton	Chordotonal organModulators	29	75	150
Monocrotophos 15% SG	Cotton	Acetylcholinesterase (AChE) inhibitors	1B	200	1333
Profenofos 50% EC	Cotton	Acetylcholinesterase (AChE) inhibitors	1B	500	1000
Spiromesifen 22.9% SC	Tomato	Inhibitors of acetyl-CoA carboxylase	23	150	625
Spiromesifen 22.9% SC	Cotton	Inhibitors of acetyl-CoA carboxylase	23	144	600
Thiamethoxam 30% FS	Cotton	Nicotinic acetylcholine receptor (nAChR) agonists	4A	3	10
Thiamethoxam 70% WS	Cotton	Nicotinic acetylcholine receptor (nAChR) agonists	4A	300	430
Thiamethoxam 12.6% + Lambda-cyhalothrin 9.5% ZC	Tomato	Nicotinic acetylcholine receptor (nAChR) agonists + Sodium channel modulators	4A + 3A	44	200

8.8.9.2 Neonicotinoid Insecticides

Neonicotinoids are nicotine mimics which bind to the nicotinic acetylcholine receptor (nAChR) of both the central and peripheral nervous systems, resulting in excitation and paralysis followed by insect death. Imidacloprid was the first commercial neonicotinoid successfully used for controlling agricultural pests.

8.8.9.3 Diafenthiuron

Diafenthiuron is a thiourea derivative which directly affects insect respiration via inhibition of oxidative phosphorylation and disruption of mitochondrial ATP synthesis.

8.8.9.4 Pyridine Insecticides (Pymetrozine)

Pymetrozine is an azomethine pyridine insecticide which affects the nerves controlling the salivary pump and thereby causes immediate and irreversible cessation of feeding due to obstruction in stylet penetration, followed by starvation and insect death.

Table 8.7 List of insecticides recommended against whitefly in the United States (Vegetable Production Handbook for Florida, 2020 and Lapidot et al. 2014)

Insecticide name	Group	Mode of action	
		Site of action	Code
Oxamyl	Carbamates	Acetylcholinesterase (AChE) inhibitors	1A
Methamidophos, Acephate	Organophosphates	Acetylcholinesterase (AChE) inhibitors	1B
Endosulfan	Cyclodiene Organochlorines	GABA-gated chloride channel blockers	2A
Beta-cyfluthrin, Bifenthrin, Esfenvalerate, Gamma-cyhalothrin, Lambda-cyhalothrin, Pyrethrins + Piperonyl butoxide, Zeta-cypermethrin	Pyrethroids Pyrethrins	Sodium channel modulators	3A
Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Thiamethoxam	Neonicotinoids	Nicotinic acetylcholine receptor (nAChR) competitive modulators	4A
Sulfoxaflor	Sulfoximines	Nicotinic acetylcholine receptor (nAChR) competitive modulators	4C
Flupyradifurone	Butenolides	Nicotinic acetylcholine receptor (nAChR) competitive modulators	4D
Pyriproxyfen	Pyriproxyfen	Juvenile hormone mimics	7C
Pymetrozine	Pymetrozine	Chordotonal organ TRPV channel modulators	9B
Afidopyropen	Pyropenes	Chordotonal organ TRPV channel modulators	9D
Novaluron	Benzoylureas	Inhibitors of chitin biosynthesis affecting CHS1	15
Buprofezin	Buprofezin	Inhibitors of chitin biosynthesis, type 1	16
Fenpyroximate	METI acaricides and insecticides	Mitochondrial complex I electron transport inhibitors	21A

(continued)

Table 8.7 (continued)

Insecticide name	Group	Mode of action	
		Site of action	Code
Spiromesifen, Spirotetramat	Tetronic and tetramic acid derivatives	Inhibitors of acetyl-CoA carboxylase	23
Chlorantraniliprole	Diamides	Ryanodine receptor modulators	28
Flonicamid	Flonicamid	Chordotonal organ Modulators	29
Lambda-cyhalothrin + chlorantraniliprole	Mixes of more than one active ingredient		3A + 28
Bifenthrin + imidacloprid	Mixes of more than one active ingredient		3A + 4A
Lambda-cyhalothrin + Thiamethoxam	Mixes of more than one active ingredient		3A + 4A
Bifenthrin + Avermectin B1	Mixes of more than one active ingredient		3A + 6
Thiamethoxam + chlorantraniliprole	Mixes of more than one active ingredient		4A+ 28
Buprofezin + flubendiamide	Mixes of more than one active ingredient		16 + 28

8.8.9.5 Ryanodine Receptor Insecticides (the Diamides)

Ryanodine receptors are a class of ligand-gated calcium channels which controls the release of calcium from intracellular stores. Cyazypyr™ is the commercially available ryanodine formulation specific for sucking pests such as whiteflies and aphids.

8.8.10 Genetically Engineered Resistance

Genetic engineering is a potent tool that broadens and enriches the resistance gene pool against virus diseases. Viral coat protein gene expression in transgenic plants confers resistance against virus. This type of induced resistance effect is categorized as 'coat-protein-mediated' protection, a part of pathogen-derived-protection (PDR) strategies. Further for plant transformations, constructs showing mutated or truncated virus genes or virus RNA sequences are used, which interfered with virus infection or silenced the expression of viral genes. Most transgenics expressing truncated virus genes are model crops and not the field or horticultural crop itself.

8.8.10.1 Replication (*Rep*)-Associated Proteins

Transgenic tomato plants expressing truncated geminivirus *Rep* genes exhibited resistance against *Tomato yellow leaf curl Sardinia virus* (TYLCSV). The *Rep* genes particularly interfered with related viral infection and not with those viral strains which showed low identities at amino acid level.

8.8.10.2 Movement Proteins (*mp*)

Transgenic tomato plants with mutated *Bean dwarf mosaic virus* (BDMV) *mp* gene conferred resistance against *Tomato mottle virus* (ToMoV). This gene provides a

wide range of resistances; however, its over-expression induces toxic effects making them impractical.

8.8.10.3 Gene Silencing

Target gene that needs to be suppressed/silenced can be achieved by blocking their expression either through transcriptional gene silencing by DNA methylation or post-transcriptional gene silencing (PTGS) by degradation of mRNA. PTGS can be triggered by the expression of dsRNAs homologous to virus sequences. PTGS was used successfully against the begomoviruses *Mung bean yellow mosaic virus* (MYMV), Tomato Leaf Curl Virus (ToLCV), etc.

8.8.10.4 Antisense RNA

In vivo base pairing of RNA molecules with sequence complementation to the viral RNA can prevent RNA translation or induce PTGS. This strategy has been successfully exploited to target and selectively suppress the expression of geminivirus genes of tomato golden mosaic virus (TGMV), ToLCV and others; however, this approach failed when tested against other geminiviruses.

8.9 Conclusion

Apart from causing direct yield loss through infestation, whiteflies also act as vector for various plant viruses inflicting 60–100% yield loss in different crops. Further, whiteflies can easily adapt to changing climatic conditions, especially in subtropical and tropical agroecosystems and in temperate regions under greenhouse conditions. Managing the vector itself can reduce the disease incidence and further spread of the same to new regions. Hence, vector management is of prime importance especially for controlling whitefly-transmitted viral diseases. Careful integration of various IPM components like host plant resistance, physical/mechanical methods, biocontrol and need-based chemical control with selective novel insecticides would discourage pest population explosion and also minimize the pesticide load at levels that pose risk to human health and the environment. Currently, whitefly management solely relies upon the predominant use of insecticides. Adopting IPM package will alleviate the numerous concerns that accompany the use of chemicals, including environmental pollution and resistance development. Moreover, uses of novel insecticides are relatively target specific and therefore pose minimal hazard to natural enemies and the environment. Further, these new molecules should be compatible with other management strategies to enhance the efficiency of the existing IPM package.

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9 Recent Advances in Management of Bacterial Diseases of Crops

M. R. Ravikumar, H. S. Mahesha, J. U. Vinay, and K. Dinesh

Abstract

The number of diseases caused by bacterial plant pathogens was less as compared to fungi, but the loss caused by these diseases is huge. In the recent past, some of the bacterial diseases which had minor importance earlier were major constraints in crop production due to changing climate, which led to lesser productivity. For the management of bacterial diseases, farmers solely rely on chemicals, i.e. antibiotics and antibacterial chemicals. Many of the antimicrobial agents currently available for agricultural use are highly toxic, non-biodegradable and responsible for causing chronic contamination of the ecosystem. In addition, an increasing number of phytopathogens develop resistance against antibiotics and even residual problems in food products. To address this problems, there is a need of novel technologies in plant protection, which includes nanotechnology, chitosan as defence elicitor, CRISPR/CAS, transgenic crops, bacteriophages and endophytes.

Keywords

Bacterial diseases · Management · Nanotechnology · Elicitors · Transgenics · Antagonists

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9.1 Introduction

India had a population of 1.21 billion in 2011 and projected population of 1.5 billion during 2030. The population had been growing at a rate of 1.76% per annum in the decade of 2001 to 2011. The projected estimate of the total food grain demand is 311 Mt. comprising 122 Mt. of rice, 115 Mt. of wheat, 47 Mt. of coarse grains and 27 Mt. of pulses by year 2030 (Kumar 2016). Every year there will be an increasing demand for food due to the growing population of the world. But the natural resources that are available for agriculture are limited: these include water, agricultural land, arable soil, biodiversity, availability of non-renewable energy, human labour and fertilizers (Smil 2001). It is possible to cut the gap between demand and supply of food grain by adopting good crop protection practices. Crop protection in general and management of plant diseases in particular, play an important role in meeting the future demand with respect to both quality and quantity of food (Strange and Scott 2005). This goal became more challenging because of crop yield losses to various bacterial and fungal diseases, which accounts to about 15% (Oerke and Dehne 2004).

In case of different plant pathogenic groups, fungi and fungi-like organisms stand first in number as well as monitory loss, followed by bacteria and viruses. Bacteria are very small, simple, unicellular microorganisms. Although considered structurally simple, bacteria are extremely diverse from a metabolic standpoint and are found almost everywhere on earth and their biological properties and predominant reproduction by binary fission relate them in prokaryotes. Plant-associated bacteria may be beneficial or detrimental. Although many bacteria are strictly saprophytes and they are very beneficial to man because of their necessarily required activities to human beings includes digestion in animals, nitrogen fixing ability in roots of certain legume crops, the decomposition of plant remains and animal carcasses, and sewage disposal systems.

However, several bacteria are responsible for causing severe fatal diseases in humans, animals and plants. Besides these, some bacterial species which generally live in and around the crop plants in which they incite various diseases of economic significance are known as plant pathogenic bacteria. In general, a rod-shaped bacterium mostly infects various plants (Mansfield et al. 2012). Bacterial diseases are more prevalent in the subtropical and tropical regions of the world (Ashbolt 2004). The first bacterial disease ever discovered was anthrax (caused by *Bacillus anthracis*) in cattle and sheep in the year 1876. This discovery was immediately followed by the discovery of fire blight of pear and apple (causal agent is *Erwinia amylovora*) by T. J. Burrill from the University of Illinois (1877–1885). More than 200 species of phytopathogenic bacteria have been identified so far and almost all of them are parasites within the plant either in soil or on the surface of plants. The survey conducted by Mansfield et al. (2012) reveals the top 10 bacterial pathogens based on their economic/scientific importance including, in rank order, (1) *Pseudomonas syringae* pathovars, (2) *Ralstonia solanacearum*, (3) *Agrobacterium tumefaciens*, (4) *Xanthomonas oryzae* pv. *oryzae*, (5) *Xanthomonas campestris* pathovars, (6) *Xanthomonas axonopodis* pathovars, (7) *Erwinia amylovora*,

(8) *Xylella fastidiosa*, (9) *Dickeya* (*dadantii* and *solani*) and (10) *Pectobacterium carotovorum* (and *Pectobacterium atrosepticum*).

The important bacterial diseases in the southern part of the Indian subcontinent include bacterial blight of pomegranate (*Xanthomonas axonopodis* pv. *punicae*), bacterial wilt of ginger, tomato, chilli and eggplant (*Ralstonia solanacearum*), black rot of cabbage (*X. campestris* pv. *campestris*), tip over of banana (*Erwinia carotovora* subsp. *carotovora*), bacterial leaf spot of betel vine (*X. campestris* pv. *betlicola*), bacterial leaf spot of grapes (*Xanthomonas* sp.), citrus canker (*X. axonopodis* pv. *citri*) and bacterial spot of tomato (*X. campestris* pv. *vesicatoria*). To address the above problems for the efficient management, new approaches need to be followed.

9.2 Recent Approaches for Management of Bacterial Diseases of Plants

9.2.1 Biocontrol and Endophytes for Management of Bacterial Plant Disease

Soil microorganisms coexist in association with plant roots and also interfere with plant physiological functions and other associated microbial community in the soil. It is estimated that bacteria occupy 7% to 15% of the total root surface area. Of these, some bacteria positively affect plants and have been designated as plant growth-promoting rhizobacteria (PGPR) (Kloepper 1978). In vivo biocontrol agent selection is not a simple task due to the diversity of agents and interactions with the host plant, and therefore, efficient search methods are required. In recent years, biocontrol of phytopathogenic organisms has been considered as one of the major and potential control strategies. The use of biocontrol strategies offers several advantages over the chemical control, since it is economical, self-perpetuating and usually free from residual side effects. However, in reality, it will not immediately nor totally replace chemicals, but the use of biocontrol agents can significantly enhance quality of life, the environment and agricultural productivity.

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the destructive diseases in rice. Phenazine antibiotic produced by some fluorescent pseudomonads has excellent antibacterial activity against *X. oryzae* pv. *oryzae*. Different *Bacillus* spp. applied to rice plants as seed treatment before sowing; a root dip prior to transplantation and two foliar sprays prior to inoculation could suppress 59% of bacterial leaf blight (Vasudevan et al. 2002). Bacterial wilt caused by *Ralstonia solanacearum* is one of the most important bacterial diseases of plants of commercial value in the tropics, subtropics and regions with warm temperature around the world. Biocontrol of *R. solanacearum* is mostly based on antagonism (antibiosis) activity. Solanaceous crops other than potato and other vegetatively propagated crops were protected biologically from bacterial wilt by dipping the root system of seedlings before transplanting. *Pseudomonas putida* strain has been developed for management of tobacco and tomato bacterial wilt. Other method to

deliver bacterial antagonist is by seed treatment, either by seed dressing, seed coating or seed pelleting. Treatment of tomato seed with water suspension of *P. putida* strain Pf-20 suppressed bacterial wilt into some degree (Asrul et al. 2004). Crown gall, caused by *Agrobacterium tumefaciens*, is distributed worldwide and is responsible for nursery and field losses among the large variety of plants especially stone fruit trees. The method of control is by inoculation of planting material with non-pathogenic *A. radiobacter* strain K84 immediately before sowing or planting. For over 15 years, crown gall on many different host plants has been successfully controlled by K84 in many countries. The inhibition of the pathogen by Agrocin 84 (a bacteriocin produced by K84), is mainly due to competition for site and certain nutrient. biological site competition and competition of a certain nutrient that common these bacteria.

Endophytes occur almost everywhere in various parts of the world with diversified climatic conditions, and their association with algae, bryophytes, pteridophytes, gymnosperms and angiosperms has been studied in detail. Information compiled from more than three decades of research on endophytic fungi could reveal that 347 host plants belonging to 119 plant families have been screened out of 17,527 angiosperms and 67 gymnosperm species reported from India (Karthikeyan et al. 2005). Pre-treatment of cotton seedlings with *B. bassiana* also resulted in reduced severity of bacterial blight disease caused by *Xanthomonas axonopodis* pv. *malvacearum* (Griffin et al. 2006; Ownley et al. 2008). Machavariani et al. (2014) reported on endophytic isolates from the medicinal plants *Aloe arborescens*, *Mentha arvensis*, *Lysimachia nummularia*, *Fragaria vesca* and *Arctium lappa*. The endophytic isolates were identified as *Nocardiopsis*, *Streptomyces* and *Micromonospora*. The assay was done by the well diffusion method. The isolates showed activity against different strains of bacteria such as *S. aureus* strain FDA 209P, *S. aureus* 209P/UF-2, *Micrococcus luteus* strain 9341, *B. subtilis* strain 6633 and *E. coli* strain 25922. Some examples of endophytes against phytopathogenic bacteria are presented in tabular form.

9.2.2 Bacteriophages

Bacteriophages (phages) are obligate intracellular parasites that multiply inside bacteria by using some or all of the host biosynthetic machinery. Phages specific to *X. oryzae* pv. *oryzae* are found in the water of rice field, irrigation canal and rivers. The population density of bacteriophage is correlated with the number of its bacterial host. However, the problem in using of bacteriophage in the biocontrol of this pathogen was its inactivation by UV light, variable bacterial sensitivity and the rapid development of resistance to the phage (Mayer 2007). Svircev et al. (2019) reported development of antibiotic resistance, popularity of organic fruit production and the consumers' desire for pesticide-free food has reinvigorated interest in biological control of *Erwinia amylovora*. Application of bacteriophages as biologicals exploits the ability of lytic phages to kill the pathogen. The results of past field trials were variable from failed trials to 50–65% efficacy. Tomato bacterial

wilt caused by *Ralstonia solanacearum* is difficult to control. The phage that had widest spectra against several isolates of *R. solanacearum* was then chosen for the next experiment. A bacteriophage from a region in Central Java showed the wide host range compared with the others. Clear phages were produced in the lawn of *R. solanacearum* in CPG medium (Arwiyanto et al. 2019).

9.2.3 Nanotechnology

Nanotechnology is leading for the development of new concepts and agricultural products with immense potential to manage the aforementioned problems. Nanotechnology has substantially advanced in medicine and pharmacology but has received comparatively less interest among agricultural applications. The application of nanotechnology in agriculture is currently being explored in plant hormone delivery, seed germination, water management, transfer of target genes, nanobarcoding, nanosensors and controlled release of agrichemicals. As agricultural nanotechnology develops, the potential to provide a new generation of pesticides and other active compounds for plant disease management will greatly increase.

Prabhu et al. (2015) have reported the antibacterial activity of iron oxide nanoparticles against *Xanthomonas* sp. and *Proteus vulgaris* at 50 mg/ml. Chikkanaswamy (2018) proved the antibacterial activities of green synthesized mango-based copper nanoparticles (CuNPs) against *X. axonopodis* pv. *citri* (citrus canker) under glasshouse condition. Vinay et al. (2018) proved the antibacterial activity of green synthesized *Pseudomonas fluorescens*-based ZnNPs against *Xanthomonas oryzae* pv. *oryzae* and *X. axonopodis* pv. *punicae* at 500–1250 ppm. Poovizhi and Krishnaveni (2015) had reported the antibacterial activity of zinc oxide nanoparticles against *Xanthomonas axonopodis* pv. *citri*. Ocsoy et al. (2013) studied the effect of DNA-directed silver (Ag) nanoparticles against bacterial spot caused by *Xanthomonas perforans* in tomato; most of the *X. perforans* strains are copper resistant. These Ag-dsDNA-GO composites had effectively decreased the cell viability of *X. perforans* in culture and on plants. At a very low concentration (16 ppm) of Ag-dsDNA-GO, composites had shown excellent antibacterial activity in culture with significant advantages in improved stability, stronger adsorption properties and enhanced antibacterial capability. Application of AG-dsDNA-GO at 100 ppm on tomato seedlings in an experiment conducted in greenhouse significantly reduced the severity of bacterial spot disease; there was no phytotoxicity observed. Silver nanoparticles synthesized from cotton stem showed antibacterial activity against *X. axonopodis* pv. *malvacearum* and *X. campestris* pv. *campestris*. Furthermore, AgNPs exhibited strong antioxidant activity and no phytotoxicity on cowpea. Overall, the findings suggest that cotton stem extract could be efficiently used in the synthesis of AgNPs and showed antimicrobial activity against plant pathogenic microbes (Vanti et al., 2019). Vinay and Narguad (2019) reported an excellent antibacterial activity of green synthesized water-soluble chitosan-based iron nanoparticles (Ch-FeNPs) against *Xanthomonas oryzae* pv. *oryzae* and

X. axonopodis pv. *punicae* at 500–1250 ppm. They noticed bacterial growth inhibition due to treatment of iron nanoparticles.

Bryaskova et al. (2011) studied the effect of three different bacteria (*Staphylococcus aureus*, *E. coli* and *P. aeruginosa*) in order to study the antibacterial potential of synthesized silver nanoparticles. Concentration, physiology, metabolism, intracellular selective permeability of membranes and the type of microbial cell are the different factors for the basis of antimicrobial activity of the nanoparticles. The significant antibacterial activity was observed in ZnO NPs against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli*. The synthesized ZnO NPs have shown antibacterial efficacy against both Gram-positive and Gram-negative pathogens (Gupta et al., 2018). Synergistic effects of ZnO NPs and streptomycin showed increased efficacy as indicated by the increased zone of clearance in comparison to their individual effects (either ZnO NPs or streptomycin). Nanoparticles synthesized from titanium dioxide (TiO_2) induced photocatalysis, resulting in antimicrobial effects against the bacterial spot pathogen *Xanthomonas perforans* (Paret et al. 2013). Interestingly, doping the TiO_2 NPs with Ag and Zn significantly increased the photocatalytic activity against *X. perforans*. Treatment of *X. perforans*-infected tomato plants with TiO_2/Zn NPs at approximately 500–800 ppm significantly reduced bacterial spot severity compared with untreated and copper controls (Paret et al. 2013). ZnO and Ag NPs also exhibited promising antimicrobial activity against *E. amylovora* with minimum inhibitory concentrations. Some NPs can exert antimicrobial activity through the release of ions, such as Ag^+, Zn^{2+} and Cu^{2+}, which are toxic to bacteria. The release of Ag^+ greatly contributed for such activity of Ag NPs (Lok et al. 2007).

9.2.4 CRISPR/Cas9- Novel Tool for Management of Plant Bacterial Diseases

CRISPR (Clustered Regular Interspaced Palindromic Repeats)/Cas9 (CRISPR-associated protein) is a recent breakthrough in gene editing technology. It can be utilized to exploit defensive mechanism in plants against pathogen attack with recognition and degradation of the invading pathogenic genes by bacterial immune system. CRISPR/Cas9-mediated genetically engineered resistance can be inherited to further generation of crops after segregation of Cas9/sgRNA transgene in F1 generation. CRISPR/Cas9 proves itself as a fascinating tool to revolutionize plant breeding for developing various disease-resistant cultivars (Ghimire 2017). Phytopathogenic bacteria are difficult to control, mainly because of undetected latent infections and also the lack of suitable agrochemicals. Generally speaking, bacteriological plant control is based on prevention and exclusion of the pathogen by using genetic resistance, agronomic practices and biocontrol agents (Kerr 2016). CRISPR/Cas9 mutagenesis of OsSWEET13 has been performed in rice to achieve resistance to bacterial leaf blight (BLB) disease caused by γ-proteobacterium *Xanthomonas oryzae* pv. *oryzae* (Zhou et al. 2015). OsSWEET13 is a susceptibility (S) gene

Table 9.1 Effective endophytes against bacterial plant pathogens

Crop	Fungal endophytes	Targeted pest/pathogen
Sugarcane	*Epicoccum nigrum*	*Xanthomonas albilineans*
Eggplant	*Pseudomonas fluorescens*	*Ralstonia solanacearum*

encoding a sucrose transporter involved in plant-pathogen interaction. *X. oryzae* produces an effector protein, PthXo2, which induces OsSWEET13 expression in the host and the consequent condition of susceptibility. In a previous work concerning OsSWEET14 promoter mutagenesis adopting a TALEN approach, the disruption of this gene rendered the *X. oryzae* effector unable to bind OsSWEET14 and ultimately resulted in disease resistance (Li et al. 2012). Zhou et al. (2015) obtained a null mutation in OsSWEET13 in order to better explore PthXo2-dependent disease susceptibility, and resultant mutants were resistant to bacterial blight.

Two recent research works have reported the employment of CRISPR/Cas9 with the aim of producing citrus plants resistant to citrus bacterial canker. Citrus canker is caused by *Xanthomonas citri* subsp. *citri* (Xcc). Jia et al. (2016) generated canker resistant mutants by editing the PthA4 effector binding elements in the promoter region of the Lateral Organ Boundaries 1 (CsLOB1) gene in Duncan grapefruit. Mutated lines showed a decrease in typical canker symptoms 4 days post inoculation with Xcc, and no further phenotypic alterations were detectable. Furthermore, no potential off-target mutations in other LOB family genes were found by PCR sequencing. Peng et al. (2017) confirmed the link between CsLOB1 promoter activity and citrus canker disease susceptibility in Wanjincheng orange (*Citrus sinensis* Osbeck). The complete deletion of the EBEPthA4 sequence from both CsLOB1 alleles induced resistance enhancement to citrus canker (Table 9.1, 9.2 and 9.3).

9.2.5 Antimicrobial Peptides: Emerging Candidates for Plant Protection

Antimicrobial peptides (AMPs) are the short polymers of amino acids with peptide bond having ≤50 amino acids or short polymers of amino acids having broad-spectrum antimicrobial activity against bacteria/fungi/viruses/nematodes. Antimicrobial peptides (AMPs) are also called as host defence peptides (HDPs) and cell-penetrating peptides (CPPs). These are isolated from many organisms, namely, insects, amphibians, humans, microorganisms and plants. AMPs are part of the nonspecific host defence system and are active against different types of microorganisms including phytopathogens.

Microscopic analysis revealed wide-scale damage to the microorganism's membrane, in addition to inhibition of pathogen growth. In *planta* potent antibacterial activity was demonstrated. Treatment with the lipopeptides of *Arabidopsis* leaves infected with *Pseudomonas syringae* efficiently and rapidly reduced the number of bacteria with no toxicity on the plant tissues (Makovitzki et al. 2007). The ultrashort

Table 9.2 CRISPR/Cas9 applications for bacterial disease resistance

Plant species	Targeted pathogen	Target gene	Gene function	Strategy	References
Oryza sativa	Bacterial blight (*Xanthomonas oryzae* pv. *oryzae*)	SWEET13	Sucrose transporter gene	Agrobacterium-mediated transformation of embryogenic callus with Cas9/gRNA expression plasmid vectors and TALEN	Li et al. (2012) and Zhou et al. (2015)
Citrus paradisi	Citrus canker (*Xanthomonas citri* subsp. *citri*)	LOB1	Susceptibility (S) gene promoting pathogen growth and pustule formation	Agrobacterium-mediated transformation of epicotyl with Cas9/gRNA expression plasmid vectors	Jia et al. (2016)
Citrus sinensis Osbeck	Citrus canker (*Xanthomonas citri* subsp. *citri*)	LOB1	Susceptibility (S) gene promoting pathogen growth and pustule formation	Agrobacterium-mediated transformation of epicotyl with Cas9/gRNA expression plasmid vectors	Peng et al. (2017)
Malus domestica	Fire blight (*Erwinia amylovora*)	DIPM-1 DIPM-2 DIPM-4	Susceptibility factor involved in fire blight disease	PEG-mediated protoplast transformation with CRISPR ribonucleoproteins	Malnoy et al. (2016)

Table 9.3 The effective concentration of native chitosan or its derivatives against bacterial plant pathogens (Rabea and Steurbaut 2010; Badawy et al. 2014)

Plant pathogenic bacteria	Chitosan/its derivative	Effective concentration (ppm)
Agrobacterium tumefaciens	N-(o,o-dichlorobenzyl) chitosan	500
Agrobacterium tumefaciens	Quaternary N-(benzyl) chitosan	500
Agrobacterium tumefaciens	N-(benzyl) chitosan	800
Xanthomonas campestris	Chitosan	500
Erwinia carotovora	Chitosan	200
Erwinia carotovora	N-(o,o-dichlorobenzyl) chitosan	480
Erwinia carotovora	Quaternary N-(benzyl) chitosan	600
Erwinia carotovora	N-(benzyl) chitosan	700
Erwinia carotovora subsp. *carotovora*	Chitosan	5000
Clavibacter michiganensis subsp. *michiganensis*	Chitosan	1000
Erwinia carotovora	N-(α-methylcinnamyl) chitosan	1025

(Xing et al. 2015)

lipopeptides could serve as native-like antimicrobial agents economically feasible for use in plant protection. The synthetic LFchimera showed potential antibacterial activities against phytopathogenic bacteria such as *Ralstonia solanacearum, Xanthomonas campestris, Erwinia amylovora, Pseudomonas syringae* and *Pectobacterium carotovorum*. LFchimera was effective against bacterial strain in a dose-dependent manner (Chahardoli et al. 2017) and can improve the potential of an antimicrobial peptide in plant disease management. The synthetic antimicrobial peptide, i.e. Shiva-1, was isolated from the silk moth and introduced in transgenic tobacco (Jaynes et al. 1993). Lactoferrin is another iron-binding glycoprotein known to have antibacterial properties. The expression of a human lactoferrin gene in tobacco delayed the onset of symptoms caused by *R. solanacearum* from 5 to 25 days. This resistance appears to be due to the truncation of lactoferrin, resulting in a smaller peptide with strong antibacterial activity (Mitra and Zhang 1994).

9.2.6 Transgenic Approaches

Plant pathogens can cause significant reduction in crop yield. Due to infection of many invasive pathogens, there is a possible threat to wipe out plant species. Hence, plant pathologists and biotechnologists trying their best to develop pathogen-resistant plants against some diseases caused by bacteria of economic importance (Wani et al. 2010). Many molecular approaches have been proposed to enhance plant resistance to bacterial pathogens like *P. syringae*; these strategies

include the use of antibacterial proteins from different insect vectors and their transformation in plants for development of disease resistance (Huang et al. 1997) and inactivation of virulence factors resulted the immunity of plants against the relevant bacterial species (Anzai et al. 1989). The resistance nonbacterial genes can also be introduced by transgenic approaches for broad-spectrum resistance against the devastating pathogens.

Recently, the utilization of RNAi has emerged as an important tool to counter the bacterial genome at transcriptional and post-transcriptional level. Small interference RNA has been proved effective against the crown gall disease in *Arabidopsis*, *Nicotiana* and *Lycopersicum* species caused by *Agrobacterium tumefaciens* by transformation of inverted repeats of this pathogen genes ipt and iaaM to encode precursors of biosynthesis for two important phytochromes auxin and cytokinins (Escobar et al. 2001). Phenolic compounds (a group of secondary metabolites) are widely distributed in plants and have shown to possess antimicrobial properties. The anti-*Xylella* activity of 12 phenolic compounds, representing phenolic acid, coumarin, stilbene and flavonoid, was evaluated using an in vitro agar dilution assay. Overall, these phenolic compounds were effective in inhibiting *X. fastidiosa* growth, as indicated by low minimum inhibitory concentrations (MICs). In addition, phenolic compounds with different structural features exhibited different anti-*Xylella* capacities. Particularly, catechol, caffeic acid and resveratrol showed strong anti-*Xylella* activities. Differential response to phenolic compounds was observed among *X. fastidiosa* strains isolated from grape and almond. Elucidation of secondary metabolite-based host resistance to *X. fastidiosa* will have a broad implication in combating *X. fastidiosa*-caused plant diseases. It will facilitate future production of plants with improved disease resistance properties through genetic engineering or traditional breeding approaches and will significantly improve crop yield (Maddox et al. 2010).

9.2.7 Organic Elicitor: Chitosan in Disease Management

Chitosan is a deacetylated derivative of chitin that is naturally present in the insect body wall, in fungal cell wall and in crustacean shells from which it can be easily extracted. Chitosan has been reported to possess antimicrobial activity. Chitosan also behaves as a resistance elicitor inducing both local and systemic plant defence responses when applied to plants directly (Orzali et al. 2017). Chitosan has been emerging as one of the most promising polymers for the efficient delivery of agrochemicals and micronutrients in nanoparticles (Kashyap et al. 2015). Chitosan and its derivatives inhibited the growth of a wide range of bacterial plant pathogens (Liu et al. 2001 (Fei Liu) Wisniewska-Wrona et al. 2007). Based on the available evidences, bacteria appear to be generally less sensitive to the antimicrobial action of chitosan than fungi. Gram-negative bacteria are more susceptible to chitosan (Park et al. 2004; Du et al. 2009). They suggested that hydrophilicity in Gram-negative bacteria is significantly higher than that in Gram-positive bacteria, which makes them more sensitive to chitosan (Chung et al. 2004). Moreover, the Gram-negative

cell envelope contains an additional outer membrane composed by phospholipids and lipopolysaccharides, which face the external environment. The highly charged nature of lipopolysaccharides will confer an overall charge of negative to the cell wall of the Gram-negative bacteria. Therefore, Gram-negative bacteria with high electronegative charge will interact more effectively with the polycationic chitosan compared with Gram-positive bacteria. Chitosan was evaluated against several bacterial pathogens and effective concentration for suppression of growth of bacteria is presented in tabular form hereunder.

The in vitro antibacterial effect of chitosan and its ability in protection of watermelon seedlings from *Acidovorax citrulli* were evaluated. The disease index of watermelon seedlings planted in soil and the death rate of seedlings planted in perlite were significantly reduced by chitosan at 0.40 mg/mL compared with the control (pathogen) (Li et al. 2013). Chitosan solution at 0.10 mg/mL inhibited the growth of *Xanthomonas* pathogenic bacteria from different geographical origins. The surviving cell numbers in the chitosan solution decreased more than 3.86 log10CFU/mL compared with the control after 6 h of incubation regardless of the bacterial strain (Li et al. 2008). Pre-treatment of tomato seedlings with 10 μg/mL chitosan before *Pseudomonas syringae* pv. *tomato* DC3000 (Pto DC3000) inoculation significantly decreased bacterial damages in cotyledons compared with control. Not only does chitosan inhibit bacterial cell growth but also it affects the already established biofilms (Mansilla et al. 2013). The polysaccharide chitosans represent a renewable source of natural biodegradable polymers and meet with the emergence of more and more food safe problems. A wider comprehensive knowledge of the mechanism of action of chitosan in pathogens and plants will increase the chance of its successful application to control disease spread in plants.

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10 Resistance Breeding and Exploitation of Wild Relatives for New Resistance Sources

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Abstract

Increasing yield from same piece of land and resources has now become more imperative since the share of resources is decreasing continuously due to increasing population. Change in environmental parameters are confronting with plants by changing dynamics as well as emergence of novel parasites. Resistance breeding though has been the traditional objective of plant breeding programme. With changing scenario, effective and diverse-resistant sources, particularly from wild relatives and from other sources, seem to be essential for durability of the resistance. Furthermore, precise tools are required for identification and transfer of genes for developing resistant genotypes. This chapter includes description of necessity of resistance breeding, types of resistance, breeding tools used in development of resistant genotypes and wild relatives that can be used as potential sources of resistant genes.

Keywords

Resistance sources · Wild relatives · Breeding tools · Specific race · Nonspecific race · Biotic stress

10.1 Introduction

Every living organism takes food in one or other form to sustain, grow and reproduce. Availability has not been an issue when production avenues were considerably high than the consumption capacity of all the dependent creatures. With the changing scenario due to anthropogenic activities, industrialization, infrastructural

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development and decline in efficiency of production avenues, the emphasis has shifted to the use of alterative and innovative approaches to minimize the gap between production and consumption. Agriculture sector has been witness to major changes not only in intensive use of resources but also in evolutionary changes of diverse cropping system with dramatic shift in method and time of crop cultivation. Thus, agriculture has grown continuously and dynamically to support the requirement of food, feed, medicines and raw materials for industries. Furthermore, growth in agriculture is highly anticipated till population growth is stabilized. In fact agriculture is considered to be a gamble of monsoon in majority of the developing countries, because monsoon rainfalls influence the availability of water and successful crop cultivation. Plants growth and development are affected by climatic conditions as favourable biotic and abiotic factors ensure positive interactions leading to optimum growth and performance of the crop. However, change in climatic conditions exert stresses not only on plants but also on all the living organisms and also disturb the dynamic balance between crop and different biotic and abiotic factors. Altered environmental parameters are not only interfering with genetic potential of crop leading to altered or abnormal growth and development but also it affects population dynamics as well as emergence of novel race of microorganisms that may or may not interact with different stages of plants. Plants being sessile are exposed continuously to good or adverse environmental conditions of varying intensity, including attack by pathogens, wounding by insects, exposure to ultraviolet radiation, low temperature and decrease in water and nutrient availability. It is quite remarkable that in spite of ever-changing environmental parameters, plants are able to adapt, continue to grow, develop and importantly remain productive. To accomplish this feat, plants rely heavily on their ability to coordinate the perception of environmental stimuli with alterations in developmental and physiological programmes required for adaptation and survival. Plant response to external signals is a very complex and highly coordinated process that involves the quick perception of stress stimuli, the activation of many signalling networks and changes in the expression of hundreds of genes and ultimately altered metabolites that probably plant needs. In addition, it is imperative that these genes are only transcribed in response to the right signal, at the right time, in the right place and for the appropriate amount of time. Since the available knowledge and system is unable to perceive and understand the language of plants, it is very difficult to know whether plants feel pain or discomfort and also it is difficult to pin-point exactly when a plant is in abnormal state. It is accepted that a plant is healthy or normal, when it can carry out its physiological functions to the best of its genetic potential.

In case we noticed that the ability of the cells of a plant or plant part to carry out one or more of essential functions is interfered with by either a pathogenic organism or an adverse environmental factor, the activities of the cells are disrupted, altered or inhibited, the cells malfunction or die, and the plant becomes diseased. Initially, the distress is localized and by and large is invisible. Their action becomes more prominent and affects many plant parts, and consequential changes are visible morphologically. In fact, these visible changes are the symptoms of the disease. The visible or otherwise measurable adverse changes in a plant, produced in reaction

to infection by an organism or to an unfavourable environmental factor, are a measure of the amount of disease in the plant. Disease in plants, then, can be defined as the series of invisible and visible responses of plant cells and tissues to a pathogenic organism or environmental factors that result in adverse changes in the form, function or integrity of the plant and may lead to partial impairment or death of plant parts or of the entire plant.

Prevost proved as early as in 1807 that diseases are caused by microorganisms. The physiological functions that are likely to be interrupted due to pathogens are dependent on the cells and tissues that are invaded by the pathogenic organism. For example, infection of roots may cause roots to rot and make them unable to absorb water and nutrients from the soil; infection of xylem vessels, as happens in vascular wilts and in some cankers, interferes with the translocation of water and minerals to the crown of the plant; infection of the foliage, as happens in leaf spots, blights, rusts, mildews, mosaics and so on, interferes with photosynthesis; infection of phloem cells in the veins of leaves and in the bark of stems and shoots, as happens in cankers and in diseases caused by viruses, mollicutes and protozoa, interferes with the downward translocation of photosynthetic products; and infection of flowers and fruits interferes with reproduction. Although infected cells in most diseases are weakened or die, in some diseases, namely in case of crown gall, infected cells are induced to divide much faster (hyperplasia) or to enlarge a great deal more (hypertrophy) than normal cells and to produce abnormal amorphous overgrowths (tumours) or abnormal organs. Microorganisms that can cause disease are generally referred to as pathogens, usually they cause disease in plants by disturbing the metabolism of plant cells through enzymes, toxins, growth regulators, and other substances they secrete and by absorbing foodstuffs from the host cells for their own use. Some pathogens may also cause disease by growing and multiplying in the xylem or phloem vessels of plants, thereby blocking the upward transportation of water or the downward movement of sugars, respectively, through these tissues. Environmental factors cause disease in plants when abiotic factors, such as temperature, moisture, mineral nutrients and pollutants, occur at levels above or below a certain range tolerated by the plants. Devastating diseases caused by pathogens and pests have threatened crop production, human health and the stability of global economies. Management of pathogenic organism is the potential option to minimize distortion and unusual changes in plants leading to normal production potential. Replacing plant genes by more efficient allelic form to incorporate resistance potential is still a more prospective option as it does not add any additional input in growing crops, and also this option is environmentally safe. The availability of gene (s) conferring higher level of resistance is essentially required either in cultivated form or use of wild relatives for exploring and domesticating wild alleles, and genes conferring resistance to pathogenic organisms is another novel avenue for successful resistance in breeding programme.

10.2 Resistance Breeding

Plant breeding has been the most successful approach for developing new crop varieties since domestication occurred, making possible major advances in feeding the world and societal development. Crops are susceptible to a large set of pathogens including fungi, bacteria and viruses, which cause significant economic losses; the enhancement of plant resistance played an important role in adjusting crop production to meet the requirement of global population growth. Approaches to disease control that depend on resistant varieties and agrochemicals are usually highly effective whenever they are deployed. Dynamic evolutionary properties of plant pathogens, however, limit the cultivation of new varieties in localized or in larger areas once a virulent race that counter the existing resistant gene is evolved. As such, disease control approach based on the existing resistant gene has become ineffective. Incorporation of novel genes and deployment of new genotypes in the areas are therefore required as a control measure of plant diseases. In fact resistant gene-based disease control strategies are like continuous war against pathogens in which genotypes equipped with different weapons that are 'resistant genes' are deployed strategically in time and geographical frames to manage plant diseases.

Following the ancient domestication of crop species, plant breeding occurred only informally for thousands of years. During that time, farmers might have chosen to save seed from the healthiest or highest-yielding plants from one generation to the next, but they lacked the scientific knowledge of inheritance to permit deliberate breeding for traits or understand the causes and effects of the widely used method of mass selection. Domestication syndrome leads to development of modern crop with high production potential and adapted to high input environments, on the one hand, but, on the other hand, consequential domestication bottleneck witnessed the loss of many adaptive alleles leading often susceptibility of crop to diseases, insects and abiotic stresses. To find resistance genes, it is often necessary to go back to their wild ancestors and close relatives. In fact this seems to be a problematic option due to the inevitable setback in optimized yields and other agronomical parameters attained when crossing to wild relatives, on one hand, however, on the other hand, it seems to have great prospect for novel resistant gene (s) and resistant breeding point of view. Pathogens are major constraints for field and horticultural crop production, impairing both yield and quality. Breeding for disease resistance is the most efficient, economical and environmental-friendly way of control. It is, therefore, a crucial component of sustainable agriculture that can be performed by using several approaches from classical breeding to genetic engineering. However, detailed understanding of pathogen biology, host–pathogen interaction and the efficient resistance mechanisms at the cellular and molecular levels are required to improve the efficiency of the breeding process.

The act of changing the genetic make-up of plants has been done by humans in distant past to fulfil their immediate needs and services. The only thing that has changed from then to now is the level of understanding we have on the subject. However, human experimentation with plant breeding has developed many of our modern crops even if the breeder didn't have much knowledge on the subject. From

the very beginning, plant breeding had been a common activity. In fact, Gregor Mendel's work on how genes behave in terms of phenotypic appearance and how they could be passed to offspring was the first major event to spark an interest in the science behind plant breeding. Until the 1900s, it had been ignored. However, once three scientists having trouble with breeding came across it, Mendel's findings had been publicized. It is in fact very tedious to trace the exact date that humans began breeding plants. Yet, it is well known that R.J. Camerarius of Germany is credited for first reporting sexual reproduction in plants in 1694. Since then, tools have been made, new plant breeds have been developed and the general knowledge humans have on this subject has grown greatly and become more and more scientific and specific.

10.3 History of Resistance Breeding

Theophrastus, in the third century BC, noted that cultivated varieties differed in their ability to avoid disease. That diseases are produced by a pathogen was conclusively shown by Benedict Prevost, he showed that wheat bunt was produced by a fungus. During the middle of nineteenth century, various workers noted that crop varieties differed for disease resistance. In 1904, Blakeslee described mating type differentiation in Rhizophus. In 1905, Biffen demonstrated that resistance to yellow rust in wheat is governed by a recessive gene showing classical Mendelian segregation ratio in F2. Breeding for disease resistance is believed to have started with the work of Orton in 1900, who selected lines of cotton resistant to Fusarium wilt by growing cotton on wilt sick soil. He also used hybridization to develop wilt (*Fusarium oxysporum*) resistant varieties of water melon.

In 1894, Erikson showed that pathogen, although morphologically similar, differed from each other in their ability to attack different related host species. Later, in 1911, Barrus showed that different isolates of a microorganism differed in their ability to attack different varieties of the same host species. This finding has formed scientific basis for physiological races and or pathotypes. Later on, Johnson and Newton (1940) established in case of black rust of wheat that the ability of a pathogen to infect a host strain, i.e. pathogenicity, is genetically determined. Thus, both the ability of a host to resist invasion by a pathogens as well as the ability of a pathogen to infect a host strain, i.e. pathogenicity, is genetically determined. The breeding for resistance to diseases and insect pests gained further momentum when Flor (1955, 1956) proposed the gene-for-gene hypothesis which states that 'for each gene conditioning rust reaction in the host, there is a specific gene conditioning pathogenicity in the parasite'. In other words, each genetic locus conditioning resistance or susceptibility in the host has a corresponding locus in the pathogen controlling avirulence or virulence. The gene-for-gene concept provides a useful working model for studying host parasite systems, even when genetic information is not fully available. Thus, gene-for-gene hypothesis added knowledge on host pathogen interaction and helped in planning effective resistance breeding programme. The gene-for-gene hypothesis has thus (a) prompted the identification of new major

genes for disease resistance, (b) enabled the development of varied and usually effective strategies for the use of major gene resistance in space and/or over time to manage diseases, (c) provided a clear understanding of the host–parasite interactions, the nature of gene action and co-evolution of host–parasite systems and (d) enabled planning of breeding programme for the development of disease-resistant varieties.

Available evidences indicate that the pathogens are more dynamic for generating new variation in pathogenicity by a variety of reproduction methods and mutation. The evolution of different races of the same pathogen is a continuous feature. Thus, the resistance breeding objective should not only be to develop varieties resistant to the prevalent pathotypes of the pathogens but also be vigilant with access of diverse alleles for resistance to face the challenges once emerged due to evolution of the new virulent pathotypes in future. Thus, resistance breeding requires continuous intervention by various classical and molecular tools along with diverse pool of resistance sources for breeding genotypes with appreciably high level of resistance along with high yield potential.

10.4 Complexities in Breeding for Resistant Varieties

The breeding for disease resistance is supposed to be more complex and complicated than the breeding for other traits. The complication is because of the dynamic nature of pathogens leading to evolution of pathogenic races or biotypes that are new and can overcome the crop resistance potential since the gene deployed in the crop is specific to existing pathogenic race. This in fact poses an additional hurdle to breeding programmes, once a new genotype for resistance to a specific pathogen race may show susceptibility to other races. Therefore, when a pathogenic race is mutated and a new race emerges, plant breeders have to initiate a new breeding effort to search an effective allelic form of a gene and to deploy the same in the crop to develop a resistant cultivar. In reality, this process is an endless battle against the pathogen. Another problem is the shift of prevalent existing pathogenic races in a region, since they may also reduce the life span of a resistant cultivar that after a few growing seasons become susceptible. It must also be recognized that the number of insects that causes yield reduction is large, including those that attack the crops during the growing season, feeding on leaves, pods, fruits and roots. An additional class of insects that causes losses by feeding on the harvested crop, like borer, weevils among others exists and causes significant losses both in terms of quantity and quality. Historical evidences show that biotic stresses occur, in high or low intensity, in all agricultural areas around the world. In some areas, the stresses caused by pests and weeds may not be relevant in a specific year, but they bounce back in another years or seasons. Migration of insect-pests is another burden on breeding for resistance cultivars. For example, Fall army worm, an exotic insect, entered in India and was reported for the first time in May 2018 from a maize field located in southern region of the country. Within a year of its appearance in India, Fall army worm spread to most of the maize-growing regions causing severe damage. Additionally,

climate change is bringing new pests and weeds to relevance in crop production, especially in the tropical regions. Climate changes are also affecting insect-pests to remain active and becoming burden for sequential crop in the field bypassing the seasonal boundary. Overall, global warming has caused and probably may cause even larger incidence by insect-pests, diseases and weeds on farmers' fields globally in general, but impact seems to be more devastating on the fields of medium and resource-poor farmers. Some biotic stresses that have been considered secondary in the past may emerge as biotic stress of major relevance with climate change. Breeding efforts for developing insect-pests resistant cultivars have not been as effective as for disease resistance because of non-availability of potential and effective gene sources in primary gene pool of the crop species. However, some resistant cultivars have been developed over the years against many insect-pests. Success for breeding insect-pests resistance has been remarkable by exploring and transferring gene(s) showing resistance across the species and genera boundary with the help of biotechnology. The contribution of plant breeding throughout history in helping agriculture to produce food, feed, fibre and fuel is very well documented in the scientific literature (Vencovsky and Ramalho 2006; Duvick et al. 2004). However, what will happen in the coming decades with the new challenging scenario will demand from breeders new and more efficient strategies to manage biotic stresses to sustain agricultural production and productivity of different crops for producing adequate amount of food, feed, fibre, industrial raw materials, etc. Thus, complexities in breeding for biotic stresses are more which seem to increase with changing climatic conditions as well as cropping patterns. Search for novel genes seems to be the foremost priority followed by efficient and precise tools to identify and incorporate resistant genes in crops from the sources beyond the primary gene pools.

10.5 Nature of Resistance

Plant breeding tools can be used to develop varieties that show resistance against specific race (race-specific, qualitative, vertical resistance) or race-nonspecific resistance (quantitative, race-nonspecific, horizontal resistance and field resistance) or integration of both kind of resistance.

10.5.1 Qualitative Resistance

It is also known as a major gene resistance and is based on one or few genes with major effect and provides race-specific, high level resistance (vertical resistance). Qualitative resistance, often associated with rapid cell death called hypersensitive response (HR) around the contact point of pathogen, is generally quickly overcome when deployed in the field, though there are exceptions. In fact, race-specific

resistance is conditioned by the interaction of specific genes in the host with those in the pathogen. The genetic principles underlying host–pathogen interaction were elegantly established by Flor (1955) while working on rust (*Melampsora lini*) of flax and elaborated that resistance or susceptibility of a cultivar is dependent on gene/allele for resistance or susceptibility in the host and the presence of corresponding gene/allele for virulence or avirulence in the race of pathogen. A similar system has been shown to exist for most of the cereal crops and their rust pathogen. The ability of the pathogen to change its racial identity into another new virulent form necessitates an on-going search for new sources and types of resistance that can be utilized in breeding for disease resistance. Qualitative resistance is generally effective against biotrophic pathogens (pathogens that derive their nutrition from living host cells). Observed start of an epidemic is delayed and effective amount of initial inoculums reduces once qualitative resistance is deployed in a variety. This type of resistance mechanism has been deployed against many pathogens in many crops namely coffee (*Coffea arabica* L.) – *Hemileia vastatrix* Berk. & Br., maize (*Zea mays* L.) – *Puccinia sorghi* Schw., oats (*Avena sativa* L.) – *P. coronata* Cda., wheat (*Triticum aestivum* (L.) Thell.) – *P. graminis* f. sp. *tritici* Pers., wheat – *P. recondita*, wheat – *P. striiformis* Westend, barley (*Hordeum vulgare*) – *Erysiphe graminis* D.C. f. sp. *hordei*, flax (*Linum usitatissimun* L.) – *Melampsora lini* (Ehrenb.) Desmaz. These resistance genes often cluster together in certain chromosome arms, sometimes so tightly that they can be considered as complex loci, and true allelic series also occur. In the flax-flax rust pathosystem, 34 R-genes have been identified in seven regions: K(2), L(14), M(7), N(3), P(6), D (1) and Q(1). Regions N and P are linked, as well as regions N and K. The N region consists of at least two closely linked loci. The M region, together with seven resistance alleles, also consists of some closely linked loci. The L region, with 14 resistance alleles, behaves as a locus with an allelic series, but intra-allelic recombination has been reported (Islam and Shepherd 1991). In barley, most of the resistance genes to powdery mildew are located on one arm of chromosome 5 and one arm of chromosome 4 (Jorgensen 1990). On the short arm of chromosome 10 in maize, at least 16 resistance genes to *P. sorghi* are found on the complex locus Rp1 and the loci Rp5 and Rp6 within three centimorgans of each other (Saxena and Hooker 1968). The three downy mildew [*Peronospora effusa*(Grev.) Tul.] resistance genes known in spinach are tightly linked.

10.5.2 Quantitative Resistance

As the name indicates, quantitative resistance has gradient of phenotypic class that is determined by many genes, each contributing small, but together become important to confer significant amount of disease and pest tolerance. Multiple genes typically form the genetic basis and generally provide a level of resistance against many races (non-race specific, horizontal). Such genetic foundation supports the durability of quantitative resistance and ultimately the durability of a variety on farmers' field. Quantitative resistance (QR) is more often associated with resistance to necrotrophic

pathogens (pathogens that derive nutrition from dead cells). The utility of horizontal resistance is more prospective in long epidemic in which disease increases with small beginnings to relatively very great heights. Quantitative resistance seems to be more effective and durable when large area is covered with crop varieties showing race-nonspecific resistance (Parlevliet 2002). Barley leaf rust (*P. hordei)* resistance showed polygenic inheritance and all cultivars in Western Europe, including the very susceptible cultivars, carry at least some QR. Most cultivars carry considerable levels of QR, thus preventing the barley leaf rust from becoming a major pathogen in Western Europe (Parlevliet 1979). In rice, cross between two very susceptible cultivars, some lines were obtained that were considerably more susceptible than either parent, while a few other progeny lines were moderately resistant to bacterial blight, *Xanthomonas campestris pv. oryzae*. The progenies performance beyond the parental values meant that both highly susceptible cultivars carried many genes with small effects for QR that differed from each other (Koch and Parlevliet 1991). Thus, even the so-called very susceptible genotypes may harbour some QR, confirming the experience with barley leaf rust. Similar observations were reported from quantitative trait loci (QTL) analyses done in the pathosystems maize/*Cercospora zeae-maydis* by Tehon & Daniels, pea (*Pisum sativum* L.)/*Ascochyta pisi* Lib. and tomato (*Lycopersicon esculentum* Mill.) /*Ralstonia solanacearum* (Smith 1896 and Yabuuchi et al. 1996). In crosses between a susceptible and a QR parent, QTLs for QR were found that originated not only from the QR parent but also from the susceptible parent when a cross was analysed where crossing between a susceptible and a QR parent was used to generate the experimental materials (Young 1996).

Varieties with clear-cut visible resistance due to genes with larger effects are required for release against major prevalent pathogens of the areas. Such varieties are generally recommended for cultivation due to high score of resistance. Under this situation, the effect of QR is not visible in spite of presence of genes with small effects on resistant phenotype. After a number of years of cultivation, resistant scores of the varieties goes down, as the major gene resistance is not effective any more. However, after the resistance "breaks down", QR becomes visible, if present. All cultivars selected for their major gene resistance appear to carry moderate to fair levels of QR hidden behind that major gene (Anonymous 1958–1998). This hidden QR is sometimes indicated as residual resistance which is due to the presence of some level of QR. Potatoes have a range of viruses which may affect them. Apart from major resistance genes, QR also exists against those viruses. This QR is often expressed through a reduced frequency of infected plants (incidence). The Dutch recommended list of potato cultivars discerns between major gene resistance and QR. All potato cultivars listed from the period 1958 to present carry low to high levels of QR to each of the four viruses assessed: Potato virus X, Potato virus Y and Potato virus A and Leaf roll virus. Therefore, QR is present almost everywhere. Cultivars without any QR are very rare. For this type of resistance, breeders do not need to look for primitive genotypes from centres of diversity nor to related wild species. The resistance is found in adapted cultivars, a fortunate situation as it makes breeding easier. McIntosh (1996) concluded that the ideal sources of resistance are

those present in closely related, commercial genotypes, and any effort to transfer resistance from related species and genera should be considered long term.

10.6 Breeding Methods for Disease Resistance

Breeding methods used to modify genetic make-up of plants by integrating gene (s) that confer resistance to biotic stresses can be broadly classified into two categories:

10.6.1 Classical Breeding Approach
10.6.2 Novel approaches
  10.6.2.1 Molecular Breeding approach
  10.6.2.2 Transgenic breeding approach

10.6.1 Classical Breeding Approach

Classical breeding approaches used for developing resistant varieties include the same approaches as used for developing high yielding varieties. In practice, resistance to existing races of prevalent pathogens is considered directly or indirectly an integral part in yield improvement programmes. In resistance breeding programme, progenies or populations are required to be screened for reaction to the targeted plant pathogens under hot spots natural conditions or under artificially inoculated conditions to identify genotypes with high level of resistance score. In fact reliable screening against the plant pathogens is essentially required to validate the parental lines planned to be used in the beginning of a resistance breeding programme or to screen and testify the level of resistance in segregating/stabilizing populations derived from a hybridization programme. In both the cases, sick plots/hot spots sites are very useful and helpful in reliable screening.

The simplest breeding approach is to search out the resistant lines, genetic stocks, advanced breeding lines, landraces by screening using enough inoculums and disease pressure either in the areas where abundant pathogenic races are available or under ambient controlled condition or in the disease screening nursery. Multi-location and multi-environment testing will be more reliable in exploring lines with true resistance potential. In fact, standard screening techniques should be used for preparation of inoculums, transfer of inoculums at right parts of the plant at right stage under appropriate environmental conditions followed by scoring of response in terms of data on appropriate scale. Based on the data of multi-location and multi-year trials, resistant genotypes are selected.

There are many sources that can be explored for resistant gene in the breeding programme. Primary gene pool sources of resistant genes are land races, farmers' varieties, commercial varieties, natural or induced mutants, exotic and indigenous germplasm, and elite lines. The primary gene pools are the best source of resistant gene, since they are ready to use materials that a breeder can use freely. The

secondary gene pool sources of resistance include those lines that are considered to be wild relatives or progenitors of cultivated crops. These sources are rather more important in terms of allelic divergence and may provide a rather strong source of resistant gene. However, crossability with cultivated plants and transfer of tightly linked undesirable genes are major hurdles in domestication of wild alleles for disease resistance. Tertiary gene pool sources include those species which are quite distantly related, and crossing between primary and tertiary gene pool sources are normally not possible and various improved and advanced tools are required to make genomic influx. Secondary and tertiary gene pools have great potential to support the biotic stress breeding programme with potential resistant genes.

Classical breeding methods based on major gene or gene with major effect towards disease resistance is simple and straightforward. In case the major resistance is available in primary gene pool, backcross breeding approach will be more pertinent to transfer a single desired character to an otherwise superior genotype (the recurrent parent) without altering the genetic make-up of recurrent parent. Success from this approach depends largely on (i) the availability of a potential genotype or variety that has good adaptability and yield potential to serve the purpose of recipient or recurrent parent, (ii) the identification of the transferred character in segregating populations and (iii) no existence of linkage disequilibrium between any undesirable trait with the desirable trait to be transferred from a genotype that possessed the resistant gene and serve the purpose of donor (non-recurrent) parent. This approach has been very useful in transferring simply inherited traits, especially genes that have clear-cut evidence of resistance/susceptibility. The backcross breeding methods can be used successfully in self-pollinated crops for development of pure line varieties. The method has same level of significance and importance in cross pollinated crops where the objective is to develop a hybrid cultivar resistant to a particular disease conditioned by a gene with major effect. In case of self-pollinated crops, product is developed at the end of backcross breeding programme, however in case of cross pollinated or hybrid breeding programme, parental lines resistance to disease is developed at the end of the programme. These improved parental lines are crossed to develop disease resistance hybrid cultivar. Backcross breeding approach can also be used for pyramiding diverse major genes for resistance to a pathogen/insect in one and the same variety. It is believed that the greater the number of major genes for resistance in a variety, greater would be its longevity. Further deployment of effective resistance gene in time and space can be another effective strategy for utilization of resistance sources in management of diseases and minimization of crop losses due to biotic stresses. Backcross breeding can also be used successfully in development of multiline variety having many resistance genes in component lines. In fact a multiline variety is a population of plants that is agronomically uniform but heterogeneous for genes that condition reaction to pathogens. This concept was first given by Jensen (1952) for use in oats. Similar approach was suggested by Borlaug (1959) in wheat for controlling stem rust. Multiline variety has genotypic diversity with respect to vertical resistance genes. Each component line of a multiline must have strong resistance gene to ensure the reduction of initial inoculums and spread of diseases

for longer duration. Except disease-resistant genes, component of multiline must have phenotypic uniformity with respect to agronomic characters, namely plant height, plant morphology, maturity and seed shape, colour and size. Pedigree and bulk methods of selection in segregating population generated by biparental or multi-parental crossing are other approaches that can be used for development of genotypes with high resistance score. Again the success of the programme depends on the availability of resistant sources used in crossing programme followed by handling of segregating population, comprehensive screening and identification of potential genotypes. These methods are heavily dependent on gene sources from primary gene pool.

In case resistance gene is not available in germplasm, induced mutation followed by mutation breeding approach can be adopted to screen the variability induced due to mutagen and subsequently to identify the individual plants showing resistance. In the subsequent generations, progeny rows are tested under adequate disease pressure to further validate and identify a stable mutant line. In case of mutation breeding, initial genetic material should be a well-established variety in case of self-pollinated crops or well-proven parental lines of a hybrid cultivar. Natural or spontaneous mutant can also be screened for identification of source of resistance to a particular pathogen. Mutagenized lines can serve the purpose of variety if other required parameters are *at par* with standard cultivars or alternately can be used as source of resistance gene (donor) in backcross breeding programme. Frequencies of desirable mutant (disease resistant) or specific mutant are extremely low, therefore large population size should be grown for increasing probability of desirable mutant.

To induce the desired genetic variability, tools and techniques for induction of variability in vitro in somatic cells is another effective way, in case resistance gene is not available or available resistance source is not as effective as required. Somaclonal variation is the variation seen in plants that have been produced by plant tissue culture. Somaclonal variation is not restricted to, but is particularly common in plants regenerated from callus. The variations can be genotypic or phenotypic, which in the latter case can be either genetic or epigenetic in origin. Typical genetic alterations are changes in chromosome numbers (polyploidy and aneuploidy), chromosome structure (translocations, deletions, insertions and duplications) and DNA sequence (base mutations). The phenomenon of high variability in individuals from plant cell cultures or adventitious shoots is called somaclonal variation. Therefore, it can be defined as the variation that occurs because of genetic mutation caused by in vitro conditions or by chimeral separation. Somaclonal variation is usually undesirable. In some cases, somaclonal variation can lead to new cultivars (e.g., disease resistance, new leaf pattern) that may have desirable ornamental characteristics or increased pest resistance. Disease-resistant somaclonal variants can be obtained by (1) Screening of plants regenerated from cultured cells or their progeny are subjected to disease test, and resistant plants are selected. (2) Cultured plant tissues are selected for resistance to the toxin or culture filtrate produced by the pathogen, and plants are regenerated from the selected cells. Cell selection strategy is most likely to be successful in cases where the toxin is involved in disease development. Resistance was first reported in sugarcane for eye spot disease caused by *Helminthosporium sacchari*.

The choice of any one method of selection depends on the breeder, stage of the breeding program, stage of germplasm development, stage of knowledge of the populations and objectives of the breeding program. In cross pollinated crops, quantitatively inherited disease resistance can be enhanced by recurrent selection (RS) – any breeding system designed to increase the frequency of desired alleles for particular quantitatively inherited characters by repeated cycles of selection (Sleper and Poehlman 2006). Number of selection cycles may be repeated as long as superior genotypes with higher score of resistance are generated. The improved population can be used as a variety per se; alternatively, it can be used as source for development of inbred lines that can be used as parents of a synthetic or hybrid cultivar. With simplest form of intra-population improvement which target only one population, the method of recurrent selection can be extended to several divergent populations. Individuals in a population can be evaluated on the basis of their phenotype or on the basis of the performance of their half-sib or full-sib progenies. In both intra- and inter-population improvement approaches, the ultimate aim is to improve the frequency of genes conferring resistance against a quantitatively inherited disease. Improved populations are in fact elite group of plants that can be used as such in bulk as an open pollinated variety for cultivation in those areas where crop is damaged by pathogens. Furthermore, such cultivars do not require seed replacement every year and the farmers' harvest can be used for sowing next year. Alternatively, improved population can support hybrid development programme in areas where seed production and supply network is well established.

10.6.2 Novel Approaches in Biotic Stress Resistance Breeding

Why Novel Approaches?

Advancement in genetic research at molecular level leads to the development of many handy but useful tools that can be integrated with classical plant breeding to achieve the specific objective of gene transfer, gene pyramiding, gene addition, gene knockout, alteration in nucleotide sequence of a gene and ease accessibility of any gene across the crossing barrier boundary for improvement in general and development of resistance cultivars against biotic stresses in particular. Novel molecular-based tools can be used successfully in resistance breeding programme, as it is advantageous over classical resistance breeding methods as depicted below:

- Can exploit horizontal variability, thus widen option for genetic manipulation.
- Precise transfer of specific functional fragment of DNA.
- Precise up and down regulation of gene.
- Reduces time required in gene transfer.
- Novelty can be introduced.
- Effective screening is easy.
- Gene can be precisely modified partly or wholly.
- Many resistant genes for a disease or for different diseases can be pyramided.

10.6.2.1 Molecular Breeding

Molecular breeding (MB) or molecular plant breeding (MPB) in broad sense includes use of genetic manipulation performed at DNA molecular levels to improve plants characteristics and covers genetic engineering or gene manipulation, molecular marker-assisted selection and genomic selection. However, often MB or MPB implies molecular marker-assisted breeding (MAB) and it is defined as the application of molecular biotechnologies, specifically molecular markers, in combination with linkage maps and genomics, to alter and improve plant characteristics on the basis of genotypic assays. This term is used to describe several modern breeding strategies, including marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genome-wide selection (GWS) or genomic selection (GS) (Ribaut et al. 2010).

MAS is the process in which a marker is used for indirect selection of a genetic determinant of biotic stresses. This method involves selection of plants carrying genomic regions that are involved in the expression of traits of interest through the application of molecular markers. The development and availability of an array of molecular markers and dense molecular genetic maps in crop plants have made application of MAS possible for traits governed by major genes as well as those governed by many genes and expression are quantitative in nature (Choudhary et al. 2008). The success of MAS depends on several factors, including the number of target genes or genomic regions to be transferred and the distance between the flanking markers and the target genomic site. With availability of different marker systems and high throughput genotyping and phenotyping along with improved statistical approaches, MAS is gaining considerable importance as it can improve the efficiency of plant breeding through precise transfer of genomic regions of interest and acceleration of the recovery of favourable alleles of the recurrent parent genome (Wijerathna 2015).

With the application of MAS, individual plants can be selected based on their genotype, during the selection procedure. For most traits, homozygous and heterozygous plants cannot be distinguished by conventional phenotypic screening. MAS can be used to assist selection of parents, increasing the effectiveness of backcross breeding and improving sex-limited traits (Zhou et al. 2007). MAS can be used to investigate heterosis for hybrid crop production (Reif et al. 2003), and there is the potential for use of DNA marker data along with phenotypic data to select hybrids (Jordan et al. 2003). There are various advantages of using MAS in rice breeding. For example, it may be simpler than phenotypic screening; therefore, it can reduce time, effort and resources. Selection of quality traits in rice generally requires expensive screening procedures that are made feasible through MAS. Additionally, MAS can be conducted at the seedling stage, and undesirable plant genotypes can quickly be eliminated (Khan et al. 2015). The advantages associated with the use of markers includes speed, consistency, efficiency, biosafety and the ability to skew the odds in our favour, even while dealing with complex traits.

Gene pyramiding is the transfer or pyramiding more than one resistance/tolerance genes/QTLs into a single genotype (Collard and Mackill 2008). Pyramiding of resistance genes into a single line for each disease is a novel strategy in resistance

breeding to prevent the breakdown of resistance against specific disease or stress. Pyramiding of genes/QTLs that confer resistance to biotic stresses and tolerance against various types of abiotic stresses is now feasible because of advancements in molecular markers (Das and Rao 2015). MAS has been found to work efficiently for transferring genes from pyramided lines into new plants and into improved varieties (Magar et al. 2014). Breeders have used marker-assisted selection to "pyramid" resistance conferred by several separate resistance genes/QTLs with the help of closely linked markers against diseases such as bacterial blight, rice blast and insect as gall midge in rice, leaf rust resistance and powdery mildew resistance in wheat, and insect pest resistance in cotton, as well as many other traits (Das and Rao 2015; Pradhan et al. 2015; Suh et al. 2015; Shamsudin et al. 2016). To get the desired population with required gene combinations without unwanted genes, backcrossing with the recurrent parent is required. The use of molecular markers, which were unlinked to the assembled genes/QTLs for back ground selection, enhances the proportion of recovery of the recipient genome. The gene pyramiding scheme can be distinguished into two parts, development of a pedigree, which is designed to accumulate all target genes in a single genotype known as the root genotype, and a fixation step, which is intended to fix the target genes into a homozygous state to derive the ideal genotype from one single genotype. The brown plant hopper (BPH), *Nilaparvata lugens*, has been one of the most devastating pests to rice crops in Vietnam and Asia. There is successful report of the use of SSR and STS markers in pyramiding two BPH resistance genes *Bph14* and *Bph15* into three elite japonica varieties Shengdao 15, Shengdao 16 and Xudao 3, using marker-assisted backcross breeding programmes (Xu 2013). Rice cultivation across tropical and semi-tropical regions of the world is affected by bacterial blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). A total of 38 R genes of BB have been identified in rice (Khan et al. 2014). Resistant cultivars with one or two major resistant genes are unsustainable in the field, and the only way to delay such a breakdown of BB resistance is to pyramid many resistance genes using MAS (Rafique et al. 2010). Rice sheath blight disease caused by *Rhizoctonia solani* Kuhn reduces trivial yield in rice-growing areas around the globe (Yellareddygari et al. 2014; Yadav et al. 2015). Genetic studies have shown that SB resistance can be controlled by polygenic QTLs. It is possible to pyramid SB resistance QTLs into rice varieties using MAS.

Marker-assisted selection (MAS)/Marker-assisted backcrossing (MAB) has been used successfully in many crop species for introgressing resistance genes. It is an important tool for pyramiding different genes for a disease or vice versa. In India, many varieties/hybrid have been developed using MAB/MAS (Table 10.1).

10.6.2.2 Transgenic Breeding for Biotic Stress Tolerance

Genetic variations are unanimously accepted as the basic necessity for improvement of any trait using classical breeding approach and so is the case with molecular or transgenic breeding programme. Resistance genes present in taxonomically different plant species or in wild relatives have no meaning since breeder or molecular breeder of other crops cannot use such variability which is referred to as horizontal

Table 10.1 MAS derived varieties in India for different characters

Crop	Varieties	Characters
Pearl millet	HHB 67 improved	Downey mildew
Rice	Pusa 1612 (Pusa Sugandh 6) Pusa 1592 Pusa Basmati 1609 Improved Pusa Basmati 1 Improved Samba Mahsuri	Blast (*Piz5* and *Pi54)* Bacterial blight (*xa13* and *Xa21)* Blast (*Piz5* and *Pi54)* Bacterial blight (*xa13* + *Xa21)* Bacterial blight (*xa5* + *xa13* + *Xa21)*
Wheat	PBW-343 improved HD 2329 WH147 HD2687	Lr37/Yr17 and Lr76/Yr70 Lr24 and Lr28 Lr24 + Lr28,Lr24 + Lr37,Lr28 + Lr37 Lr24 + Lr28 + Yr15

variability. Transfer of genes between plant species has played an important role in crop improvement for many decades. Genes expected to confer disease resistance are isolated, cloned and transferred into many crop species. Useful traits, viz., resistance to diseases, insects and pest, have been transferred to crop varieties from noncultivated plants. Transfer of useful alleleic variants or novel gene (s) across the species and genera trans-boundary requires identification, introduction, validation followed by transfer of the gene into desired plant species. Once a gene known to confer resistance is identified from any sources or even chemically synthesised and is finally cloned, its transfer become easy and in principle it can be transferred to any plant species using different plant transformation techniques. The overall process of genetic transformation involves introduction, integration and expression of foreign gene in the recipient host plant. Plants that carry additional stably integrated and expressed transferred gene (transgenes) from other genetic sources are referred to as transgenic plants. The capacity to introduce and express diverse foreign genes in plants was first described in tobacco by *Agrobacterium* mediated and vectorless approach (Horsch et al. 1984) Development and deployment of strong major genes over time and space may check epidemics in alternate years and geographic regions so that the virulent pathotype against any one of them doesn't evolve and doesn't survive even if it did evolve. Based on the principle of crop rotation to control certain soil-borne pathogens, transgenic crops with different resistant gene can be of great help in management of pathogens. Refugia approach can also be integrated while recommending cultivation of transgenic crop based on single major gene to delay the emergence and also manage the population of virulent strains. The term 'refugia' is classically defined as an area in which a population of organisms can survive over a period of unfavourable conditions. Exploiting refugia as a means of delaying the evolution of resistance has been explored in biological systems, in particular in the management of agricultural pests.

The transgenic breeding approach can use many strategies to incorporate genes responsible for disease resistance. Diverse genes reported to confer different function related directly or indirectly to disease resistance can be used for development of GM crops. Targets that can be used for modification in host for enhancing heritable tolerance against different pathogens can be based on:

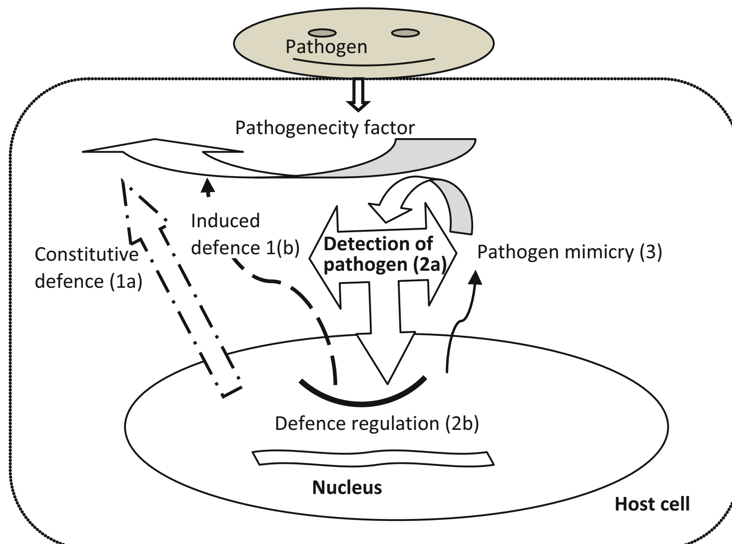

Fig. 10.1 A simplified model of defence illustrating successful transgenic strategies. Strategy 1 concerns direct interference with pathogenicity or inhibition of pathogen physiology. Thus, *1a* involves constitutive expression of antimicrobial factors and *1b* involves pathogen-induced expression of one or more genes in the transgenic plant. Strategy 2 concerns the regulation of the natural induced host defences. *2a* concerns altering recognition of the pathogen (e.g. R-genes) and *2b* concerns downstream regulatory pathways (e.g. SAR) and includes transcription factors. Strategy 3 is pathogen mimicry: the manipulation of the plant to prime recognition of a specific pathogen through pathogen-derived gene sequences. (Adapted from Collinge et al. 2008)

1. Expression of antifungal compounds—PR proteins, phytoalexins, defensins
2. Enzymes that destroy or neutralize components of pathogen arsenals polygalactouronase inhibitors, oxalic acid (toxin) oxidase
3. Alterations of structural components-peroxidase for lignin production, callose synthase
4. Resistance genes
5. Components of signalling pathways

A simplified model of defence in host illustrating successful transgenic strategies that can be adopted for deployment of genes conferring resistance to pathogens has been proposed (Fig. 10.1).

Transgenic breeding approach has been used for introgression of resistant gene (s) in many plant species. The diverse sources have been used to isolate the genes that confer resistant property directly or indirectly. Both physical and biological approaches of genetic transformation have been used to transfer gene into recipient plants. Though there are many successful examples of introgressing resistance gene (s) against various plant pathogens, it has been more successful in cases of diseases caused by viruses (Table 10.2). Use of transgenic variety in control of papaya ring spot virus is an excellent example of successful use of transgenic breeding approach.

Table 10.2 Genetically engineered biotic stress-resistant varieties

Crop/Even/ Trade name	Gene introduced	Gene source	Product	Function
Common bean (*Phaseolus vulgaris* L.)				
EMBRAPA 5.1	**ac1 (sense and antisense)**	Bean Golden Mosaic Virus (BGMV)	Sense and antisense RNA of viral replication protein (Rep); no functional viral replication protein is produced	Inhibits the synthesis of the viral replication protein of the Bean Golden Mosaic Virus (BGMV), thereby conferring resistance to the BGMV
Papaya (*Carica papaya*)				
Rainbow, SunUp	**prsv_cp**	Papaya ringspot virus (PRSV)	Coat protein (CP) of the papaya ringspot virus (PRSV)	Confers resistance to papaya ringspot virus (PRSV) through "pathogen-derived resistance" mechanism
63–1	**prsv_cp**	Papaya ringspot virus (PRSV)	Coat protein (CP) of the papaya ringspot virus (PRSV)	Confers resistance to papaya ringspot virus (PRSV) through "pathogen-derived resistance" mechanism
Huanong no.1	Replicase domain of the papaya ringspot virus (PRSV)	Papaya ringspot virus (PRSV)	Confers resistance to papaya ringspot virus (PRSV) through gene silencing mechanism	Papaya ringspot virus (PRSV)
X17–2	**prsv_cp**	Papaya ringspot virus (PRSV)	Coat protein (CP) of the papaya ringspot virus (PRSV)	Confers resistance to papaya ringspot virus (PRSV) through "pathogen-derived resistance" mechanism

(continued)

Table 10.2 (continued)

Crop/Even/ Trade name	Gene introduced	Gene source	Product	Function
Plum (*Prunus domestica*)				
C-5	**ppv_cp**	Plum pox virus (PPV)	Coat protein of plum pox virus (PPV)	Confers resistance to plum pox virus (PPV) through "pathogen-derived resistance" mechanism
Potato (*Solanum tuberosum L.*				
Hi-Lite NewLeaf™ Y potato	**cry3A**	*Bacillus thuringiensis* subs. *tenebrionis*	cry3A delta endotoxin	Confers resistance to coleopteran insects by selectively damaging their midgut lining
	pvy_cp	Potato Virus Y (PVY)	Coat protein of the potato virus Y (PVY)	Confers resistance to potato virus Y (PVY) through "pathogen-derived resistance" mechanism
New Leaf™ Y Russet Burbank potato	**cry3A**	*Bacillus thuringiensis* subs. *tenebrionis*	cry3A delta endotoxin	Confers resistance to coleopteran insects by selectively damaging their midgut lining
	pvy_cp	Potato Virus Y (PVY)	Coat protein of the potato virus Y (PVY)	Confers resistance to potato virus Y (PVY) through "pathogen-derived resistance" mechanism

(continued)

Table 10.2 (continued)

Crop/Even/ Trade name	Gene introduced	Gene source	Product	Function
New Leaf™ Plus Russet Burbank potato	**cry3A**	*Bacillus thuringiensis* subs. *tenebrionis*	cry3A delta endotoxin	Confers resistance to coleopteran insects by selectively damaging their midgut lining
	cp4 epsps (aroA: CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
	plrv_orf1	Potato Leaf Roll Virus (PLRV)	Putative replicase domain of the potato leaf roll virus (PLRV)	Confers resistance to potato leaf roll virus (PLRV) through gene silencing mechanism
	plrv_orf2	Potato Leaf Roll Virus (PLRV)	Putative helicase domain of the potato leaf roll virus (PLRV)	Confers resistance to potato leaf roll virus (PLRV) through gene silencing mechanism
SP951	**RB**	*Solanum bulbocastanum*	Late blight-resistant protein	Broad spectrum resistance against *Phytophthora infestans* races
W8	**asn1**	*Solanum tuberosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate Asn1 transcripts which lowers asparagine formation
	ppo5	*Solanum verrucosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate Ppo5 transcripts which lowers black spot bruise development

(continued)

Table 10.2 (continued)

Crop/Even/ Trade name	Gene introduced	Gene source	Product	Function
	Rpi-vnt1	*Solanum venturii*	Late blight-resistant protein	Confers resistance to potato late blight
	PhL	*Solanum tuberosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate PhL transcripts which lowers reducing sugars
	R1	*Solanum tuberosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate R1 transcripts which lowers reducing sugars
	Vlnv	*Solanum tuberosum*	Double-stranded RNA	Downregulates VInv transcripts which lowers reducing sugars
Innate® Acclimate	**asn1**	*Solanum tuberosum*	Double-stranded RNA	Designed to generate dsRNA to down-regulate Asn1 transcripts which lowers asparagine formation
	ppo5	*Solanum verrucosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate Ppo5 transcripts which lowers black spot bruise development
	Rpi-vnt1	*Solanum venturii*	Late blight-resistant protein	Confers resistance to potato late blight
	R1	*Solanum tuberosum*	Double-stranded RNA	Designed to generate dsRNA to downregulate R1 transcripts which lowers reducing sugars
	Vlnv	*Solanum tuberosum*	Double-stranded RNA	Downregulates VInv transcripts which lowers reducing sugars

(continued)

Table 10.2 (continued)

Crop/Even/ Trade name	Gene introduced	Gene source	Product	Function
Squash (*Cucurbita pepo*)				
CZW3	**cmv_cp**	Cucumber Mosaic Cucumovirus (CMV)	Coat protein of cucumber mosaic cucumovirus (CMV)	Confers resistance to cucumber mosaic cucumovirus (CMV) through "pathogen-derived resistance" mechanism
	zymv_cp	Zucchini Yellow Mosaic Potyvirus (ZYMV)	Coat protein of zucchini yellow mosaic potyvirus (ZYMV)	Confers resistance to zucchini yellow mosaic potyvirus (ZYMV) through "pathogen-derived resistance" mechanism
	wmv_cp	Watermelon Mosaic Potyvirus 2 (WMV2)	Coat protein of watermelon mosaic potyvirus 2 (WMV2)	Confers resistance to watermelon mosaic potyvirus 2 (WMV2) through "pathogen-derived resistance" mechanism
ZW20	**zymv_cp**	Zucchini Yellow Mosaic Potyvirus (ZYMV)	Coat protein of zucchini yellow mosaic potyvirus (ZYMV)	Confers resistance to zucchini yellow mosaic potyvirus (ZYMV) through "pathogen-derived resistance" mechanism
	wmv_cp	Watermelon Mosaic Potyvirus 2 (WMV2)	Coat protein of watermelon mosaic potyvirus 2 (WMV2)	Confers resistance to watermelon mosaic potyvirus 2 (WMV2) through "pathogen-derived resistance" mechanism

(continued)

Table 10.2 (continued)

Crop/Even/ Trade name	Gene introduced	Gene source	Product	Function
Sweet pepper (*Capsicum annuum*)				
PK-SP01	**cmv_cp**	Cucumber Mosaic Cucumovirus (CMV)	Coat protein of cucumber mosaic cucumovirus (CMV)	Confers resistance to cucumber mosaic cucumovirus (CMV) through "pathogen-derived resistance" mechanism
Tomato (*Lycopersicon esculentum)*				
PK-TM8805R (8805R)	**cmv_cp**	Cucumber Mosaic Cucumovirus (CMV)	Coat protein of cucumber mosaic cucumovirus (CMV)	Confers resistance to cucumber mosaic cucumovirus (CMV) through "pathogen-derived resistance" mechanism

Source: ISAAA's GM Approval Database (2019)

Expression of toxic protein gene in many plant species for control of insect is another successful example of transgenic approach in resistance breeding.

Transgenic breeding approaches have been successful in transferring different genes conferring resistance to different pathogens in plant species. In addition, great stride has been made in developing insect-pest resistant genotypes in different crop species. Molecular genetic studies over past decades have originated new tools for breeding programmes of crop plants. Innumerous genetic engineering techniques were developed and applied to generate genetically modified crop varieties with superior agricultural characteristics, including new traits that do not occur naturally in the species. In the further refinement of GM technology, molecular techniques promising to develop 'transgene free' crops have been introduced in plant breeding programmes. These new techniques are collectively called Genome Editing (GE). GE is changing the way of producing genetically modified organisms since it produces specific genetic changes within a genome, with no transgene manipulation. GE refers to platforms that use site-specific nucleases (SSNs) that can introduce DNA lesions at a specific genomic position. Several novel GE systems based on SSNs were developed: Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/ Cas9). Because of its high efficiency and relatively low cost, CRISPR/Cas9-based genome editing system have become the most popular choice of plant molecular biologists for functional studies of plant genes. The

CRISPR-Cas9 system is a plant breeding innovation that uses site-directed nucleases to target and modify DNA with great accuracy. Developed in 2012 by scientists from the University of California, Berkeley, CRISPR-Cas9 has received a lot of attention in recent years due to its range of applications in modifying plant genomes for altering yield, architecture and tolerance/resistance to biotic and abiotic stress. Advances in genome editing tools have opened new ways to achieve the improvement of resistance in crops. In the recent past, the CRISPR/Cas system has been employed to respond to several agricultural challenges, including improved biotic stress resistance (Arora and Narula 2017). The application of CRISPR/Cas tools has mainly been explored against virus infection, followed by efforts to improve fungal and bacterial disease resistance. Recent experimental findings of many research groups indicate genome editing technology as an effective tool in developing tolerant genotypes without altering genetic make-up of plants (Borrelli et al. 2018; Langner et al. 2018; Andolfo et al. 2016).

10.7 Wild Relatives in Biotic Stress Tolerance

10.7.1 Wheat

The species belonging to primary, secondary and tertiary gene pools of *Triticeae* species are rich source of genes for improvement of traits pertaining to biotic stress tolerance. Introgression of alleles from nearly 52 related species have already been done for improvement of wheat for different traits (Wulff and Moscou 2014).

Fusarium head blight resistance has been reported by Oliver et al. (2007) in *Triticum dicoccoides*. A diploid progenitor of bread wheat *Triticum monococcum* has also been found to be the source of resistance genes to a number of fungal diseases of wheat. Yao et al. (2007) discovered the genes for traits responsible for imparting resistance against powdery mildew in *Triticum monococcum,* while Sodkiewicz et al. (2008) identified resistant genes for leaf rust. An adult plant resistance (APR) gene for stripe rust and leaf rust have been transferred from T. *monococcum* to bread wheat by means of marker-assisted selection, and a leaf rust-resistant gene have also been transferred to PBW343 (Singh et al. 2007). A race specific stem rust resistance gene, *Sr35*, has also been identified in *T. monococcum*. Moseman et al. (1984) identified *T. monococcum* as a source of powdery mildew resistance. *Aegilops tauschii*, another diploid progenitor contributing D genome to hexaploid wheat, serves as donor for Russian leaf rust resistance gene (*Lr21*) (Yumurtaci 2015), race specific yellow rust resistance gene *Yr28* and an adult plant resistance gene *Lr22a*. Additionally, Bockus et al. (2012) identified *A. tauschii* as a source of blast resistance in wheat. Introgression of stem rust resistance genes found in *T. turgidum* and *T. dicoccum* into bread wheat have also been reported as early as the 1930s by McFadden. *T. dicoccum* have also been identified as donor source for *Rmg7* gene conferring resistance to *Triticum* isolates of *Pyricularia oryzae* by Tagle et al. (2015). *Aegilops geniculata*, an allotetraploid relative of wheat, was found to be the source of barley yellow dwarf virus and

powdery mildew resistance of wheat (Yumurtaci 2015). The 6P chromosome of *A. cristatum* is responsible for improving resistance to powdery mildew and barley yellow dwarf virus besides enhancing some yield-contributing traits as number of kernels and grain weight in wheat (Wang et al. 2011). Cruz et al. (2016) found that 2NS/2AS translocation from *Aegilops ventricosa* conferred resistance to wheat blast disease. *T.dicoccoides* and *Triticum carthlicum* have also been reported as source of genes conferring resistance to powdery mildew by Moseman et al. (1984). Vertical and horizontal resistance genes have also been identified from diverse sources, viz., *Yr5* from *Triticum spelta*, *Lr9* from *Aegilops umbellulata* conferring vertical resistance and *Yr36* from *Triticum diccocoides* conferring horizontal resistance against rust disease of wheat. Multiple disease resistance have been found in a number of wild relative as *Sr36/Pm6* from *Triticum timopheevi, Pm8/Sr31/Lr26/Yr9* from rye and *Lr19/Sr25*, *Sr24/Lr24*, *Sr26* from *A. elongatum* and *Pch1* and *Sr38/Lr37/Lr17* from *Aegilops ventricosa* (Wulff and Moscou 2014). Wan et al. (1997) identified Fusarium head blight resistance genes in different genera, viz., *Agropyron, Elymus and Hystrix* as well as in related wheat species, namely *T. monococcum, T. timopheevi and T. militinae* (Cai et al. 2005). Introgression of segments of alien chromosome from wild relatives into wheat genetic background has been found to improve tolerance to different disease. The *7DL.7Ag* translocation from a wild relative *Lophopyrum elongatum* carrying *Lr19* gene was done by Monneveux et al. (2008) which conferred leaf rust resistance to wheat. Similarly, genes for stem rust resistance (Waines and Ehdaie 2007) and powdery mildew (Yediay et al. 2010) have also been successfully introgressed from rye into wheat germplasm lines.

10.7.2 Rice

Genus *Oryza* of the graminae family constitutes a total of 24 species. Out of these 24 species, *O. sativa* L. and *O. glaberrima* are the only cultivated species of genus, Oryza while the remaining 22 are wild species distributed worldwide (Khush 1997; Vaughan 1989). Depending on the ease of transfer of genes to their cultivated counterparts, the wild species are divided into three complexes, i.e., *O. sativa* complex, *O. officinalis* complex and *O. meyeriana* and *O. ridleyi* complex (Morishima and Oka 1960). Khush (1997) later renamed these complexes as the primary, secondary and tertiary gene pool of rice. Wild species of rice are a rich source of economically valuable traits on account of being grown in diverse climate and lack of artificial selection. Some of the donors of these traits are mentioned in forthcoming section.

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is reported to be one of the most destructive diseases of rice worldwide. However, the utilization of two resistant genes namely, *Xa3* and *Xa4*, have enhanced BLB resistance, but due to continuous evolution of new pathogen strains, the need to discover new sources of resistance became inevitable. Two new resistant genes (*Xa21* and *Xa 23*) were identified in wild rice (Song et al. 1995; Zhou et al. 2011). *Xa21* have been identified

in *Oryza longistaminata* and was transferred to rice variety IR72 in 1990 to mark the first ever example of utilization of rice wild relative for crop improvement. Positional cloning of *Xa21* gene was done, and it was found to encode receptor kinase-like protein (Song et al. 1995). The gene was soon after tagged with molecular markers and transferred into various famous rice cultivars by means of marker-assisted breeding to produce BLB-resistant varieties. The resistance conferred by *Xa21* was soon broken down as many new virulent strains of BLB pathogen evolved in China. Therefore, the need to identify novel resistant genes providing durable resistance against BLB emerged. *O. longistaminata* along with *O. rufipogon* have also been identified as a potential source of rice tungro virus and have been utilized to produce a number of virus-tolerant rice lines. Rice blast caused by *Magnaporthe oryzae* is yet another economically important fungal disease of rice. The disease was first reported from the United States and since then has been reported in about 85 rice-growing countries worldwide (Wang et al. 2014). The source of resistance for this disease has been searched in wild relative of rice and to date, about 100 resistance genes and more than 350 QTLs have been identified providing resistance against rice blast (Wang et al. 2014; Ashkani et al. 2015; Vasudevan et al. 2015). 96% of the total identified R genes have been found in japonica and indica cultivars while only 4% of them are reported to be contributed by crop wild relatives (Wang et al. 2014) including *O. rhizomatis*, *O. minuta* and *O. autraliensis*. Resistance genes have been introgressed into susceptible lines to breed for blast tolerance (Sharma et al. 2012; Wang et al. 2014; Ashkani et al. 2015). Another blast resistance gene, *Pi33*, identified in *O. rufipogon* has also been transferred into IR64, producing a blast-resistant variety (Ballini et al. 2007). Three R gene clusters viz., *Piz, Pik* and *Pita*, were discovered on chromosomes 6, 11 and 12, respectively. Besides providing blast resistance, *O. rufigon* has also been identified as a source of a single, dominant BLB-resistant gene *Xa23* which provides efficient broad spectrum resistance at all crop growth stages and was responsible for providing resistance against all 20 known strains of the pathogen (Zhang et al. 2001; Zhang and Xie 2014). *O. nivara* also serves as source of a novel BLB-resistant gene, *Xa38* (Ellur et al. 2016). Rice crop is affected by more than 20 viral disease with the most damaging being grassy stunt disease; caused by rice grassy stunt virus (RGSV), and rice tungro disease; caused by infection of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). BPH acts as vector for transmission of RGSV, while green leafhopper serves as vector for rice tungro disease. A single dominant gene, *Gs* was identified in *O. nivara* responsible for imparting resistance against RGSV. Resistance against RGSV has been introgressed into a number of germplasm lines from *O. nivara* (Leung et al. 2002).

10.7.3 Maize

Quantitative trait loci (QTLs) responsible for providing resistance against a number of diseases were discovered in maize wild relatives. Lennon et al. (2016) conducted a study where he utilized a population of near isogenic lines derived from cross

between maize and teosinte (*Zea mays* ssp. *parviglumis*) for QTL mapping of gray leaf spot (GLS). A GLS resistance QTL was thus identified in bin 4.07. Chavan and Smith (2014) reported that teosinte can also be used as donor of resistance to corn smut disease. *Z. diploperennis* was reported to be a source of resistance to southern corn leaf blight, northern corn leaf blight and corn leaf spot disease. Wei et al. (2001) studied maize × *Z. diploperennis* crosses and using genomic in situ hybridization identified specific segments derived from *Z. diploperennis* imparting resistance to above mentioned three fungal diseases. A higher level of resistance was reported against a number of viral and mycoplasmal diseases of maize viz., maize chlorotic mottle virus, maize bushy stunt mycoplasma, maize streak virus, maize stripe and rayadofino virus and maize chlorotic dwarf virus in *Z. diploperennis* by Nault and Findley (1982). Maazou et al. (2017) reported *Z. mays* ssp. *mexicana* to be the donor for Fusarium and downy mildew resistance. *Tripsacum dactyloides* is also reported to be a source of many resistance alleles which when transferred into maize genetic background have historically helped to overcome various disease epidemics in maize. One of the examples was the transfer of blight-resistant alleles into commercial corn lines which resolved the problem of corn blight in the United States (Maxted and Kell 2009). The introgression of *Ht3*, a northern leaf blight resistance genes (Hooker 1981) and *Rp1td*, a novel rust resistance genes (Bergquist 1981) from eastern gamagrass into maize genetic background remain the success stories of utilization of wild relatives to breed for disease resiliency in crop plants. Hajjar and Hodgkin (2007) have also reported the utilization of introgression from *Tripsacum* to breed for *Helminthosporium* and *Puccinia* resistance in maize.

10.7.4 Soybean

Genus Glycine was divided into two subgenera, Glycine and Soja. Cultivated soybean *Glycine max* and its wild progenitor *G. soja* make up the subgenus Glycine. The subgenera Soja is composed of seven perennial wild, diploid, perennial species, *G. canescens*, *G. clandestine*, *G. falcate*, *G. latifolia*, *G. latrobeana*, *G. tabacina* and *G. tomentella*. Although only *G. soja* has been utilized in past for manipulation by plant breeders, now wild relatives belonging to subgenus Soja are also being actively utilized as donors of different agronomically useful traits in soybean breeding programmes.

Soybean rust is one of the major diseases affecting soybean production worldwide. It is caused by pathogen *Phakopsora pachyrhizi*. Hartman et al. (1992) screened 294 accessions belonging to 17 Glycine species and identified numerous rust-resistant sources, viz., *G. clandestine*, *G. canescens, G. argyrea, G. tabacina, G. microphylla, G. latifolia* and *G. tomentella*. Partial resistance to Sclerotinia stem rot or white moulds have also been reported in *G. tabacina* and *G. tomentella* (Hartman et al. 2000). Several accessions of *G. tomentella* and *G. canescens* were also reported showing resistance to powdery mildew disease and sudden death syndrome (Mignucci and Chamberlain 1978; Hartman et al. 2000).

10.7.5 Chickpea

Genus *Cicer* is composed of a total of 43 species which includes 34 wild perennial species, 8 wild annual species and 1 annual cultivated species. All the species are self-pollinated and diploid (Ladizinsky and Adler 1976; Singh and Ocampo 1997). Two wild species, *C. microphyllum* and *Cicer songaricum,* and one cultivated species, *C. arietinum,* are reported to be grown under Indian conditions.

Phytophthora root rot- (*Phytophthora medicaginis)* resistant germplasm lines in chickpea have been developed using *C. echinospermum* as a donor source of resistant alleles (Knights et al. 2008). Resistance to botrytis grey mould has been derived from *C. echinospermum and C. reticulatum* (Singh et al. 1984; Jaiswal et al. 1986; Singh and Ocampo 1997; Singh et al. 2005), *C. judaicum* (Chaturvedi and Nadarajan 2010) and *C. pinnatifidum (*Kaur et al. 2013). Some species of wild relative, *C. bijugum, C. reticulatum, C. pinnatifidum* and *C. echinospermum* also show resistance to multiple stress conditions (Kumar et al. 2011) as *C. echinospermum* harbours resistance for both pod borer and Phytophthora root rot (Knights et al. 2008).

10.7.6 Pigeonpea

The genus *Cajanus* comprises of 32 species (van der Maesen 1986). Mallikarjuna et al. (2010) divided pigeon pea wild relatives into primary, secondary, tertiary and quaternary gene pools. Primary gene pool of pigeonpea constitutes *Cajanus cajan* and its land races. The secondary gene pool comprises of 10 wild species, *C. cajanifolius, C. lineatus, C. lanceolatus, C. laticepalus, C. albicans, C. reticulatus, C. sericeus, C. scarabaeoides, C. trinervius* and *C. acutifolius. Tertiary* gene pool is composed of *C. goensis, C. heynei, C. kerstingii, C. mollis, C. rugosus, C. volubilis, C. platycarpus, C. niveus, C. gandiflorus, C. crassicaulis, C. rugosus, C. elongates, C. villosus, C. confertiflorus, C. visidus, C. aromaticus, C. crassicaulis, C. lanuginosus, C. pubescens, C. cinereus, C. marmoratus, C. mareebensis.C. lanuginosus* and *C. pubescens,* while quaternary gene pool is composed of Flemingia, Rhynchosia, Dunbaria, Erisema Paracalyx, Adenodolichos, Bolusafra, Carissoa, Chrysoscias and Baukea.

Pigeonpea is prone to attack by a number of diseases. Resistance in cultivated gene pool is limited (Pande et al. 2011), therefore wild relatives need to be investigated. With the objective to investigate wild relatives for identification of sources resistant to different diseases so as to utilize prebreeding to introgress genes from wild *Cajanus* species belonging to secondary and tertiary gene pool in order to expand the primary gene pool of pigeonpea, a number of experiments were undertaken at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, India (Sharma and Upadhyay 2016). The experiments involved advanced backcross population derived from interspecific crosses between *C. cajanifolius, C. acutifolius and C. scarabaeoides* belonging to cross-compatible secondary genepool and *C. platycarpus* belonging to cross-incompatible tertiary

gene pool as donors and cultivated pigeonpea as recipient. Embryo rescue technique was utilized to recover population involving tertiary gene pool species. The populations derived from interspecific crosses were screened for resistance to wilt and sterility mosaic disease. Two inbred lines derived from *C. platycarpus and* 15 inbred lines derived from *C. acutifolius* displayed combined resistance to both diseases. Phytopthora-resistant inbred lines were also recovered from the population derived from cross between *C. acutifolius* and cultivated chickpea. Additionally, a number of other wild species belonging to secondary and tertiary gene pool of pigeonpea were also reported to be having resistant traits against a number of other destructive diseases (Sharma and Upadhyay 2016). For example *C. albicans, C. cajanifolius, C. ineatus, C. scarabaeoides* and *C. sericeus* belonging to secondary gene pool; *C. platycarpus, C. volubilis* belonging to tertiary gene pool and *C. sericeus* belonging to secondary gene pool were found to be resistant to alternaria blight and phytophthora blight, respectively.

Apart from the crops described above, wild relatives have also been used as excellent sources of resistance against various biotic stresses in many crop species (Table 10.3).

10.8 Conclusion

Plants are the most important creatures on the earth, working like processing machine that convert one form of 'solar' energy into another form on which all the living organisms depend directly or indirectly for food, shelter and other requirement. Thus, higher productivity in terms of quantum and quality and easy and adequate access to everyone is the only objective of growing plants. High yielding genotypes have been created using classical as well as molecular breeding approaches that are under cultivation. Genetic potential of the improved genotypes are actually not harnessed at farmers' field due to many reasons, among those biotic stresses being one of the major causes. Due to excess and extreme nature of parasitic load, total crop failures have been noted in many crops in the past which led to large scale damage and consequently famines in many parts of the world. Uses of synthetic chemicals have been effective in control of pathogens across the different crops species. This approach is however adding additional burden and also it causes many environmental and health hazards, in addition to polluting natural resources. Gene-based resistance strategy seems to be the most economical and also environment friendly. Dynamic nature of the pathogens however reminds about breakdown of resistance which requires availability of diverse pool of resistance sources that can be used as potential source of donor in resistance-breeding programmes. Wild relatives though have been used in the past, now more systematic investigation is needed for identification of novel sources of resistant genes considering the change in environmental parameters due to climate change. Furthermore, other species can

Table 10.3 Crop species, their wild relatives and resistant traits contributed

Crop	Wild relative	Trait	References
Pearl millet	*P. glaucum* subsp. *monodii*	Rust resistance	Hanna et al. (1985)
	P. glaucum subsp. *monodii*	Leaf spot	Hanna et al. (1985)
	P. orientale	Pest	Dujardin and Hanna (1987)
	P. pedicellatum Trin. and *P. polystachion*	Downy mildew	Dujardin and Hanna (1989)
	Pennisetum glaucum subsp. *monodii*	*Striga* spp.	Hanna et al. (1985)
Barley	*Hordeum spontaneum*	*Fusarium spp.*	Chen et al. (2013)
	Hordeum spontaneum	Leaf stripe	Biselli et al. (2010)
	Hordeum spontaneum	Powdery mildew	Schmalenbach et al. (2008)
	Hordeum spontaneum	Leaf rust	Schmalenbach et al. (2008)
	Hordeum spontaneum	Leaf scald	Friedt et al. (2011)
	H. bulbosum	Powdery mildew, leaf rust and leaf scald	Morrell and Clegg (2011)
Oat	*Avena strigosa*	Leaf rust resistance	Lehnhoff et al. (2013)
	Avena barbata	Powdery mildew	Swarbreck et al. (2011)
Common bean	*Phaseolus coccineus*	Anthracnose, rootrots, white mould, and bean yellow mosaic virus	Sharmaand Rana (2012)
	P. coccineus and *P. dumosus*	Angular leaf spot resistance	Mahuku et al. (2003)
	P. coccineus and *P. dumosus*	Anthracnose resistance	Mahuku et al. (2002)
	P. acutifolius	Common blight resistance	Singh and Muñoz (1999)
	P. coccineus	Root rots resistance	Silbernagel and Hannan (1992)
	P. coccineus	*Bean golden yellow mosaic virus*	Osorno et al. (2003)
Vigna spp.	*V. vexillata*	Cowpea pod sucking bug, bruchids	Kaur et al. (2018)
	Vigna tribolata, *V. mungo* var. *sylvestris,* *V. radiate* var. *sublobata*	Yellow mosaic virus	Kaur et al. (2018)

(continued)

Table 10.3 (continued)

Crop	Wild relative	Trait	References
Lentil	*Lens orientalis,* *L. odomensis* and *L. ervoides*	Rust, powdery mildew	Singh et al. (2013)
	Lens ervoides	Anthracnose	Tullu et al. (2013)
Groundnut	*Arachis stenosperma*	Root-knot nematode	Leal-Bertioli et al. (2016)
	F_2 (*A.duranensis* x *A. stenosperma*)	Late leaf spot resistance	Leal-Bertioli et al. (2009)
Brassica	*B. fructiculosa*	Cabbage aphid, cabbage root fly	Pink et al. (2003)
Linseed	*L. grandiflorum*	Linseed bud fly, *Alternaria* blight	Kaur et al. (2018)
Sesame	*Sesamum laciniatum*	Leaf phyllode	Kaur et al. (2018)
	S. *malabaricum*	Powdery mildew	Kaur et al. (2018)
	S. mulyanum	Powdery mildew	Kaur et al. (2018)
	S. alatum	Powdery mildew	Kaur et al. (2018)

also be evaluated for either allelic divergence or for entirely new gene. Effective use of wild and wide genetic resources in resistance-breeding programme is possible only by integrating genomic tools in resistance-breeding programmes. Different genomic tools will be helpful in identification of genes and their validation followed by their introgression in desired genotypes. Minor changes in nucleotide sequence of a gene, such as single nucleotide change, addition/deletion of few nucleotide sequences, have now been known to make large differences in expression profile of one or many associated genes. Recently developed genome editing technologies have entered with great stride in the boundary of plant breeding and have been proved to be effective experimentally in precise modification of genome affecting different functions including disease resistance. Furthermore, perfection and precision of genome editing (GE) followed by resolving other unresolved issues like inheritance and recombination of edited sequence may help in the future in development of better resisting genotypes. Genotypes loaded with resistance function developed either by classical approaches or molecular approaches are great input to the farmers in increasing production and productivity of crops. Resistance function of the genotypes can be elevated probably by using biological and physical measures of disease control.

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New-Generation Fungicides for Sustainable Production and Disease Suppression 11

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Abstract

In modern-day agriculture, with the population increasing at an alarming rate, it's difficult to increase the production with the ever-decreasing land and water resources. The plant diseases alone account for more that 15–20% of the yield loss caused by the various biotic and abiotic factors. Efficient management of these plant pathogens will help in increasing the productivity of the crop and lead to enhanced production. Among the various disease management strategies, the chemical control using the fungicides has been the most widely adopted method for fungal disease management. There have been many adverse effects on the environment and human health associated with the use of the traditional fungicides that were less efficient and broad spectrum. In order to overcome the limitation of these fungicides, many new fungicides with novel mode of action have been developed in the past two decades. These so-called new-generation fungicides are highly efficient even at low doses, are more target specific, and leave no or very less residue on the produce. This chapter reiterates the different new-generation fungicides along with their mode of action and target pathogens.

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New-generation fungicides · Novel · Mode of action · Disease management

11.1 Introduction

Agriculture has provided the pertinent origin of subsistence for human societies over the years, and this has provided means of survival to about 50% of the population of the world till now. Greater percentage of the world populations are directly or indirectly dependent on agricultural produce and allied activities for their sustenance and survival. With the ever-increasing population, it has become increasingly difficult to enhance our production with limited area. There are many factors which influence the food production like biotic and abiotic factors. The major factor which influences food production is plant diseases. Crop losses due to plant diseases constitute the most significant constraint worldwide to increasing productivity and total food production. Crop loss due to diseases, weeds, and pest is about 10–30% of crop production (Kumar and Gupta 2012; Kumar 2009). In the history of plant diseases, there were many diseases which took form of epidemic such as the Irish Famine and Bengal Famine which occurs due to rice brown spot. Like these there are many diseases which are of historical significance and cause impact on human life. To minimize these crop losses, chemical methods for management of plant diseases using fungicides are more preferred by the farming community.

11.2 Key Methods for Management of Plant Diseases

1. Regulatory measures
2. Cultural methods
3. Biological methods
4. Chemical control

There are different management strategies which can be adopted from sowing to harvesting stages of crop. Although different management strategies are present, still we rely more on chemicals for crop disease management due to their high efficiency and comparatively lower CB ratio. Diseases in plants are caused by a number of microorganisms which include fungi, bacteria, viruses, viroids, and phytoplasma; among all these, a large number of diseases are caused by fungi, and to manage these fungal diseases, different fungicides are used (Dehne et al. 2007). Use of chemical fungicides in agriculture was started in the nineteenth century when effectiveness of copper fungicides was discovered against seed-borne bunt by B. Prevost. Since the nineteenth century, many fungicides were discovered for plant disease management (Tables 11.1, 11.2, 11.3 and 11.4). In the past two decades, several novel fungicides were also discovered which are better than earlier fungicides in their mode of action and are more effective under low doses (Sajad et al. 2017).

Table 11.1 History of fungicides

Year	Fungicides
1637	Brine
1755	Arsenic
1760	Copper sulfate
1824	Sulfur (dust)
1833	Lime sulfur
1885	Bordeaux mixture
1934	Dithiocarbamates
1951–1960	Captan
1971–1980	Dicarboximides, sterol biosynthesis inhibitors, phenylamides, alkyl phosphonates, carbamates, isoxazoles, tricyclazoles, cyanoacetamide-oximes, melanin biosynthesis inhibitors
1991–2005	Strobilurins, phenylpyrroles, anilinopyrimidines, spiroxamines, probenazole, benzothiadiazole, phenylpyridylanines, quinolines

Table 11.2 First-generation fungicides

Fungicide class	Active ingredient
Dithiocarbamate	Thiram, zineb, nabam, maneb, mancozeb
Phthalimide	Captan, captafol
Triazine	Anilazine
Guanidine	Dodine
Tin compounds	TPTA, TPTH
Chloronitro benzenes	PCNB

Table 11.3 Second-generation fungicides (1966–1976)

Fungicide class	Active ingredient
Oxathiins	Carboxin and oxycarboxin
Benzimidazoles	Thiabendazole, benomyl
Hydroxypyrimidines	Ethirimol, dimethirimol
Dicarboximides	Iprodione, vinclozolin, procymidone

Table 11.4 Third-generation fungicides (1977–1990)

Fungicide class	Active ingredient
Phenylamides	Metalaxyl, ofurace, oxadixyl
Sterol biosynthesis inhibitors	Triazoles, imidazoles, piperazines, piperidines, pyrimidines, morpholines
Carbamates	Prothiocarb, propamocarb

11.3 Classification of Fungicides on the Basis of Generations

11.3.1 Fourth-Generation/New-Generation Fungicides (Novel Modes of Action)

1. Strobilurins
2. Melanin biosynthesis inhibitors
3. Phenylpyrroles
4. Anilinopyrimidines
5. Phenoxyquinolines
6. Spiroketalamines
7. Benzamides
8. Oxazolidinediones

11.3.1.1 Strobilurins

Strobilurins are new class of fungicides which are isolated from wood-decaying basidiomycete fungus *Strobilurus tenacellus*. Strobilurins' group of fungicides inhibits mitochondrial respiration in fungi. At Q_o site of the cytochrome bc_1 complex, oxidation of ubiquinol is blocked which is situated at the inner mitochondrial membrane of fungi (Knight et al. 1997). These fungicides are now referred to as Q_oI fungicides. These are world's biggest selling fungicides (Bartlett et al. 2002). These classes of fungicides are of broad-spectrum activity and are active against many pathogens. The application of these classes of fungicides is recommended for seed treatment, foliar treatment, and furrow application. The commercial strobilurins are azoxystrobin, kresoxim-methyl, metominostrobin, trifloxystrobin, pyraclostrobin, picoxystrobin, etc. Azoxystrobin was patented by Zeneca in 1988 which was followed by other two compounds which are kresoxim-methyl and trifloxystrobin. Kresoxim-methyl exhibits excellent eradicant properties against powdery mildew (Gold et al. 1995). One advantage of this class of fungicide which makes it more important is that it is effective against the fungal strains which developed resistance against phenylamide, DMIs, dicarboximide, and benzimidazoles.

11.3.1.2 Melanin Biosynthesis Inhibitors

This group of fungicides prevents melanin biosynthesis in appressoria of *Pyricularia oryzae* and prevents penetration of pathogen. MBI are effective against rice blast disease (Koichiro et al. 2003). They act on enzymes dihydroxynaphthalene (DHN) melanin in biosynthesis pathway through fusion of five isoprenyl units, two sets of alternating reduction, and dehydration steps and polymerization of 1,8-dihydroxynaphthalene. In this process, fungicides like phthalide, tricyclazole, and pyroquilon inhibit the reduction step, and carpropamid inhibits the dehydration step. MBI prevents pathogen to enter into the host epidermis; these are commonly anti-penetrants in their mode of action. Melanization of appressorial walls is very important for successful penetration of pathogen into the host epidermis. MBI inhibition in melanin synthesis provides excellent control of pathogens *P. grisea* and *Colletotrichum* spp. also.

11.3.1.3 Phenylpyrroles

Phenylpyrroles are based on pyrrolnitrin, which are secondary metabolites produced by *Pseudomonas pyrrocinia* having antifungal properties (Floss et al. 1971). Pyrrolnitrin is instable under light; therefore, it is unsuitable to use practically in disease control (Corran et al. 2008). Phenylpyrroles inhibit all stages of fungal development like spore germination, germ tube elongation, and mycelial growth (Leroux et al. 1992). Optimization of photostability of this compound results in the development of two commercial fungicides fenpiclonil and fludioxonil which are used as seed dressing and foliar spray, respectively. Primary targets of phenylpyrroles are uncertain; they appear to affect glucose phosphorylation.

11.3.1.4 Anilinopyrimidines

Anilinopyrimidines are also known as pyrimidinamines, which are broad-spectrum fungicides. This class of fungicide can be used in a variety of crops. Mepanipyrim and pyrimethanil are active against *Botrytis cinerea* on grapevine and other fruits and *Venturia inaequalis* on apples (Neumann et al. 1992). Another such compound, cyprodinil, of this class has additional activity against *Pseudocercosporella herpotrichoides*, *Erysiphe graminis*, *Helminthosporium gramineum*, *Pyrenophora teres*, and *Septoria nodorum* on cereals (Heye et al. 1994). Anilinopyrimidines are considered to be involved in methionine biosynthesis inhibition; biochemical mode of action is still uncertain (Leroux 1996). They are single-site inhibitors in the amino acid biosynthesis pathway and also affect the secretion of hydrolytic enzymes during penetration of the target pathogens into plant tissue. In fungi *Neurospora crassa* and *Aspergillus nidulans*, the cystathionine pathway has been established as the major route of homocysteine and methionine biosynthesis; within this pathway, cystathionine β-lyase which catalyzes the synthesis of homocysteine from cystathionine was identified as a target site (Masner et al. 1994).

11.3.1.5 Phenoxyquinolines

Phenoxyquinolines consist of protectant fungicide (Longhurst et al. 1996). Quinoxyfen was introduced in 1996 for control of powdery mildew of cereals and grapevine. Quinoxyfen disrupts signaling processes which are crucial for growth and development; this fungicide changes in the early development stages of powdery mildew. It also interferes with conidia germination and appressorium formation in the life cycle of target fungi. Phenoxyquinolines act on dihydroorotate dehydrogenase in pyrimidine biosynthesis pathway (Knight et al. 1997).

11.3.1.6 Spiroketalamines

In spiroketalamines, the representative compound is spiroxamine which was introduced in 1996. Spiroxamine is a novel ergosterol biosynthesis inhibitor (that is essential for the organization and functions of structure) (Dutzmann et al. 1996). It controls powdery mildew of cereals and grapes. Spiroketalamines shows cross-resistance with morpholines and piperidines.

11.3.1.7 Benzamides

The benzamide class is also called anti-tubulin fungicide which includes zoxamide. Zoxamide fungicide is a β-tubulin inhibitor. Benzamides have demonstrated their potential for the control of oomycete pathogens and interfere with microtubule skeleton and arrest nuclear division similar to benzimidazoles (Young and Richard 2001). It exhibits high activity against a broad spectrum of oomycetes such as *Phytophthora infestans*, *Plasmopara viticola*, and various *Pythium* spp. (Chao et al. 2011). It also acts against certain non-oomycete fungi such as *Venturia* spp., *Sclerotinia* spp., *Mycosphaerella* spp., *Botrytis* spp., and *Monilinia* spp. (Young and Richard 2001).

11.3.1.8 Oxazolidinediones

This class of fungicide is represented by famoxadone. This class has a broad-spectrum activity and belongs to bc_1 complex Q_0I family (Abrue et al. 2006). Famoxadone acts as protectant compound. It is used against late blight of potato and downy mildew of grapes (Thind 2012). This compound inhibits pathogen by inhibiting activity of ubiquinol cytochrome C oxidoreductase (Sajad et al. 2017). Famoxate is a newly developed fungicide useful for preventative and curative control of fungal diseases in crops. This new class of fungicide acts on the catalytic function of mitochondrial cytochrome of bc1 (Douglas et al. 1999).

11.3.1.9 Recently Developed New-Generation Fungicides in Nigeria

In Nigeria, some of the recently developed new-generation fungicides/active ingredients on crops and farmlands have been registered for use, while some others are currently under evaluation for different diseases. Few of the new-generation fungicides registered in Nigeria are for use against major diseases of cash and annual crops, fruits, and vegetables. And some of these new-generation fungicides are as follows:

1. Cabrio Duo (pyraclostrobin and dimethomorph), Pergado (metalaxyl-M + mandipropamid), Ridomil Gold Plus (metalaxyl-M, cuprous oxide), and Red Force (copper(I) oxide + metalaxyl-M) against black pod disease of cocoa caused by *Phytophthora* species
2. Camazeb (carbendazim + mancozeb) against twig and inflorescence dieback of cashew and pod rot of cacao
3. Ridomil Gold MZ (metalaxyl-M + mancozeb) against oomycete diseases in beans, cucurbit, lettuce, onions, spinach, pepper, pineapple, rubber, oil palm, citrus, potatoes, soybeans, sugar beet, tobacco, and tomatoes
4. Ortiva Top (azoxystrobin, difenoconazole) against early blight, black scurf, black dot in potatoes, powdery mildew, leaf spots, early blight, anthracnose in tomatoes, leaf and neck blast in rice, and gray leaf spot, smuts, and rusts in grains and sorghum
5. Fungicare (azoxystrobin, difenoconazole) against rust disease; powdery mildew in rice, maize, and wheat; leaf spot; early and late blight in potato, tomato, cucumber, pepper, onion, and carrot; black spot; leaf rust; stem rot and powdery

mildew in soybeans, cowpea, and groundnut; and disease complexes in mango, citrus, cashew, guava, banana, and ornamental plants

There is significant improvement in the formulations of the new fungicides, which are also less phytotoxic and produce safe crops. Due to their novel mode of action and specificity, their demand and use are increasing day by day.

11.4 Conclusion

For plant disease management, chemicals are very important because they prevent plant diseases. In spite of the advantages of chemicals in food production, there are also disadvantages of their use. Due to inadequate use of chemicals, many pathogens had developed resistance against many chemicals. Moreover, another major disadvantage of using these chemicals is their negative effects on the environment, humans, and other living organisms. So to overcome these negative effects, some new classes of fungicides are developed, which are better than those old chemicals in many ways in that these new compounds which have originated from different approaches such as traditional random screening and from natural products are expected to provide better disease control options. These are ecologically safe and show good efficacy at much lower doses. These require lesser treatment per season compared to earlier compounds. Since they possess novel mode of action, there are fewer chances of cross-resistance to previous fungicides. These are some of the advantages of new-generation fungicides which will help in food production without causing harm to the environment.

11.5 Future Prospects

For management of plant diseases, there are many methods which are adopted in the field, and fungicides will always play a very important role in this management. Nowadays, it is very important to use chemicals wisely because there are many chemicals which have developed resistance against many pathogens, so it will be always challenge for us to use chemicals in a selective and judicious manner. New-generation fungicides which are derived from natural origin are less harmful to the environment and are novel in their action, so in the future more new novel fungicides should be developed so that we can overcome the problems of resistance and environment pollution from earlier chemicals. Development of ecologically safer molecules with broader range of target pathogens can be done. To minimize the resistance risk development and use of combination products with multisite action can be a good option for disease management. These novel action fungicides will be obtained by screening of natural-based products.

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12 Toxicity of Organophosphate Pesticide on Soil Microorganism: Risk Assessments Strategies

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Abstract

Synthetic organophosphate pesticide (Op) is used extensively in modern agriculture by farmers to enhance crop yield under a limited agriculture land resource as well as food for the increasing population worldwide. Further, OP is increasing due to more adaptation and resistance of varieties of pests in agricultural crops. Recently, the toxicity of OPs has increased in the soil ecosystem so that some microbes may be survived and some may be lost. Therefore, soil fertility and health may be disturbed due to more toxicity of OPs. Currently, pesticide-resistant microbes can be one alternative for boosting the agricultural productivity as well as enhancing soil fertility and health. Therefore, in this chapter, we attempt to explore the risk assessment methodology and techniques as well as challenges regarding toxicity of OPs on the soil ecosystem and plant-microbe interaction function. Also, we attempt to explore the advance analytical techniques and molecular tools, like culture-dependent and culture-independent methods for measuring tolerance of multiple pollutants at the microbial community level and ecological disturbance.

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Organophosphate pesticide · Plant growth-promoting microbes (PGPM) · Toxicity assessment methodologies · Culture-independent and -dependent analyses

12.1 Introduction

Soil microbes are the most important factor in determining good soil and plant health. The microbial diversity of the soil helps in carrying out several vital functions necessary for the well-being of plants. Microbes act as mediators and feed the plants in the very literal sense. Many nutrients present in the soil are in unavailable form, where these microbes come to the rescue of plants. Based on the type of environment they inhabit, they can be classified into various types. There are many free-living microbes, symbiotic microbes, soil inhabiting microbes, endophytic microbes, and so on. Many microbes that live in association with the plants or around them may not function at all until its requirement comes. There are many microbes, which help mitigate various plant stresses and promote plant growth; also, they are called as plant growth-promoting microbes (PGPM) (Table 12.1). There are many microbes that live in association with the plants to provide beneficial effects to them. The most commonly known are the *Rhizobium* and *Bradyrhizobium* (mostly belonging to α-proteobacteria), which live in symbiotic association with the root nodules of the leguminous plants and carry out nitrogen fixation (Appelbaum 2018). These help in fixing the atmospheric nitrogen unavailable to plants to organic forms of nitrogen, which is readily available to them. These microbes facilitate plant growth, by providing nitrogen to plants, and in turn are benefitted from the plants. This is vital as it provides substantial economic and environmental benefits. Mineral solubilization is also an important function performed by the soil microbes, which facilitate plants with the uptake of essential nutrients, such as phosphate solubilization, potassium solubilization, and many others. Phosphate-solubilizing microbes (bacteria and fungi) play an important role in P bioavailability and are used to increase the phosphorous uptake in plants. These microbes help in solubilizing inorganic P into soluble forms. This process is achieved through many mechanisms, the usual of which is the production of organic acids by the phosphate-solubilizing bacteria (mostly Gram-negative), which leads to the dissolution of mineral glucose to gluconic acid. These organic acids act as good chelating agents of Ca^{2+} ions and thus release phosphates from calcium phosphate (Shenoy and Kalagudi 2005).

These bacterial species include *Enterobacter* sp., *Pantoea* sp., *Klebsiella* sp., and many others. Apart from the solubilization of inorganic calcium phosphates, iron and aluminum phosphates are also solubilized by phosphate-solubilizing microbes. Potassium, another essential nutrient for the plants, is also facilitated to plants by some capable soil microbes. The main sources of potassium in soil include K-feldspar, muscovite, biotite phlogopite, and mica. The microbes, such as *Pseudomonas* spp., *Bacillus* spp., and *Burkholderia* spp., can be potassium-solubilizing

Table 12.1 List of beneficial soil microbes (BSM) with their plant growth–promoting activities and activity against different stresses

Name of microbe	Objective	Application	References
Biofertilizer			
B. amyloliquefaciens Bs006 and *P. fluorescens Ps006*	Screening, plant growth promotion, and root colonization pattern of two rhizobacteria on banana cv. Williams (*Musa acuminata* Colla)	Promoted banana growth similarly or even slightly superior to 100% chemical fertilization	Gamez et al. (2019)
Bacillus licheniformis (BHUJP-P3) and *Bacillus cereus* (BHUJP-P4)	Molecular characterization of monocrotophos- and chlorpyrifos-tolerant bacterial strain for enhancing seed germination of vegetable crops	Strains BHUJP-P4 and BHUJP-P3 showed higher EPS, IAA, PSB, HCN, and ammonia production	Jaiswal et al. (2019)
Achromobacter, Bacillus, Lysinibacillus, Paenibacillus, Pseudomonas, and *Stenotrophomonas,*	Purple corn-associated rhizobacteria with potential for plant growth promotion	N_2 fixation, phosphate solubilization, indole acetic and siderophore production, 1-aminocyclopropane-1-carboxylic acid deaminase activity and biocontrol abilities, significant increases in root and shoot dry weight, total C and N contents of the plants	Castellano-Hinojosa et al. (2018)
Bacillus spec. strain Bt04, *Pseudomonas, Lysinibacillus fusiformis* strain Lf89	Isolation and characterization of the three new PGPR and their effects on the growth of *Arabidopsis* and *Datura* plants	All strains significantly improved plant growth of the plant species tested, and some strains produce a shift in the C/N ratio in *A. thaliana*	Rahmoune et al. (2017)
Pseudomonas fluorescens spp.	Evaluation of fluorescent *Pseudomonas* spp. with single and multiple PGPR traits for plant growth promotion of sorghum in combination with AM fungi	Phytohormones, siderophores, HCN, proteases, chitinases, cellulases, ammonia, and exopolysaccharide production and phosphate solubilization or antagonistic activity	Kumar et al. (2012)
Pantoea, Serratia, Enterobacter, and *Pseudomonas*	Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina	Produce siderophores and indoles	Viruel et al. (2014)
Pseudomonas fluorescens	Organic acid production in vitro and plant growth promotion in maize	Production of organic acids during inorganic phosphate solubilization	Vyas and Gulati (2009)

(continued)

Table 12.1 (continued)

Name of microbe	Objective	Application	References
	under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*	and influence on plant growth	
Bacillus megaterium	Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling	Promoted growth of *A. thaliana* and *P. vulgaris* seedlings	Ortíz-Castro et al. (2008)
Bacillus subtilis and *Pseudomonas aeruginosa*	Comparison of plant growth promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables	*B. subtilis* and *P. aeruginosa* increased 31% for tomato, 36% and 29% for okra, and 83% and 40% for African spinach, respectively	Adesemoye et al. (2008)
Biocontrol agent			
Stenotrophomonas sp. str. S33 and *Pseudomonas* sp. str. S85	Endophytic bacteria from *Datura metel* for plant growth promotion and bioprotection against *Fusarium wilt* in tomato	Suppress tomato *Fusarium wilt* disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and to enhance plant growth	Rania et al. (2016)
Bacillus amyloliquefaciens subsp. *plantarum* strains	Multiple effects of *Bacillus amyloliquefaciens* volatile compounds: plant growth promotion and growth inhibition of phytopathogens	Dose-response studies with UCMB5113 on MS agar with or without root exudates showed significant plant growth promotion even at low levels of bacteria. *Bacillus* VOC antagonized growth of several fungal pathogens in vitro	Asari et al. (2016)
Pseudomonas aeruginosa and *Trichoderma harzianum*	Evaluation of plant growth-promoting activities of microbial strains and their effect on growth and yield of chickpea (*Cicer arietinum* L.) in India	Suppressed growth of phytopathogen, e.g., *Fusarium oxysporum* and *Rhizoctonia solani*	Verma et al. (2014)
Bacillus subtilis CAS15,	The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium wilt* and promotes the growth of pepper	*B. subtilis* CAS15 induced systemic resistance to *Fusarium wilt* in pepper. Iron supplementation reduced this biocontrol effect	Yu et al. (2011)

(continued)

Table 12.1 (continued)

Name of microbe	Objective	Application	References
B. amyloliquefaciens FZB42	Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42	Stimulates plant growth and produces secondary metabolites	Chen et al. (2007)
Bacillus thuringiensis	*Bacillus thuringiensis* beyond insect biocontrol: plant growth promotion and biosafety of polyvalent strains	Induces plant growth promotion and can also be used as a biofertilizer	Raddadi et al. (2007)
Abiotic stress			
Bacillus subtilis strain GOT9	*Bacillus subtilis* strain GOT9 confers enhanced tolerance to drought and salt stresses in *Arabidopsis thaliana* and *Brassica campestris*	The application of strain GOT9 led to the enhancement of drought and salt stress tolerance in *Arabidopsis thaliana* and *Brassica campestris*	Woo et al. (2020)
Trichoderma koningiopsis (NBRI-PR5)	Phosphate solubilization by *Trichoderma koningiopsis (NBRI-PR5)* under abiotic stress conditions	*T. koningiopsis* employs different mechanisms of P solubilization in different stress conditions, and therefore, it can be used in the management of stressed soils	Tandon et al. (2020)
P. mendocina, A. beijerinckii, P. stutzeri, B. subtilis	Saline soil microbiome: a rich source of halotolerant PGPR	All are indole acetic acid and ammonia producers, and they also solubilize the phosphate under 4% NaCl concentration	Hingole and Pathak (2016)
P. simiae AU	Putative bacterial volatile-mediated growth in soybean (*Glycine max* L. Merrill) and expression of induced proteins under salt stress	Lower Na accumulation, higher proline contents	Vaishnav and Choudhary (2019)
Burkholderia, Variovorax, Bacillus, Pseudomonas and *Ralstonia* species	Resistance to drought stress, cold, nutrient starvation, 2,4-DNT (dinitrotoluene) stress	Enhance root length of *Arabidopsis* under 2,4-DNT stress, doubling of the main root length	Thijs et al. (2014)

bacteria, potassium-dissolving bacteria or potassium-solubilizing rhizobacteria (Pandey et al. 2019). The bioavailability of potassium is induced by the production of organic acids, such as citric acid, oxalic acid, succinic acid, tartaric acid, and malic

acid (Saritha and Tollamagudu et al. 2019). These microbes can be used as bioinoculants under the prospect of sustainable agriculture practice.

Further, there are many soil microbes, which help plant mitigate different types of abiotic stresses majorly drought and salinity stress. There many microbes not only help in mitigating the stress but also in promoting the growth of plants through plant growth-promoting (PGP) activity (Vurukonda et al. 2016). Drought stress coping is achieved by the production of phytohormones, which leads to adaptations, such as partial closure of stomata, to prevent excessive transpiration. Likewise, for salinity stress, there are many bacterial strains, which produce IAA, I3B, ABA, ACC, and many other chemicals, which directly or indirectly lead to help in mitigating the adverse effects of the stress (Table 12.1).

12.1.1 Plant Growth-Promoting Microorganism: As Biofertilizer

As has been discussed briefly that the soil microbes help convert insoluble and unavailable form of minerals to soluble, organic, and available forms. The major soil enrichment products or fertilizers use the addition of NPK (nitrogen, phosphorus, and potassium) to the soil to promote any kind of plant growth and the desired product yield. Though the NPK is abundantly present in the surrounding environment of the plant, they are unable to acquire it. Hence, with soil microbes converting the unavailable forms to available forms, this problem can be solved and are called as biofertilizers. These microbes show plant growth-promoting properties and hence are called as PGPR. The nitrogen requirement is fulfilled by the nitrogen-fixing microbes, which may either be free-living (nonsymbiotic) or in symbiotic relationship with the plants. The microbes living in symbiotic relationship with the plants are generally *rhizobia* (Mus et al. 2016). The free-living nitrogen fixers include *Azotobacter, Clostridium*, and *cyanobacteria.* The process includes conversion of atmospheric nitrogen to ammonia, which is then converted into amino acids and other nitrogenous compounds. Phosphorous is another major nutrient required for the growth of plants. It is involved in various key mechanisms of the plants, such as cell division and development, photosynthesis, signal transduction, and biosynthesis of macromolecules (Sengupta and Gunri 2015).

The presence of insoluble phosphates in soil allows soil microbes to solubilize it and make it available to plants, a process called as phosphate solubilization, and the microbes involved phosphate-solubilizing microbes. The mechanism of solubilization of the insoluble phosphates, such as tricalcium phosphate $(Ca_3PO_4)_2$, aluminum phosphate (Al_3PO_4), and iron phosphate (Fe_3PO_4), to soluble forms includes the production of organic acids, such as malic acid and gluconic acid (*Enterobacter* sp. FS-11) (Shahid et al. 2012.), citric acid, fumaric acid, formic acid, oxalic acid, acetic acid, isobutyric acid, valeric acid, isovaleric acid, and tartaric acid, produced by bacterial species, such as *Serratia marcescens, Delftia, Chryseobacterium,* and *Phyllobacterium myrsinacearum* (Khan et al. 2014). The fungal species producing such acids include *Aspergillus niger* FS1, *Penicillium canescens* FS23, *Eupenicillium ludwigii* FS27, *Penicillium islandicum* FS30, *Aspergillus awamori*

S19, *T. flavus, T. helices, P. purpurogenum,* and *P. janthinellum*, which release organic acids for solubilizing inorganic phosphates (Mendes et al. 2013; Jain et al. 2012; Scervino et al. 2010). Other mechanisms through which insoluble P can be converted to soluble P, i.e., without the production of organic acids, are chelation and reduction processes, which are useful in the management of plant pathogens. Furthermore, H^+ pump in *Penicillium rugulosum* and inorganic acids, such as HCl, HNO_3, and H_2SO_4, by some bacterial species is also known to cause solubilization of inorganic phosphate. Next is the uptake of available phosphorous by plants mediated by the enzymes of soil microbes.

The complex organic P compounds are further mineralized by soil enzymes, such as acid phosphatase (commonly found in fungi) and alkaline phosphatase, which are considered as the principal mechanism for mineralization of soil organic P. An enzyme called phytase leads to the production of P from phytic acid. Phytate is a complex organic compound in soil, and hence its degradation is common in phosphate-solubilizing microbes. After nitrogen and phosphorous, potassium is the third macronutrient essential for the growth of the plants. Absorbed in the form of K^+, it is required in the early developmental stages of the plant. Out of the total whose concentration generally exceeds 20,000 ppm, only 1–2% is readily available to plants. This is where potassium-solubilizing microbes come to the rescue. Soil microbes, such as *Acidithiobacillus*, *Burkholderia*, and *Pseudomonas*, have the ability of solubilize phosphate from the fixed forms in the soil. Though the exact mechanism of solubilization remains lagging, activities like acid hydrolysis, through the production of organic acids, and chelation and reduction reactions are attributed to be the reason behind the process. The mechanism through which potassium is taken up by plants is either through high-affinity transport system (HATS) or low-affinity transport system (LATS). In high-affinity mechanism, the influx of K^+ is driven by the outflux of H^+ over the plasma membrane mediated by proton ATPase. In the LATS mechanism, electrogenic uniport of K^+ takes place by the ATP-driven efflux of H^+ (Sharma et al. 2016). In the plant cell, the cytosol concentration of K^+ ranges between 40 and 200 mM, which presents a challenge for K^+ uptake by plants when low concentrations in the soil are present. The low potassium concentration in the soil demands ATP-driven process as the flux needs to be driven against the gradient. The solubilization of K from different insoluble minerals in soil is dependent also on the proton concentration of the soil or soil solution. Other factors include lowering of pH, enhancing chelation of cations bound to K, and acidolysis around the microorganism's present.

12.1.2 Biocontrol Agents

Biopesticides refer to the use of biocontrol agents for the management of the crops and controlling pests rather than using chemical pesticides. The major difference between the biopesticides and biocontrol agents is that the former is passive in nature while the latter is active in nature and seeks out pest to kill them, such as parasitoids, predators, and many species of entomopathogenic nematodes. Biopesticides cover a

wide range of agents, such as microbial pesticides, entomopathogens, plant-incorporated protectants, and biochemical pesticides. Here, we will be focusing on the microbial pesticides that are used to manage and control pests. The microbial pesticides include bacteria, virus, and fungi, which attack specific pests. Many *Bacillus* and *Pseudomonas* strains are known to act biopesticides. *Bacillus thuringiensis* (Bt) is considered to have a market share of about 95% of the total microbial pesticides. The mechanism through which microbial pesticides act are through the formation of spores and crystals, which are used against several agricultural and horticultural crops. The effectiveness of the of microbial pesticides depends upon the broad range of conditions that they survive in, such as temperature, shelf life during storage, and moisture content. Considering these parameters, the *Bacillus thuringiensis* dominates the market of microbial pesticides as it is more effective at high temperatures and has higher shelf life during storage (Vimala Devi et al. 2019).

12.1.3 Alleviating Abiotic Stress

There are many microbial species which help in alleviating the abiotic stress, such as drought and salinity stress. Plant growth-promoting bacteria are known to produce plant hormones, like gibberellins, auxin, and precursor of several hormones, such as ethylene and other volatile organic compounds. Under abiotic stress conditions, two major plants hormones are secreted as a primary response which are ethylene and abscisic acid. Ethylene is secreted primarily as a response toward salinity stress and abscisic acid as a response toward drought stress. Ethylene retards plant growth and development, while abscisic acid induces the formation of lateral roots in the plants. While the formation of lateral roots in plants is beneficial, the effect of ethylene is not beneficial for the plant in any sense. Several microbes are also known to produce ACC (aminocyclopropane-1-carboxylic acid), which is a precursor of ethylene and thus reduces the retardation of growth in plants under stress. The ACC deaminase activity is also known to induce modifications in the root tip and its surface area, thereby making it resistant to stress conditions by promoting nutrient acquisition. Other plant growth-promoting activities of bacteria under salinity stress include the production of auxins, IAA (indole acetic acid) and I3B (indole-3-butyric acid), and other beneficial substances, such as extra-polymeric substances and antioxidants. The microbial species include *Bacillus* spp., *Pseudomonas* spp., *Frankia* spp., *Nocardia* spp., *Streptomyces* spp., and many others.

12.2 Organophosphates

The organophosphate (OP) pesticides, likewise called organophosphorus pesticides, are a group of chemical substance, which are all esters of phosphoric acid with aliphatic, phenyl, and heterocyclic utilitarian gatherings (Kumar et al. 2016). Organophosphate compounds are characterized in the main groups of herbicides, insecticides, and fungicide. In the 1970s, first-time organophosphate pesticides

were introducing after banned of organochlorine due to high toxicity and their persistent rate high in soil. Grube et al. (2011) reported OPs widely used pesticide in the world. American agriculture witnessed consumption of 334 million pounds of organophosphate insecticides from 2001 to 2007.

The commonly used organophosphates in India are chlorpyrifos, phorate, profenofos, dichlorvos, quinalphos, acephate, triazofos, monocrotophos, and malathion, (Table 12.2). The responsible authority for registering pesticides for use on crops to control pests and weeds is the Central Insecticides Board and Registration Committee (CIBRC), which falls under the Ministry of Agriculture and Farmer Welfare.

Organophosphate is liable for a large number of deaths worldwide due to their unregulated utilization and their easy accessibility (Buckley et al. 2004; Gunnell et al. 2007). So, also, toxicity of pesticides could be a lot higher for developing countries like India, where countless farmers don't have any information about the toxicity of applies of pesticides, and they don't use any defensive gear during the spraying of pesticide (Banerjee et al. 2014). Therefore, farmers face occupational risk while applying pesticides onto the farming field (Mamane et al. 2015; Muñoz-Quezada et al. 2016; Rastogi et al. 2010). The occupational hazard of pesticides isn't limited to just the farmers and furthermore influences the family members of farmers and individuals living close to farming field. In a study by Rastogi et al. (2010), the

Table 12.2 Most consumed organophosphate insecticide in the country (during 2017–2018)

S. No.	Organophosphate insecticide	Quantity (in metric tons)
1	Chlorpyrifos	477.90
2	Phorate	480.24
3	Profenofos	300.25
4	Dichlorvos	287.11
5	Quinalphos	242.37
6	Acephate	168.77
7	Triazofos	151.21
8	Monocrotophos	140.30
9	Malathion	103.00
10	Dimethoate	91.94
11	Phosphamidon	45.15
12	Ethion	20.73
13	Methyl parathion	19.27
14	Temefos	18.00
15	Fenitrothion	16.50
16	Oxydemeton-methyl	13.80
17	Phenthoate	11.20
18	Phosalone	3.56
19	Fenthion	3.00
20	Diazinon	1.00

Source: http://ppqs.gov.in/statistical-database

high recurrence of neurologic manifestations was found in kids, having a place with the groups of farmers dealing with and utilizing OPs bug sprays, which presented occupationally to different organophosphate bug sprays (chlorpyrifos, diazinon, dichlorvos, ethyl parathion, fenthion, malathion, methyl parathion).

12.2.1 Organophosphate Insecticide Pollution and Contamination Status

Amidst the growing Indian population and proportional food demand till 2024, under the pressures of limited agricultural land and attack of pest infestation, the need of food grains, vegetables, and fruits is foreseen to be multiplied from 2.5 to 5 times. Therefore, to achieve the projected goal, it is forecasted that pesticide application will also increase by at least 2–3 times in the forthcoming years (Dar et al. 2020). The widespread contamination of OP, due to their excessive use, has been revealed in soil, sediments, water, fruits, vegetables, tea, and human fluids by residual analysis of these pesticides (Table 12.2). Pesticide contamination of water, air, and food chain to ultimately reach the human body occurs mainly due its transport through the soil via means of leaching, runoff, transfer, interflow, and subsurface drainage (Abrahams 2002).

Vegetables, being an essential dietary component with higher consumption rates across the world, have been under extensive OPs application due to their higher vulnerability to pest infestations. Thus, a higher application rate of pesticides in agriculture has resulted in residual contamination of vegetables and fruits with OPs. Several studies to assess pesticide contamination in vegetables, like beetroot, bitter gourd, brinjal, cabbage, capsicum, carrot, cauliflower, chili, cucumber, French bean, jackfruit, knolkhol, ladyfinger, mustard, okra, onion, pea, potato, radish, spinach, tomato, etc., have reported residual concentration of different OPs, such acephate, anilofos, chlorpyrifos, diazinon, dichlorvos, dimethoate, fenitrothion, phosphamidon, profenofos, phorate, malathion, quinalphos, and monocrotophos, to be above their respective MRL levels by application of different techniques, viz., GC-ECD, GC-NPD, FPD, and LCMS/MS techniques (Mukherjee 2003; Kumari et al. 2003, 2004; Srivastava et al. 2011; Gowda and Somashekar 2012; Sinha et al. 2012). On the contrary, some studies have reported residual concentrations of different OPs, such as methyl parathion, chlorpyrifos, malathion, monocrotophos, and others, to be below their respective MRL in vegetables (Bhanti and Taneja 2007; Mandal and Singh 2010; Chandra 2014); however, continuous consumption of these vegetables may result in the bioaccumulation of these pesticides with fatal consequences. Singh and Gupta (2002) found different vegetables collected from agricultural farms and vegetable markets, having OP contamination (e.g., chlorpyrifos, dimethoate, quinalphos, and monocrotophos) from below to above their residual limits. Apart from pesticide contamination in vegetables, fruits were also found to be contaminated with different OPs. Harinathareddy et al. (2014) found different fruit samples obtained from an agricultural farm in Andhra Pradesh, India, to be contaminated with various OPs. Another

study by REF showed contamination of different OPs, such as chlorpyrifos-methyl, diazinon, malathion, monocrotophos, profenofos, and pirimiphos-methyl, in fruits and vegetables (apple, grapes, strawberry, watermelon, bell pepper, cabbage, carrot, cucumber, eggplant, potato, tomato, and zucchini consumed commonly in Kuwait). Contamination with different OPs is not just limited to fruits and vegetables; they have also been detected in other food items, such as butter, ghee, tea, honey, soft drinks, juices, etc., and in specific animal tissues. A pesticide, chlorpyrifos, was found to be above its maximum residual limit in butter and ghee samples, collected on a random basis from different rural and urban locations in Haryana, India (Kumari 2005). Contamination with highly hazardous, class Ib pesticide monocrotophos, classified by the WHO, which is also likely to be mutagenic and neurotoxic, was observed in tea samples manufactured by different companies, like Tata, Hindustan Unilever, Golden Tips, Goodricke, Royal Girnar, Kho Cha, and Wagh Bakri, from all across India with a concentration ranging from 0.026 to 0.270 mg/kg, even when its application on tea is not allowed (Greenpeace India report 2014). Another pesticide, ethion, was also detected in 22 tea samples, resulting from its direct application on tea (Greenpeace India report 2014). Seenivasan and Muraleedharan (2011) reported contamination of only 0.5% tea samples out of the collected 912 samples, from various districts of Tamil Nadu, India, with residues of pesticides ethion, quinalphos, and others, however, below their respective MRL values.

The presence of organophosphate residues in fish, bovine milk, human blood, urine, and breast milk has been reported across the world, even though OPs are less persistent with a lesser potential of bioaccumulation as compared to other classes of pesticides. Several studies have shown contamination of OPs in humans and animals due to their exposure to contaminated water, air, soil, food, etc. Detection of chlorpyrifos, phosphamide, monocrotophos, and malathion has been reported in human blood samples obtained from different villages of Punjab, India, indicating toward their longer persistence in human body (CSE report 2005). Residues of another OP, chlorpyrifos, have also been detected in blood samples of chick (*Gallus gallus*), fish (*Rita rita* Ham.), goat (*Capra hircus* Linn.), and humans in Uttar Pradesh, India, due to their transport to other ecosystems. The maximum level of the residue was found in fish followed by chick, goat, and humans (Singh et al. 2008). Sanghi (2003) reported contamination of breast milk samples with chlorpyrifos, malathion, and methyl parathion residues in poor women belonging to the age group of 19 to 45 years from Madhya Pradesh, India. The presence of OP residues in bovine milk samples is linked to the consumption of fodder or herbaceous vegetation contaminated with pesticides, by the cows (Fagnani 2011). Contamination of bovine milk with residues of methyl parathion, a highly hazardous (class Ia) pesticide, was reported in samples collected from rural and urban dairies of Allahabad, India (Srivastava et al. 2008). The presence of methyl parathion residues was higher in samples obtained from the diary in rural area, possibly due to extensive agricultural activities and unsafe handling methods in rural areas.

12.2.2 Fate of Organophosphosphate Insecticide

After pesticides are applied to the crops in the agricultural field, it is not utilized completely; instead, they get distributed into the biosphere (i.e., atmosphere, lithosphere, and hydrosphere), depending on various physical and biological factors. Some portion of pesticide may volatize into the atmosphere, depending on the temperature, wind speed, humidity, and atmospheric stability (Van Den Berg et al. 1999). Other pesticide residues may reach to the water bodies due to spray drift, volatilization and precipitation, surface runoff, and groundwater leaching (Carter 2000). Their distribution on land is determined by factors, such as surface preparation, soil structure, clay content of soil, moisture content of soil, type of irrigation, pesticide group, time of application of pesticide, and rainfall (Flury, 1996). The factors controlling the fate of pesticides in the environment are either biotic or abiotic in nature. The physical processes, like transportation, adsorption, and desorption, control the transformation of pesticide once it enters into the soil. Finally, pesticides also affect flora and fauna, decrease biological diversity, and contaminate the food chain (Ribeiro et al. 2005).

12.3 Impact of Pesticide and Its Toxicity

12.3.1 Soil Microbe's Biomass

The stability and their toxicity of pesticide in soil are determined by its chemical composition and soil structure (Laabs et al. 2007). The presence of pesticide residue pollution in soil are available to contact with soil rhizospheric and non-rhizospheric microbial community and disturbed their biochemical and physiochemical behavior (Singh and Walker, 2006). The foremost essential marker of microbial activities in soil is microbial biomass, which shows the dimension microbial growth, nutrient availability, and its biotransformation and different ecological phenomena (Schultz and Urban 2008). Many studies on the toxicity of pesticide on microbial biomass are positive, negative, or neutrals. It is depending on the different nature of pesticide structure (Pampulha and Oliveira 2006). According to Wang et al. (2006), some soil microbes can utilize the pesticide as a carbon source for their growth but for other soil beneficial microbes (like, PGPMs) may decrease their population. Also, it can disturb the microbial and their functional diversity. But, in some studies, pesticides induce the fictional diversity but reduce the microbial diversity (Wang et al. 2006; Pampulha and Oliveira 2006). Also, pesticides periodically show reversible positive or reversible repressing effects on soil microbes (Pampulha and Oliveira 2006). This implies either evoked the biodegradation of pesticide by soil microorganism or change in microbial diversity.

12.3.2 Soil Enzymatic Activities

Severable-biological reactions in soil are catalyzed by different soil enzymes, and also, they act as a critical ecological indicator and marker of various ecological processes of soil ecosystem (Antonious 2003; Jaiswal and Verma 2018). Soil enzymes have defined the status of agricultural field soil, like fertility (Antonious 2003), quality (Bucket and Dick 1998), and pollution status (Kucharski et al 2010; Schaffer 1993). Soil enzymes, presented within the live microbial cells, remotely appended to the cell layer and discharged extracellularly in soil system or bounded with the dead microbial cells, soil matrix, soil colloids, or humic materials (Mayanglambam et al. 2005). The activity of the enzyme is depending upon the soil physico-biological properties such as organic matter content (Kandeler and Gerber 1988), moisture (Bergstrom et al. 1998), and temperature (Tscherko et al. 2001), and pollutants present in the soil. But, changes in the trends of pesticide application, under conventional agricultural practices, are responsible for increasing the ratio of pesticide residues in the soil as compared to the recommended dose. Therefore, the presence of pesticide residues in the soil can interact with soil enzymes, and it's characterized by their response as positive, negative, and neutral effects. Many studies on the interaction of pesticide and soil enzymes observed negative responses on soil enzymes, such as alkaline phosphatase, hydrolase, phosphomonoesterase, acid phosphatase, urease, and dehydrogenase (Caceres et al. 2009; Monkiedje and Spiteller 2002). Another study showed the positive response of pesticides on the soil enzyme properties (Fragoeiro and Magan 2008; Defo et al. 2011). Bolton et al. (1985) have been reported the dehydrogenase soil enzyme, characterized in intracellular microbial enzyme, and it is a significant marker of soil microbial activity. According to Jastrzebska (2011), pesticides show all types of impacts that are neutral, positive, and negative on dehydrogenase. There is an immense range of research considered that reports the effect of pesticides on dehydrogenase activity. Mayanglambam et al. (2005) observed 30% inhibition in the activity of the dehydrogenase enzyme after 15 days, under the toxicity of organophosphate insecticide quinalphos. Additionally, the loss of enzyme activities shows recovery after 90 days of treatment; the conceivable reason for this could be the acclimatization of soil microorganisms toward quinalphos. Similarly, Bayer et al. (1982) observed that dehydrogenase activities reduced in alluvial soil treated with a recommended dose of different organophosphate insecticide methidathion, methoxatin parathion, after six weeks of incubation. Pozo et al. (1995) found that the application of chlorpyrifos at 2–10 kgha-1 reduced the dehydrogenase activity; however, the activity recovered after 14 days to control levels. Another most important soil enzyme phosphatase is a group of enzymes – mono-, di- and, tri-esterase, pyrophosphatases, and phosphamides, which hydrolyze both esters and anhydrides of phosphoric acid and convert the organic phosphate to inorganic phosphate (Hussain et al. 2009). Phosphomonoesters are the most common group of phosphatases in the soil (De Cesare et al. 2000), which include two subgroups, enzyme-like acid phosphatase and alkaline phosphatase, that work at different pH range (Hussain et al. 2009). In the phosphorus cycle, the phosphatase enzyme is playing a significant role in

increasing the availability phosphorus to plant (Schneider et al. 2001). The rhizospheric zone is rich in a high concentration of phosphate, along with low content of humus (Tarafdar et al. 2001). The toxicity of insecticides on acid and alkaline phosphatase activities affects in a different manner due to the structural difference between soil microbial communities (Klose et al. 2006; Srinivasulu and Rangaswamy 2014). Sikora et al. (1990) observed the negative, positive, or neutral effect on the acid phosphatase activity in the loamy soil under the toxicity of organophosphate groups of insecticides chlorpyrifos, terbufo, and fonofos. But in the case of fungicide, toxicity showed positive effect on acid phosphatase and negative effect on alkaline phosphatase. In the nitrogen cycle, urease plays a vital role and hydrolyzes urea into CO2 and ammonia. Urease enzyme is abundantly present intra- and extracellularly in both plants and soil microbes but it rapidly degrades in soil, when extracted from plant and microbes. This surely shows urease activities in soil fundamentally contributed by extracellular urease immobilized on organic soil (Beri et al. 1978; Zantua and Bremner 1977). Urease, especially, has seen a broad concentration because of the essentialness of its substrate urea in agriculture farming. Urease activity is a significant marker to evaluate the soil contamination condition, since urease function in soil diminishes as pesticides meddle with the urease enzyme and decline urea hydrolysis (Antonious, 2003; Srinivasulu and Rangaswamy 2014). Several studies have indicated the influence of different pesticides on urease in a positive, negative, or unbiased way (Antonious 2003;Ingram et al. 2005). Lethbridge and Burns 1976 observed that the urease activity decline by 40–50% after 60 days in a sandy clam soil and silt loam soil, due to the presence residues of organophophate groups pesticide phosphorothioates, fenitrothion, malathion, and phorate at the concentration range 50 to 1000 mg kg^{-1}. In contrast, another study showed that urease activity increased in profenofos-treated soil (6.4 and 38.4 μg g^{-1}) for 6 weeks (Abdel-Mallek et al. 1994). In outline, the physicochemical properties of both soil and pesticide residues and natural conditions govern the toxicity of pesticide on the soil beneficial microbes (Dick et al. 2000).

12.3.3 Plant Growth-Promoting Microorganism and Biofertilizer

Repeated application of pesticide on vegetable and crops against the control of pest infestation, as results unwanted pesticides residues reached to the soil, where that impose their toxicity on rhizospheric microbes like PGPMs (plant growth promotion microorganism) and also suppress their beneficial properties (IAA, production, phosphorus and Zn solubilization, etc.) (Ahemad and Khan 2012a). PGPMs are defined and categorized on the based totally over the feature and their mechanism (direct and indirect) that consists of out; like inoculum or consortium of PGPMs are used as biofertilizer, which improve the bioavailability soil nutrients (N, P, K, Ca, Zn & S) to plants by solubilizing and mineralizing natural organic compounds; phytostimulators, that stimulate the plant and root growth or development by released plant hormones; biocontrol agent, which act as a biopesticide against different pest and weed infestation by producing antibiotics and antifungal

metabolites. Several soil microbe's species, such as *Bacillus* (Jung and Kim, 2003), *Pseudomonads* (Lemanceau and Alabouvette 1993; Raaijmakers et al. 2002), *Rhizobacteria* and *Bradyrhizobium* (Chaintreuil et al. 2000), *Acetobacter* (Sevilla et al. 2001), and *Klebsiella* (El-Khawas and Adachi, 1999), showed the plant growth-promoting properties, like fixing biological nitrogen, producing of plant hormones (auxins, cytokinins, gibberellins, abscisic acid), facilitating the availability of soil nutrients, and tolerance toward toxicity of pesticides (Upadhyay et al. 2009; Yasouri 2003). However, several studies (in vitro and in vivo) have reported the interference and toxicity of organophosphate groups of pesticide on plant growth-promoting microorganisms and also on their interaction with the plant (Verma et al. 2014). Several studies have found the toxicity of pesticide on plant growth-promoting microorganisms and their properties; Shaheen and Sundari 2013 showed that PGPM strains are more sensitive at 100 ppm of monocrotophos insecticide. Ahemad and Khan (2012b), found that under pesticide stress (metribuzin and glyphosate, imidacloprid and thiamethoxam, hexaconazole, metalaxyl, and kitazin), all plant growth-promoting traits of *Mesorhizobium* sp. strain MRC4 have progressively declined with an increase in their concentration. Khan et al. (2009) observed that nodulation in chickpea and rhizobacterial population suppressed under the toxicity of chlorpyrifos (Pyrifos 40% EC). Sepperumal et al. (2016) showed that the growth of *Klebsiella pneumoniae* declined by increasing the dose of phorate. Kumar et al. 2019 found that siderophore properties of strains *P. fluorescens* (NCIM-5096), *R. leguminosarum* (NCIM-2749), *B. brevis* (NCIM-2532), *A. vinelandii* (NCIM-2821), and *S. typhimurium* (NCIM-2501) decreased under the dose toxicity of phorate, acephate, monocrotophos, and glyphosate.

12.3.4 Human Health

According to Bajgar (2004), organophosphate showed as highly toxic nerve agents, which inhibit the cholinergic nerve signaling, and simultaneously, it also catalyzes the neurotransmitter cholinesterase, resulting in an increased level of acetylcholine. The chemical acetylcholine is responsible for the lesions of the different parts of the brain. The clinical sign of organophosphate (OP) poisoning is frequently characterized into receptor-specific manifestation, time-onset-precise manifestation, and organ-specific manifestation. In serious insecticide poisoning, patients have muscarinic signs, like nausea, reflex, drooling, blurred vision, etc. (Agrawal and Sharma 2010). There are a variety of cases of insecticide chemical toxicity in India (Gupta et al. 2006; Shivakumar et al. 2013; Parmar et al. 2014). The first case of insecticide toxicity of organophosphate insecticide in India was reported from the state of Kerala in 1958, wherever quite more than a hundred individuals affected to the poisoning of parathion by overwhelming of contaminated wheat flour (Gupta 2004). The occupational toxicity of OPs was reported as symptoms and sign of neurotoxic poisoning in the state of Andhra Pradesh, by accidentally exposed of the farmer in a cotton-filled during mixing and refiling spraying tank (Mancini et al. 2005). Likewise, another poising of OP case was reported in Saran District of Bihar,

in 2013, in which 23 children were dead after taking the foods of midday meal that were contaminated with monocrotophos (BBC Report 2013).

12.4 Analytical Techniques for the Determination of Organophosphate Pesticides in Environmental Samples

The happening to different coherent explanatory methods has enabled us to accumulate the information and choose the structure of the recently orchestrated atoms. Eventually, incessant assessment is being coordinated to uncover the ligand and detailing media for organophosphate pesticide identification by means of various spectroscopic systems like UV-Vis (Elgailani and Alghamdi 2018), Fourier-transform infrared (FTIR) spectroscopic (Buvaneswari et al. 2017.), NMR spectroscopy (Dahiya et al. 2020), X-ray diffraction (Wang and Liu 2016) mass spectrometry (Alnedhary et al. 2020; Liu et al. 2005), and electrochemical (Gong et al. 2009). The detection and estimation of organophosphate pesticides have been reported in water and vegetables by using analytical methods (Tables 12.3 and 12.4).

12.5 Assessments of Organophosphate Pesticide Toxicity to Microorganisms

Soil is a living entity which has diverse micro- and macrofauna as well as flora. These soil organisms are an integral part of agricultural ecosystems, essential for the maintenance of healthy productive soils. Among the soil microflora, plant growth-prompting rhizobacteria (PGPR) promotes plant growth and development in different ways (Krishna et al. 2019; Verma et al. 2018). Nevertheless, extensive use of plant protection chemicals had led to an accumulation of a huge amount of residues, adversely affecting the environmental health as well as ecosystem services. Among the different classes of pesticides used, organophosphate (OP), a phosphoric acid derivative, is widely used in highly toxic heterogeneous compound. Globally, about 140 OP compounds are being used as pesticides and plant growth promoters (Kang et al. 2006). These agents cause a specific and irreversible inhibition of acetylcholinesterase, a vital enzyme responsible for the degradation of acetylcholine in the nerve terminal (Bakry et al. 2006). Though pesticides have been developed with target organism specificity, often nontarget species are affected by their application. Being persistent, its accumulation in the environment had led to a substantial soil health hazard and toxicity to soil microflora, resulting in altered soil microbial diversity and biomass. Hence, assessment for soil toxicity of OP compounds is a vital component of agricultural environment pollution monitoring. Assessment of biological effects by utilizing a quick, sensitive, and economic method can indicate precise information on OP ecotoxicity. In recent decades, toxicity of OP compounds, by assessing the microbial diversity and biomass, has gained momentum due to advantages, like short life cycles, inexpensiveness, less time-consuming, and sensitivity to various toxic chemicals (Hassan et al. 2016; Su et al. 2011; Tothill and

Table 12.3 List of organophosphate pesticide residues in vegetable samples in India

Environmental sample	Crops	Filed location	Detected organophosphate	Rang of organophosphorus residues (μg g^{-1})	Technique used	References
Vegetable	Brinjal, chili, cauliflower, bitter gourd, tomato, cucumber, okra	Jaipur, Rajasthan	Monocrotophos, quinalphos, dimethoate, chlorpyrifos	0.03–0.10; 0.02–0.08; 0.08–0.09; 0.01–0.06	GC-NPD	Singh and Gupta (2002)
	Brinjal, cabbage cauliflower, pea grain, tomato, potato	Hisar, Haryana	Dimethoate, malathion, fenitrothion, monocrotophos, phosphamidon, quinalphos, chlorpyrifos	0.002–0.208; 0.003–0.016; 0.013–0.024; 0.033–0.051; 0.045–1.284; 0.009–0.048; 0.001–0.047	GC-ECD, GC-NPD	Kumari et al. (2003)
	Brinjal, okra, cauliflower, cabbage, pea	Hisar, Haryana	Monocrotophos, cypermethrin, quinalphos, malathion, chlorpyrifos, methyl parathion,	0.005–0.435; 0.005–0.275; 0.003–0.278; 0.103–0.309; 0.001–0.094; 0.018–0.026	GC-ECD and GC-NPD	Kumari et al. (2004)
	Cauliflower	Punjab	Quinalphos, methyl parathion, chlorpyrifos, monocrotophos, cypermethrin, deltamethrin	0.00–0.20; 0.00–0.24; 0.00–0.20; 0.00–0.20; 0.00–0.40; 0.00–0.20;	GC-ECD, GC-FTD, GC-MS	Mandal and Singh (2010)
	Eggplant, cabbage, tomato, cauliflower, chili, okra, mustard,	Delhi	Cypermethrin, fenvalerate, malathion	0.008–0.025; 0.005–0.075; 0.011–0.015;	GC-ECD	Mukherjee (2003)
	brinjal, okra, cauliflower, cabbage, and green chili	Samastipur, and Nalanda	Cypermethrin, deltamethrin, quinalphos, fenvalerate, chlorpyrifos,	0.014–0.450; 0.032–0.162; 0.010–0.33; 0.00–0.294;	GLC-ECD and GLC-TID	Singh and Singh (2006)

(continued)

Table 12.3 (continued)

Environmental sample	Crops	Filed location	Detected organophosphate	Rang of organophosphorus residues (μg g^{-1})	Technique used	References
		districts, Bihar	ethion malathion	0.00–0.058; 0.015–5.064; 0.00–0.040		
	Okra, brinjal, cauliflower, cabbage	Muzaffarpur District, Bihar	Dimethoate, cypermethrin, quinalphos, fenvalerate, chlorpyrifos methyl parathion malathion	0.560–0.058; 0.112–0.407; 0.024–0.462; 0.081–0.588; 0.057–0.121; 0.00–0.462; 0.279–0.713;	GC- ECD; GC-TID	Sah et al. (2018)
	Onion, cucumber, beetroot, spinach, radish, cauliflower, cabbage	Lucknow City, Uttar Pradesh	Anilofos, dichlorvos, dimethoate, and malathion	0.01–0.014; 0.011–0.020; 0.008–0.042; 0.090–0.151	GC-ECD/ NPD	Srivastava et al. (2011)
	Eggplant, ladyfinger, cauliflower, cabbage, tomato, chili	Hyderabad	Chlorpyrifos, Triazofos, fenitrothion, acephate	2.49–178.87; 0.491–3.014; 12.10–53.90; 2.48–2.48	LC-MS/ MS	Sinha et al. (2012)
	Brinjal, cucumber, okra, ridge gourd, and tomato	Kothapalli, Andhra Pradesh	Monocrotophos, chlorpyrifos, cypermethrin,	0.001–0.044; 0.001–5.154; 001 - 0.352	GC-MS	Ranga Rao et al. (2009)
	Beans, brinjal, cabbage, carrot	Kolar District, Karnataka	Acephate, chlorpyrifos, dichlorvos, monocrotophos phorate profenofos	80.7–89.2; 83.4–94.6; 87.2–96.6; 86.9–92.7; 83.4–92.1; 80.0–88.5;	GLC-ECD; GLC-	Gowda and Somashekar (2012)

			cypermethrin fenvalerate	83.4–93.6; 77.4–90.3		
	Brinjal, okra, green chili, crucifers, cucurbits	Andaman Islands	Chlorpyrifos, profenofos, monocrotophos, triazofos, ethion, dimethoate, acephate	0.019–1.379; 0.042–1.136; 0.023–1.696; 0.023–1.140; 0.083–0.509; 0.00–0.298; 0.00–0.345	GC/MS	Swarnam and Velmurugan (2013)

FAO/WHO MRL values (μg g^{-1}): Monocrotophos, 0.2; phosphamidon; 0.2–0.5; chlorpyrifos, 0.01; cypermethrin, 0.2; quinalphos, 0.1–0.2; malathion, 3.0; methyl parathion, 1.0; dimethoate, 2.0

Table 12.4 List of analytical techniques for the determination of organophosphate pesticides in environmental samples

OPs	Analytical methods	Samples	Objective	References
Acephate	UV-VIS spectrophotometry	*Coriandrum sativum* (leaves), *Petroselinum crispumand, Eruca sativa*	Analytical methods for the determination of acephate pesticide residues in some vegetables	Elgailani and Alghamdi (2018)
Chlorpyrifos		Waste discharges	Validation of UV spectrophotometric and HPLC methods for quantitative determination of chlorpyrifos	Zalat et al. (2014)
Malathion		Water, cauliflower, potato, spinach	Spectrophotometric determination of malathion (an organophosphorus insecticide) with potassium bromate	Venugopal et al. (2012)
Malathion, dimethoate, phorate		Cauliflower, cabbage, spinach	A rapid spectrophotometric assay of some organophosphorus pesticide residues in vegetable samples	Mathew et al. (2007)
Monocrotophos	Fourier-transform infrared (FTIR) spectroscopic	Paddy field soil	Screening of efficient monocrotophos degrading bacterial isolates from paddy field soil of Sivaganga District, Tamil Nadu, India	Buvaneswari et al. (2017)
Dimethoate		Aqueous solution of gold nanospheres and nanorods	Adsorption of organophosphate pesticide dimethoate on gold nanospheres and nanorods	Momić et al. (2016)
Chlorpyrifos, monocrotophos		Liquid broth	Simultaneous degradation of organophosphorus and organochlorine pesticides by bacterial consortium	Abraham et al. (2014)
Chlorpyrifos		Liquid broth	Analysis of chlorpyrifos degradation by *Kocuria* sp. using GC and FTIR	Neti and Zakkula (2013)
Acephate		Commercial acephate	Extraction, UV-visible, FTIR, NMR spectroscopic study of acephate and effect of pH	Upadhay et al. (2013)
Organophosphate pesticides		Biological sample	Mandal nanoparticle-based electrochemical immunosensor for the detection of phosphorylated acetylcholinesterase: an exposure biomarker of organophosphate pesticides and nerve agents	Liu et al. (2008)

Chlorpyrifos and parathion	Nuclear magnetic resonance (NMR)	Biological sample	In vitro interaction of organophosphate metabolites with bovine serum albumin: a comparative 1H NMR, fluorescence, and molecular docking analysis	Dahiya et al. (2020)
Organophosphate pesticides		Biological sample	1H nuclear magnetic resonance (NMR) metabolomic study of chronic organophosphate exposure in rats	Alam et al. (2012)
Methyl parathion		Sorbed on clays	NMR investigation of the behavior of an organothiophosphate pesticide, methyl parathion, sorbed on clays	Seger and Maciel (2006)
Dichlorvos		Lab study	Dichlorvos degradation studied by 31 P-NM	Benoit-Marquié et al. (2004)
Chlorpyrifos		Lab study	Removal of chlorpyrifos, an insecticide using metal-free heterogeneous graphitic carbon nitride (g-C3N4) incorporated chitosan as catalyst: photocatalytic and adsorption studies	Vigneshwaran et al. (2019)
Methyl parathion	X-ray diffraction (XRD)	Lab study	Methyl parathion detection in vegetables and fruits using silver@ graphene nanoribbons nanocomposite modified screen printed electrode	Govindasamy et al. (2017)
Diazinon		Lab study	Diazinon degradation by a novel strain *Ralstonia* sp. DI-3 and X-ray crystal structure determination of the metabolite of diazinon	Wang et al. (2006)
Chlorpyrifos		Lab study	Photocatalytic degradation of organophosphate pesticides (chlorpyrifos) using synthesized zinc	Khan et al. (2015)

(continued)

Table 12.4 (continued)

OPs	Analytical methods	Samples	Objective	References
			oxide nanoparticle by membrane filtration reactor under UV irradiation	
Organophosphorus compounds		Lab study	Crystallization and preliminary X-ray diffraction analysis of the organophosphorus hydrolase OPHC2 from *Pseudomonas pseudoalcaligenes*	Gotthard et al. (2013)
Methyl parathion and parathion		Lab study	Computational interaction analysis of organophosphorus pesticides with different metabolic proteins in humans	Sharma, et al. (2011)
Dimethoate	Gas chromatography-mass spectrometry (GC-MS)	Lab study	Optimization and efficiency comparison of dispersive and cartridge solid phase extraction cleanup techniques in the analysis of pesticide residues in some vegetables using gas chromatography-mass spectrometry	Alnedhary et al. (2020)
Organophosphate esters		*Citrus aurantium*	Selective pressurized extraction as single-step extraction and cleanup for the determination of organophosphate ester flame retardant in *Citrus aurantium* leaves by gas chromatography-tandem mass spectrometry	Santos et al. (2020)
Parathion, methyl parathion, disulfoton		Soil samples	Chemometric-assisted ultrasound leaching-solid phase extraction followed by dispersive-solidification liquid-liquid microextraction for determination of organophosphorus pesticides in soil samples	Ahmadi et al. (2015)
Organophosphate esters (OPEs)		Dust and soil	Analysis of organophosphate esters in dust, soil, and sediment samples using gas chromatography coupled with mass spectrometry	Jian-Xia et al. (2014)
Organophosphate esters		Soil	Application of Twisselmann extraction, SPME, and GC-MS to assess input sources for organophosphate esters into soil	Mihajlovic et al. (2011)

Malathion, isoprocarb	Liquid chromatography-mass spectrometry (LC-MS)	Tomato, apple, carrot, and cabbage	Simultaneous determination of carbamate and organophosphorus pesticides in fruits and vegetables by liquid chromatography-mass spectrometry	Liu et al. (2005)
Methyl parathion	Electrochemical Bioassay	Lab study	Electrochemical biosensing of methyl parathion pesticide based on acetylcholinesterase immobilized onto Au-polypyrrole interlaced network-like nanocomposite	Gong et al. (2009)
Paraoxon, methyl parathion, and fenitrothion		Lab study	Electrochemical stripping analysis of organophosphate pesticides and nerve agents	Liu et al. (2005)
Parathion methyl		Food samples	Screening of food samples for carbamate and organophosphate pesticides using an electrochemical bioassay	Del Carlo et al. (2004)

Turner 1996b). Moreover, most microorganisms have similar biochemical pathways as that of higher organisms and hence quickly respond to the changes in soil ecosystem. Consequently, for screening pesticide toxicity, numerous microbial techniques have been evolved and utilized. According to Tothill and Turner (1996a), microbial-mediated bioassays utilized various mechanisms based on:

- The ability of these microorganisms to convert carbon, sulfur, and nitrogen
- The enzymatic activity like ATP utilization, dehydrogenases, acid phosphatase and alkaline phosphatase, etc.
- Growth, mortality, and photosynthetic activity of microbes
- Glucose uptake
- Oxygen consumption
- Luminescence output

For example: Dehydrogenase-based bioassays utilize specific dyes like methylene blue, resazurin, and triphenyltetrazolium chloride, as acceptors of electron, and change color in their reduced form Tothill and Turner (1996a). Adenosine triphosphates (ATPs) are the basis of all cellular activities, which are found in all living cells and quickly break down upon microbial death. ATP toxicity assay is based on the change in ATP content under pesticide exposure. ATP luminescence as a toxicity detection method is established on luciferin-luciferase enzyme-mediated reaction, wherein luminescence produced is proportional to the amount of ATP present (Dalzell and Christofi 2002). In order to evaluate the toxicity of chemicals, activated sludge respiration inhibition was found to be an effective method (ISO 2007; OECD 2014). Bacterial respiration is commonly measured as the oxygen uptake rate. The respiration rates of samples of activated sludge fed with synthetic sewage within an enclosed cell containing oxygen electrode are measured after 3 hours of contact period (OECD, 2014). Liao et al. (2001) identified biosensor based on respiration rate inhibition for wastewater toxicity using oxygen-sensitive bacterial isolates obtained from activated sludge. When bacterial respiration was inhibited due to toxicity, more oxygen was able to cross the biosensor membrane, which resulted in change in the oxidation-reduction rates. The response time of the biosensor was shown to be approximately 8 min. dos Santos et al. (2002) developed an activated sludge respirometry-based assay using 96-well microplates. In this micro-assay, respiration was indirectly quantified by using a dye (tetrazolium violet) mixed with sludge bacteria in the wells of the microplate. As the result of redox reaction, tetrazolium violet dye was chemically reduced by bacterial respiration and produced deep purple color. Hence, the dye color was used as an indicator of sludge respiration. An incubation period of 24 h was needed, which limits its use for effluent toxicity monitoring, wherein a quick response is required. Likewise, to evaluate nitrification-inhibiting substances in industrial wastewaters, similar bioassay was used (Svenson et al. 2000). Conversion rates of ammonium-nitrogen and nitrite-nitrogen were used to calculate the nitrification inhibition levels in *Nitrosomonas* and *Nitrobacter* assays, respectively. Luminescent microorganisms have been used in the production of several toxicity test biosensors. The bioluminescence inhibition

assay is based on marine Gram-negative bacteria, *Vibrio fischeri* or *Photobacterium phosphoreum*. The specific strain, *V. fischeri* NRRL B- 11177, has been widely used to estimate acute toxicity. Several commercial test kits, i.e., Microtox, LUMIStox, and ToxAlert, based on this strain are available (Shijin, 2004). The test relies on the change in bacterial luminescence upon the exposure to toxic chemicals. The bioluminescence inhibition of *V. fischeri* has been standardized (OECD, 2014), and the test kits are commercially available in different versions. The advantages of these toxicity tests include short analyzing period and simplicity of operation. The following are the methodology in brief, which can be used to analyze the pesticide toxicity on microorganisms.

12.5.1 Culture-Independent Analyses

The microbial community diversity in pesticide-contaminated soil can be analyzed by a culture-independent approach. This is achieved by cloning of small subunit of ribosomal RNA (SSU rRNA) gene (Zhang et al. 2006). In this technique, microbial DNA isolated from both the pesticide-contaminated and non-contaminated soil sample and PCR are performed using 16S rRNA gene-specific primers. The amplicons are sequenced, and sequence phylogenetic analysis is carried out, comparing both samples. The indication of dominant phylotypes demonstrates considerable change in bacterial communities.

12.5.1.1 Biomass Estimation: Counting

To evaluate the impact of pesticide on the bacterial population, biomass estimation technique is applied. According to the method of Frostegård and Bååth (1996), the bacteria from soil are isolated following the method of Bååth et al. (1992), which is the modification form of Faegri et al. (1977). The collected 2.5–10 grams of powered soil sample add in 100ml of distilled water and mixed properly by Omni mixer (1 rain, 80% of full speed) and after centrifugation of soil solution at 5°C for 10 min at 750 g. The supernatant was collected and filtered with the help of glass wool; the sediments redissolved in 100 ml of distilled water and further centrifuged at 5 °C for 10 min at 750 g. From this, a 2 ml of sample was taken out to count the bacteria from the resulting 200 ml solution containing bacteria. After this, the remaining solution was centrifuged at 13000 g for 20 min. The pellet was collected and redissolved in 3 ml of citrate buffer. The sonication of dissolved pellets for 15 min from different soil samples was conducted to break up the bacterial aggregates. After this, a 100 μl bacterial solution was taken to microscopical count. The bacterial count values were calculated per gram organic matter (measured as loss on ignition at 600 ~ for 4 h). For the microscopical count, bacterial suspension was diluted using filter sterilized HAc buffer (0.15 M; pH 4.0), and acridine orange dye is mixed (0.001% working concentration). After 5 min of incubation, the suspension is filtered via a 0.2 μg black nucleopore filter, and with the help of fluorescence microscope, stained bacteria are counted. The bacterial suspension and pellet can be stored in formalin to count the bacteria after adding an appropriate amount of HAc buffer and acridine orange dye.

12.5.1.2 Phospholipid Fatty Acid (PLFA) Analysis

The extraction of phospholipid fatty acid (PLFA) can be carried out by using the method developed by Frostegård et al. (1993); in this method, 1.0 g of soil (wet mss) is extracted in a one-phase mixture having chloroform, methanol, and citrate buffer (1: 2:0.8, v/v/v). After this, the extract gets separated into two phases; the phase containing lipid is dried under N_2 stream and kept at −20 °C. The lipid component is fractionated on columns having silicic acid into neutral, glycolipid, and phospholipid, including polar lipids. The fraction of phospholipid is dried under N_2 stream and stored for fatty acid methyl ester preparations. Methyl nonadecanoate is mixed to the fractions of phosphor lipid as standard (internal). After this, the samples are carried out for alkaline metanalysis as by Dowling et al. (1986), and the separation of fatty acid methyl esters are carried out by using gas chromatograph (GC), having a flame ionization detector. Various types of capillary columns having different polarities can be used. The programming of temperature can be set as follows: initially, 80 °C for 1 min increases with 20 °C per min to 160 °C, and after this, increase at 5 °C per min to 270 °C final temperature for 10 min. The fatty acid methyl ester's relative retention times are compared with standards.

12.5.2 Culture-Dependent Analyses

There are several established conventional methods which can be used for the quantitative analysis of soil microbial characteristics, including mineralization and respiration tests (Iyyemperumal et al. 2007; Kähkönen et al. 2007), microbial biomass quantification (Brookes et al. 1985), growth analysis and patterns of substrate use by utilizing standardized Biolog® plates (e.g., Stefanowicz et al. 2009; Wainwright 1978), and community-level physiological profiling (CLPP) (e.g., Yang et al. 2006). Soil microbial metabolic activities can be quantified by recording the production of CO_2 (Iyyemperumal et al. 2007; Kähkönen et al. 2007) and also with the help of different enzyme assay (Wainwright 1978), Wallenstein and Weintraub 2008). Pesticide toxic impact on microbes is generally analyzed by measuring the functional responses in monitored soils, like the overall microbial activity rates (Wainwright 1978) or respiration (Burton and Lanza 1985), or individual bacterium test in laboratory (Bitton and Koopman 1992). However, key culture-dependent techniques for analyzing pesticide impact like compatibility in petri dish, technical grade pesticide in soil and plating, ester-linked fatty acid methyl esters, and fluorescein diacetate hydrolyzing activity are described here. In these techniques, various specific enzymes, or combinations of different enzymes have to apply, access to the impact of pesticides on bacterial communities. Previously, amidase, alkaline phosphatase, asparaginase protease, dehydrogenase, and urease activities have been characterized to analyze mancozeb fungicide (Rasool and Reshi 2010); activity of catalase for pyrethroids and organophosphorus pesticides (Shiyin et al. 2004); and dehydrogenase, phosphatase, and urease for the insecticides propiconazole, profenofos, and pretilachlor (Kalam et al. 2004).

12.5.2.1 Technical Grade Pesticide in Soil and Plating

In agriculture, the pesticide is used in different dose or concentrations and also in combination with other pesticides; these pesticides affect the microbial population, their interactions, and microbial biomass. The impact of different concentration of pesticide on soil microbes can be analyzed by the method earlier developed by Radivojević et al. (2011). In this method, the soil is collected from top to 10 cm and dried; after drying, the soil sample is sieved with a sieve having a mesh pore size 5 mm and kept at 4 °C. At the time of use, the soil is dried for 24 hours at room temperature. The different pesticide solutions of technical grade substances are prepared in distilled water. After this, the solution concentration was pipetted to 1 kg of soil surface followed by rotation for 30 min at rotator stirrer prior to homogenization. The homogenized soil is portioned into pots. Soil without treatment is kept as control. After this, the pots are incubated in a controlled chamber at 20 ± 2 °C, 50% air and soil humidity, and 12/12 h photoperiod. The sample is being collected after the application of pesticide at the desired intervals (e.g., 7, 14, and 30 days). The culture-dependent microbes are analyzed by soil dilution plate method, with the help of tryptic soy agar and Czapek agar, respectively, for bacteria and fungi and incubated at 28 °C for 3 and 5 days, respectively, for bacteria and fungi; after this, the colonies are being counted. The determination of microbial biomass C is carried out by the fumigation-extraction as described by Vance et al. (1987). The fumigation of samples is done with non-alcohol chloroform for 24 hours under wet conditions. The carbon is extracted with a 100 ml 0.5 M of potassium sulfate solution (K2SO4) and its titration performed with 0.0333 M Mohr salt ($(NH4)_2Fe(SO4)2$) solution containing phenylantranil acid to determine the contents. Control soil sample has also followed the same procedure. The microbiological biomass carbon (MBC) is calculated by the formula given by Jenkinson et al. (1979): MBC = C extracted × 0.33, where C was extracted from fumigated and *non-fumigated samples. The results are expressed in μg C g^{-1} soil.

12.5.2.2 Ester-Linked Fatty Acid Methyl Esters (EL-FAME)

Ester-linked fatty acid methyl esters (EL-FAME) also applied to analyze the impact of pesticides on microbial diversity; this analysis can be performed by ester-linked fatty acid methyl ester (EL-FAME) extraction method. In this process, as described by Schutter and Dick (2000), alkaline methanolysis of ester-linked fatty acid carried out leaving the free fatty acid, which involves four reagents and steps. In the El-FAME analysis, firstly taken 3 gram of soil sample and kept in a 35 mL Teflon-coated, caped glass centrifuge tube and mixed with the 0.2 M KOH prepared in methanol and stored for 1 hour at 37°C. And then intermittent vortexing done after every 10 min to release the ester-linked fatty acids and its methylation. In the second step, 3 ml of acetic acid (1.0 M) is mixed for pH neutralization in the tube. The FAME is separated into an organic phase by mixing 10 ml hexane and centrifuging at 480Xg for 10 min. After this, in the third step, the hexane layer is collected in a fresh glass test tube, and evaporation of hexane is carried out under N_2 stream. In the final, fourth step, FAME is being dissolved in 0.5 mL of 1:1 hexane-methyl tert-butyl ether and transferred to a GC vial for analysis. The gas chromatography can be

carried out in a Hewlett-Packard 5890 Series II (Palo Alto, CA) equipped with an HP Ultra 2 capillary column (5% diphenyl-95% dimethylpolysiloxane, 25 m by 0.2) and an ionization detector flame. The ramping temperature setup ranges from 170 to 270 °C at 5 °C per min, with 2 min at 270 °C between samples to clean the column. The identification of fatty acid being carried out and their relative peak can be determined by using MIS Aerobe chromatographic program and peak naming table as supplied by the supplier. To describe FAMEs, the standard nomenclature should be used.

12.5.2.3 PCR-Denaturing/Temperature Gradient Gel Electrophoresis

DNA fragments of similar length and different base-pairing sequence can be analyzed using either denaturation gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE). From the soil sample, DNA is isolated and PCR amplified with sequence-specific 16S or 18S rRNA universal primers (Krishna et al. 2019; Ferreira et al. 2009). Further based on the mobility of DNA fragments on partially melted acrylamide gels, amplicons are separated along with gradients of linear DNA denaturants, such as urea and formamide. Change in the melting property of DNA is attributed to any variation in the amplicon sequence, due to which separation takes place in denaturing gradient gel. Within the nucleotide strand, melting occurs at different points having the same melting temperatures. Based on the concentration of denaturants, sequence variation of fragments will prevent migration at different points in the gel. Theoretically, DGGE can separate DNA fragments, which shows single-base-pair difference (Miller et al. 1999). Also, TGGE works on the same principle; the only difference is the use of temperature gradient for denaturation instead of chemical. Both DGGE and TGGE techniques are quick, reliable, reproducible, and cost-effective compared to other techniques and can analyze numerous samples at the same time and also track the changes in microbial population under any harsh conditions or stimuli.

12.5.2.4 Terminal Restriction Fragment Length Polymorphisms (T-RFLP)

Terminal restriction fragment length polymorphisms (T-RFLP) is a modified version of RFLP, an alternate technique for rapid microbial diversity analysis in different ecosystems (Thies et al. 2007). This technique works on a similar principle of RFLP except for fluorescent dye labeling of one PCR primer, such as TET (4,7,20,70-tetrachloro-6-carboxyfluorescein) or 6-FAM (phosphoramidite fluorochrome 5-carboxyfluorescein). The PCR is performed using l6S rDNA universal primers, one of which is labeled with fluorescent dye. The fluorescent-labeled terminal RFLP (FLT_RFLP) patterns can be prepared by digesting the labeled PCR product with restriction enzymes. The fragments are then separated using gel electrophoresis in an automated sequence analyzer. The operational taxonomic unit (OTU) is prepared by counting the unique DNA fragments, and OTU frequency was computed. To measure species diversity and similarities as well as evenness between samples, banding patterns were used. However, this technique might mislead the actual microbial diversity, because in most case the most dominant species are detected

because of the availability in large amount of DNA template (Liu et al. 1997), whereas the incomplete or partial digestion of DNA by restriction enzymes may result in diversity overestimation. Further, universal primers are unable to amplify the whole sequences from bacterial and archaeal domains, and the primers are designed from the existing 16S rRNA and internal transcribed spacer (ITS) sequence databases, which have sequences from culturable microbes. Hence, it doesn't represent the true diversity of the microbial sample. Moreover, different restriction enzymes will generate dissimilar fingerprints of microbial community. T-RFLP is a unique technique used for comparing relationships among different microbial samples and has also been used for the measurement of spatial as well as temporal changes in the communities of bacteria to analyze complicated microbial communities, detect and monitor populations, and assess arbuscular mycorrhizal fungi diversity in rhizosphere.

12.5.2.5 Single-Strand Conformation Polymorphisms (SSCP)

The single-strand conformation polymorphism (SSCP) technique works on electrophoresis-based discrimination of DNA sequences and allows differentiation of DNA fragments of equal length but with varied nucleotide sequences. This technique was initially developed to distinguish point mutations or novel polymorphisms in DNA fragments. In this technique, single-stranded DNA fragments were separated in a polyacrylamide gel, wherein variation in the mobility of DNA fragments was due to its secondary structure (i.e., heteroduplex). This technique is helpful in analyzing the genetic diversity of microbes. Sometimes, multiple bands are produced for the same DNA sequence on the gel caused by the presence of multiple stable conformations of some single-stranded DNA. However, this technique does not require a GC clamp or gradient gel construction. It has been also used for the study of rhizosphere community diversity of bacteria and fungi (Stach et al. 2001).

12.6 Future Perspectives and Challenges

Food security for a rapidly growing global population and consequent higher rates of food consumption has resulted in increased production and application of pesticides in agricultural sector. Different modes of pesticide classification are generally based on their chemical composition, characteristics, target pest and mode of action, and entry. Organophosphates find a wide usage as compared to other classes of pesticides due to their efficiency and degradation. In spite of several number of studies to decipher the effect of pesticide application on soil ecosystem, there is lesser clarity in understanding the role of pesticides to cause disturbance in the soil environment due to a wide variation in the results obtained from these studies. It may be that certain pesticides' residues serve as a source of carbon or energy for the microorganisms following their microbial degradation and assimilation, while many reports state adverse effects of these residues on soil microflora also. Therefore, the effect of pesticides on microbes and associated soil nutrient transformations, enzyme

activities, and other biochemical processes is not definitely conclusive because of the differences in the levels of toxicity exhibited by the different classes of pesticides. Additionally, the effect of pesticides on soil biological activities is also defined by other contributing factors, such as soil physicochemical properties, nature, concentration and activity of pesticide used, and metabolites produced in soil from the resulting metabolic activities. Disturbance in biochemical equilibria affecting the fertility and productivity of soil occurs mostly due to the long-term application of pesticides. Thus, the understanding of fundamental mechanisms behind the different microbial responses at the molecular level due to the application of pesticides could provide great help in explaining the risk of pesticide contaminations. It will also help to understand the subsequent adverse effects on microbial diversity, enzymatic activities, and biochemical processes in soil. Therefore, to gain a comprehensive understanding and quantify the net effect of pesticide application on soil biology and biochemistry, induction of molecular techniques is very important in contrast to the traditional methods.

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'Cu-Chi-Tri', a New Generation Combination for Knowledge-Based Management of Oomycete Pathogen, *Phytophthora infestans*

13

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Abstract

Globally, an estimated 35% of the potential crop is lost annually to diseases, pest and weeds, while decreases in arable land and increases in world population, global climate change and increased production of energy crops continue to enhance the pressure. Chemical plant protection is too expensive for resource poor farmers, and it is potentially unsafe to both environment and consumer. However, as an alternative to chemical plant protection, biological plant protection is less consistently reliable. Nevertheless, a combination of different strategies could make biological plant protection more reliable. The present chapter focuses on developing a novel combination product for economically viable and ecologically safe plant protection, with emphasis on devastating diseases caused by oomycete plant pathogens, particularly *Phytophthora infestans*, the dreaded late blight causative in potato. Late blight disease management has largely relied on the use of chemical fungicides, with the above-mentioned problems and the added threat of the development of chemical resistant strains of the pathogen. Therefore, the need of alternative approaches for late blight management without compromising benefits as attained by the use of chemicals has been variously flagged. To this end, a new generation fungicide involving a low-dose fungicide ($Cu(OH)_2$), a biocontrol agent (*Trichoderma*) and a plant defence activator (chitosan) has been developed and tested under field conditions for the management of potato late blight. The 'triple combination' evokes newer avenues of application of biocontrol agents for safer and sustainable management of oomycete plant pathogens.

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Copper hydroxide · Chitosan · *Trichoderma* · Triple combination · *Phytophthora infestans*

13.1 Introduction

Extreme and unrestricted use of synthetic pesticides and chemical fertilizers in agriculture to prevent crop yield losses or product damage leads to alarming consequences of toxicity. Green revolution technologies have inadvertently increased dependence on harmful chemicals, pesticides and fertilizers, not only damaging the ecosystems but also threatening human health. Further, climate change and faulty practices have aggravated the problem, making the situation even worse. Pests and diseases cause about 35% losses to crop plants, and their management contributes to a huge quantum of pesticide use (Flood 2010).

Since the beginning of recorded history, plant disease outbreaks have had a significant impact on human society. Among these devastating outbreaks, the 'late blight disease' that caused the Irish potato famine of the 1840s triggered the official beginning of the science of plant pathology and was the first plant disease for which a microorganism was proved to be the causal agent (Schumann and D'Arcy 2000). Late blight caused by the oomycete *Phytophthora infestans* (Mont.) de Bary is affecting cultivation of potatoes and tomatoes. The disease poses very serious economic threats to the vast majority of potato production systems as well as many tomato production systems worldwide (Madden 1983) and has been estimated in 2012 to be responsible for causing $6.7 billion yield losses annually (Nowicki et al. 2012).

Management of the late blight pathogen under field conditions is a challenge since the pathogen has the ability to develop new races and is able to adapt to a wide range of environmental conditions (Njoroge 2019). The control of the disease has traditionally relied on foliar applications of fungicides. These fungicides include copper salts, dithiocarbamates, bis-dithiocarbamates, cyanoacetamide oxime and metalaxyl. Excessive use of these chemicals poses great threats at a global level (Ragunanthan and Divakar 1996) and also risks several components of agro-ecosystem (Ghorbani et al. 2004).

The only effective fungicides currently permitted for blight management even in organic agriculture are copper-based products (Mizubuti et al. 2007). FRAC (Fungicide Resistance Action Committee) has grouped copper-based fungicides in the group of 'low resistance risk' (Clark and Yamaguchi 2002). Nevertheless, appearance of fungicide-resistant pathogen strains and negative environmental impacts associated with use of chemicals has intensified the need for reducing chemical use and for alternative disease management methods.

Biocontrol agents (BCAs) could be one of the most promising and effective means of alternative disease management strategies, and plant defence inducers, such as chitosan, could also be utilized in plant disease management resulting in less

impact of the chemicals on the environment. *Trichoderma* is a widely used biological control agent in organic and integrated nutrient management approaches; however, it lacks efficacy in field condition (Monte 2001). Chitosan is a naturally occurring compound that has a potential in agriculture with regard to controlling plant diseases (Hadwiger et al. 1986). This biopolymer has antimicrobial activities against plant pathogens (viruses, bacteria and other pests) and is well known to have resistance eliciting activities in plants, leading to a variety of defence responses in host plants in response to microbial infections, including the accumulation of phytoalexins, pathogen-related (PR) proteins and proteinase inhibitors, lignin synthesis and callose formation (Oh et al. 1998). Based on these and other properties that help strengthen host plant defences, chitosan is widely used as an antimicrobial agent either alone or blended with other natural polymers (No and Meyers 1997). However, these management methods do not prevent late blight infection alone and therefore might profit from the integration of copper fungicides that already play an essential role in the control of oomycete pathogens, particularly under organic production systems.

13.2 Role of Copper in Management of Late Blight Disease of Potato

Copper is a vital element for all living organisms as it acts as a cofactor for a range of enzymes, though excessive use of copper can generate highly reactive oxygen radicals (Zapotoczny et al. 2007) and can lead to mutation (Anand et al. 2006). In recent years, due to widespread and long-term use of copper-containing pesticides in agriculture against phytopathogenic microorganisms, its toxicity has become an agricultural and environmental concern (Cornejo et al. 2013). Use of copper fungicides has been restricted in Europe due to increasing concerns about long-term build-up in soil in areas that have a history of high application rates (EU 2018). Repeated application of copper leads to Cu accumulation in the soil, affects soil biology and causes damage to non-target organisms such as earthworms (*Lumbricus terrestris*) (Homa et al. 2003).

Based on these concerns, use of Cu fungicides had been restricted in Europe to 6 kg Cu ha^{-1} (Anonymous 2008). In Denmark and the Netherlands, copper fungicides were completely banned, while in Germany, most of the organic farmer associations allowed up to 3 kg Cu ha^{-1} in potato production. Copper hydroxide is a frequently used formulation permitted in Germany and thus is authorized for use in plant protection products (Anonymous 2009). Though application of Cu fungicides was allowed until 2016, yet research groups and farmers' associations imposed clear Cu input reductions in the EU. Recent EU regulations restrict the use of plant protection products containing copper compounds to a maximum application rate of 28 kg/ha of copper over a period of 7 years (i.e. on average 4 kg/ha/year) in order to minimize the potential accumulation in soil and the exposure for non-target organisms while taking into account agro-climatic conditions occurring periodically in Member States leading to an increase of the fungal pressure (EC 2018).

Accordingly, alternative, copper-free or copper-reduced management practices including treatments based on biocontrol agents (BCAs) for controlling late blight in potato have been intensively investigated (Tamm et al. 2004). Several alternative preparations tested in recent years showed potential against the late blight pathogen in vitro but had only insignificant effects on infections of potato crops in the field. For example, sage essence (*Salvia officinalis*) reduced zoospore release of *P. infestans* (Blaeser et al. 1998) but revealed no reproducible effects on late blight severity or tuber yield in vitro (Neuhoff et al. 2006). Beneficial microorganisms, namely, fungi, bacteria or compost extracts, were found less effective in reducing foliar development of late blight on detached leaves (Ghorbani et al. 2005). Twenty-seven copper-free preparations in field trials had been tested; however, only 17% efficacy was recorded in control of the late blight pathogen. Apparently, stability under existing environmental conditions remained the main reason for the failure (Dorn et al. 2007a, b). Finally, recent findings using a 4% extract of *Frangula cortex* reduced disease progress similar to 3 kg Cu ha^{-1}, but with no significant yield effects (Krebs et al. 2013).

Today, Cu-based fungicides appear to be the best treatment in organic potato production (Zarb et al. 2002), but efficient application is required, and it should be applied only after the failure of alternative, non-chemical methods. Reducing the rates of copper per application or the frequencies of applications may significantly reduce the annual Cu input. However, insufficient information is available about field performance of formulations with reduced Cu doses (e.g. Krebs et al. 2013). Therefore, development of alternative or combination products that can naturally induce resistance in the plants and reduce the doses of copper is quite desirable.

13.3 Role of *Trichoderma* in the Management of Late Blight Disease of Potato

Trichoderma spp. are ubiquitous and often predominant components of the microflora as saprophytes in soil, litter, organic matter and rhizospheric ecosystems of all climatic zones. Recent discoveries show that they are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi (Vinale et al., 2008). Strains of *Trichoderma* spp. are endophytes and establish robust and long-lasting colonization of root surfaces and penetrate into the epidermis. However, the ability of these fungi to sense, invade and destroy other fungi has been the major driving force behind their commercial success as biopesticides (Benítez et al. 2004). *Trichoderma* defend the plants by their direct and indirect effect on plant-pathogen-soil environment interactions. These fungi do not only protect plants by killing the pathogens, mainly other fungi and nematodes, but they also induce resistance against plant pathogens (Hermosa et al. 2011), impart abiotic stress tolerance and improve plant growth, vigour and nutrients' uptake, and they are significantly involved in bioremediation of soils from heavy metals and environmental pollutants (Tripathi et al. 2013). In addition, this genus comprises fungi that produce secondary metabolites of clinical significance and enzymes with widespread industrial application. They produce

and/or release a variety of compounds that induce localized or systemic resistance responses in plants. These root-microorganism interactions cause significant changes in the plant metabolism. Plants are protected from numerous pathogens by responses that are similar to systemic acquired resistance and rhizobacteria-induced systemic resistance. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, uptake and use of nutrients and resistance to biotic and abiotic stresses (Harman et al. 2004; Hermosa et al. 2011).

Various organisms like bacteria, fungi, algae and plants have been utilized for efficient bioremediation of heavy metals (Vidali 2001). Numerous strains of *Trichoderma* have also been utilized in bioremediation, waste management and biotechnology. Binding of heavy metal ions by cell wall polysaccharides is an important detoxification mechanism in fungal systems (Leung 2004). Bioremediation can be defined as the use of organisms to break down harmful environmental contaminants, to clean the environment to a healthier state. Extensive attention has been paid to the management of environmental pollution in recent investigations and to minimize heavy metal contamination (Leung 2004). Heavy metals are part of human life for thousands of years. Although their ill effects on health have been known for a long time, exposure to heavy metals continues by different ways and is even increasing as it is a vital part of chemicals used in fields for plant disease management (Järup 2003). One such heavy metal is copper, which is a highly polluting metal, mostly used as fungicide in plant disease management.

It has been reported that *Trichoderma inhamatum* can significantly tolerate Cr (VI) and is able to reduce its concentrations. Thus, it may have potential applications in bioremediation of Cr(VI)-contaminated wastewaters (Morales-Barrera and Cristiani-Urbina 2008). The ability of immobilized *T. viride* biomass and cell-free Ca-alginate beads for biosorption has been investigated (Bishnoi and Garina 2005). *Trichoderma atroviride*, *T. harzianum* and *T. pseudokoningii* directly link the soil to plants via solubilization of phosphates and micronutrients while promoting plant growth and reducing metal toxicity (Pascale et al. 2019; Altomare et al. 1999). However, research is limited on the utilization of heavy metal-tolerant *Trichoderma* spp. in plant disease management either alone or in combination with lower doses of heavy metal-based fungicides, particularly copper. It is widely recognized that species of *Trichoderma* protect plants against pathogens by competition, mycoparasitism and antibiosis and by inducing systemic acquired resistance (Lorito and Woo 2015; Druzhinina et al. 2011).

Various studies have reported the potential use of biocontrol fungi against potato late blight (Jindal et al. 1988; Marrone 2002), either preventing the germination of sporangia or inhibiting the development of late blight. Chitosan is a biopolymer consisting of β-1,4-linked glycopyranoses, namely, glucosamine and *N*-acetylglucosamine. Enzymes that hydrolyse the β-1,4-glycosylic bonds of this biopolymer (chitinases and chitosanases) have been found in many microorganisms, such as bacteria and fungi. *Trichoderma* spp. also possess a battery of lytic enzymes and have the ability to degrade chitosan, producing chito-oligomers which are active elicitors of defence reactions in plants (Lin et al. 2005; Vander et al. 1998).

Trichoderma harzianum produces fungal cell wall degrading enzymes that are strong inhibitors of spore germination and hyphal elongation of a number of phytopathogenic fungi. Chitin – together with β-(1,3)-glucan – is the major constituent of the cell walls of asco- and basidiomycetes, whereas cellulose is mainly found in the cell wall of oomycete pathogens (Peberdy 1990). Its enzymatic hydrolysis is catalysed by the action of exo- and endochitinases, N-acetyl-β-D-glucosaminidases and hexosaminidase (Carmen et al. 1995; Peterbauer et al. 1996). Several enzymes degrading chitinous material (endochitinase, exochitinase, exo-β-D-N-acetylglucosaminidase) have been reported from *T. harzianum*, and some of those have also been cloned (Harman et al. 1993; Garcia et al. 1994; Carmen et al. 1995; Peterbauer et al. 1996). NAG and HEXO are important genes responsible for the production of *N*-acetyl-β-D-glucosaminidase and hexosaminidase enzymes, respectively, which may degrade chito-oligosaccharides to *N*-acetylglucosamine and glucosamine (Peterbauer et al. 1996). Chitosanases have been reported from a number of organisms including fungi and bacteria (Alfonso et.al. 1992; Sakai et.al. 1991; Shimosaka et al. 1993).

Exposure of fungi and yeasts to elevated copper concentrations can lead to a rapid decline in membrane integrity, which is generally manifested as leakage of mobile cellular solute (e.g. K^+). *Trichoderma* strains can persist in ecosystems with high concentrations of heavy metals. Copper tolerance in fungi has also been ascribed to diverse mechanisms involving trapping of the metal by cell wall components, altered uptake of copper, extracellular chelation or precipitation by secreted metabolites and intracellular complexing by sulphur compound, namely, metallothioneins and phytochelatins (Cervents and Corona 1994; Scheck 1996). Cu^{++} can bind to the cell wall surface of *T. asperellum*, a mechanism of metal tolerance making it less available in the medium (Erayya 2014).

Extensive metal-induced disruption of membrane integrity inevitably leads to loss of cell viability. However, even relatively small alterations in the physical properties of biological membranes can elicit marked changes in the activities of essential membrane-dependent functions, including transport protein activity, ion impermeability (Keenan et al. 1982; Borel 1996) and phagocytosis (Avery et al. 1996). The physical properties of a membrane are largely determined by its lipid composition.

The fatty acid composition of microbial membranes is highly variable and is influenced by both environmental and intrinsic factors. For example, the unsaturated fatty acid contents of microorganisms generally increase at low temperatures. The low melting temperatures and large physical volumes occupied by unsaturated fatty acids are thought to partially compensate for the lipid-ordering effect of chilling (Cossins 1994). Also, the fatty acid composition of membranes differs between microbial groups. Indeed, microbial fatty acid profiles have proven to be useful taxonomic criteria (Thompson et al. 1993) and can be indirectly correlated with other phenotypic characteristics, including pathogenicity (Harbige and Sharief 2007).

Singh and Erayya (2018) confirmed that a copper-tolerant *T. asperellum* isolate had the ability to modulate the fatty acid composition of its plasma membrane which prevented the penetration/absorption of copper ions inside the cell, so that copper accumulated only outside of the cell membrane. Copper tolerance of *T. asperellum*

increased considerably with increased plasma membrane fatty acid. Fatty acids such as octadecenoic acid derivatives (9.67%), octadecadienoic acid derivatives (17.46%), hexadecanoic acid (4.37%) and petroselinic acid (2.51%) were found to be significantly higher in copper-treated *Trichoderma* as compared to untreated (control) in fatty acid profiling study (Singh and Erayya 2018). Petroselinic acid peak was found in CuOH-amended *T. asperellum* culture only. Phospholipids which play an important role in metal ions movement across the plasma membrane were observed in less amount (1%) in CuOH-500 (ppm)-treated *T. asperellum* as compared to control (3.73%). Therefore, CuOH-tolerant *T. asperellum* modify total fatty acid content in the presence of CuOH which made the plasma membrane stable in copper-amended media (Singh and Erayya 2018). Nischwitz et al. (2007) evlauted differences in fatty acid methyl ester (FAME) profiles among copper tolerant and copper sensitive strains of *Pantoea ananatis* and reported that higher concentrations of myristic acid and oleic acid were found in copper tolerant starins.

Two unsaturated fatty acids (linoleic acid and oleic acid) were markedly increased at high copper concentration in copper-tolerant fungi (Abboud and Alawlaqi 2011). However, due to the aggressive nature of the pathogen and the typically explosive disease development, management of *P. infestans* is rather difficult with biocontrol agents when they are applied alone (Li et al. 1995; Dorn et al. 2007a, b). Therefore, a new generation of combinations of biopesticides are required for safe management of oomycete plant pathogens.

13.4 Antifungal Activity of Chitosan Against *Phytophthora infestans* and Activation of Defence Mechanisms in Late Blight Management

Chitosan is a linear amino polysaccharide consisting of glucosamine and *N*-acetylglucosamine units. It is obtained commercially by chemical deacetylation of chitin. It exhibits a range of biological activities that are potentially useful in agriculture. Chitosan can promote plant growth and development (Malerba and Cerana, 2016), or it can inhibit the growth of fungi (Oliveira Junior et al. 2012) and bacteria (Benhabiles et al. 2012), and it has been reported to activate genes involved in defence responses in plants (Hadwiger et al. 1986; Oh et al. 1998; Povero et al. 2011). Moreover, chitosan can bind heavy metals due to the free electron pair of the nitrogen atom in the amino group of glucosamine at neutral or alkaline pH, and this ability may be useful in combination with copper fungicides (Verma et al. 2004).

Chitosan also proved to be an effective inhibitor of spore germination and germ tube elongation and caused morphological changes in hyphal growth of several phytopathogenic fungi including *P. infestans*, the causal agent of potato late blight (Oh et al. 1998). Foliar or root applications of chitosan before inoculation can enhance protection against pathogenic fungi (Muzzarelli et al. 1990; Vasyukova et al. 2005). Vasyukova et al. (2005) also reported 50% reduction in late blight severity with application of low molecular mass (5 kDa) chitosan and, thus, induced

systemic resistance in potato tubers due to increased rishitin synthesis. The level of rishitin accumulated was directly related to the chitosan concentrations applied. In chitosan-treated leaves, an increase in endogenous salicylic acid (SA) as well as of intercellular chitinase and glucanase activities was observed. In general, chitosan treatment can induce various biochemical changes in plants, such as DNA damage, chromatin alterations (Hartney et al. 2007; Hadwiger 2008), activation of MAP kinases, oxidative bursts and callose apposition (Paulert et al. 2010), pathogenesis-related protein synthesis (Loschke et al. 1983), phytoalexin accumulation, hypersensitive response (Hadwiger and Beckman 1980), synthesis of jasmonic acid and abscisic acid and accumulation of hydrogen peroxide (Iriti and Faoro 2009) and increase in cytosolic Ca^{2+} (Zuppini et al. 2003). Chitosan has been shown to exhibit elicitor activity and to induce both local and systemic resistance. Spraying and soil drench with chitosan suppressed late blight (*P. infestans*) and *Fusarium* wilt (*Fusarium oxysporum* f. sp. *lycopersici*) of tomato (Oh et al. 1998). It has been reported that chitosan also induced an increase in the activity of phenylalanine ammonia lyase (PAL), the formation of phenolic compounds and lignification, which may play a significant role in induction of resistance mechanisms (Loschke et al. 1983; Moerschbacher et al. 1990; Vander et al. 1998; Menden et al. 2007).

Chitosan as an antifungal agent is extremely successful. Soil amendment with chitosan has been shown to control fungal diseases in numerous crops, especially *Fusarium* wilts and grey mould (Rabea et al. 2003; Laflamme et al. 1998). It is also important to note that these studies show chitosan to be fungistatic against both biotrophic and necrotrophic pathogens. The control of oomycete pathogens has also been achieved with chitosan treatment, with *Phytophthora capsici* controlled on peppers (Xu et al. 2007) and *P. infestans* in potato (O'Herlihy et al. 2003).

Stimulation of antagonistic biocontrol agents is also an important activity of disease control performed by chitosan. Antagonistic microbes employ a number of methods to attack plant pests and pathogens. This includes, but is not limited to, the production of chitinases (Maksimov et al. 2011), the production of toxins (e.g. antibiotics and toxins), direct parasitism, competition for nutriment, and the induction of defence responses in the plant. Therefore, adding chitin-based products to the growing environment may aid beneficial antagonists by stimulating the production and activation of chitinases that can then be used to attack pests and pathogens or be used as a stable nitrogen-rich polysaccharide food source that boosts the population to the level where other mechanisms control the plant pathogens. El Mohammadi et al. (2014) studied the effect of individual or combined application of *Trichoderma harzianum* and chitosan against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) causing Fusarium crown and root rot (FCRR) under in vitro and in vivo conditions. They found that *T. harzianum* significantly reduced the mycelial growth of the five FORL tested isolates. Chitosan applied at different concentrations (from 0.5 to 4 g/l) also significantly decreased the mycelial growth of the pathogen, and a total inhibition was obtained at a concentration of 4 g/l. Under greenhouse conditions, application of *T. harzianum* and chitosan (1 g/l) as root dipping treatment combined with chitosan (0.5 g/l) as foliar spray has reduced FCRR incidence and severity by 66.6 and 47.6%, respectively. Treatments based on *T. harzianum* alone

or in combination with chitosan led to an increase in the total phenols and to an enhancement of chitinase and β-1,3-glucanase activities in leaves of treated tomato plants compared with the untreated ones.

Yu et al. (2012) evaluated the antifungal activity of chitosan in combination with the biocontrol yeast *Candida laurentii* against the *Penicillium expansum* of pear. The results showed that the most effective concentration of chitosan able to enhance blue mould control was 5 g/l when combined with *C. laurentii*. Infections of fruit by fungal pathogens often occur in the field, prior to harvest; preharvest treatment with BCAs would therefore be advantageous in order to reduce initial infection and to suppress pathogens in storage (Tiexido et al. 1998). However, the combination of preharvest treatment with *C. laurentii* and chitosan coating of table grapes enhanced the control of fruit decay to a greater extent than the preharvest treatment alone (Meng et al. 2010). The bacterium *Bacillus subtilis* is a pathogen of fungi and is one of the most widely used biopesticides in agriculture. Chitosan addition also improved the action of *B. subtilis* against powdery mildew in strawberry (Lowe et al. 2012). The beneficial effect of chitin-based treatments to antagonistic bacteria is not restricted to *B. subtilis*, with both chitin and chitosan improving the control of *Fusarium* wilt in both tomato and cucumber (Singh et al. 1999) when applied to the soil with a range of different species of chitinolytic microbes. The use of chitin/chitosan to encapsulate microbes also assists with the practicalities of storing and applying microbes on farms and nurseries, which has been one of the major restrictions to the use of biopesticides in recent times (John et al. 2011).

Chitosan oligosaccharide (COS), a deacetylated and depolymerized derivative of chitin, has higher antimicrobial properties than chitosan and is presumed to act by disrupting/permeabilizing the cell membranes of bacteria, yeast and fungi (Jaime et al. 2012). The gene targets of COS identified in this study indicate that COS's mechanism of action is different from other commonly studied fungicides that target membranes, suggesting that COS may be an effective fungicide for drug-resistant fungal pathogens.

Chitosan synergy with other biocontrol agent is well known for plant disease management. Rahman et al. (2014) compared the antifungal activity of chitosan with DPn (average degree of polymerization) and FA (fraction of acetylation) and of enzymatically produced chito-oligosaccharides (CHOS) of different DPn alone and in combination with commercially available synthetic fungicides, Teldor (fenhexamid), Switch (cyprodinil + fludioxonil), Amistar (azoxystrobin), Signum (pyraclostrobin) and Delan (dithianon), against *Botrytis cinerea*, the causative agent of grey mould in numerous fruit and vegetable crops. CHOS with DPn in the range of 15–40 had the greatest anti-fungal activity. The combination of CHOS and low dosages of synthetic fungicides showed synergistic effects on antifungal activity in both in vitro and in vivo assays. Their study shows that degree of polymerization (DP) is an important factor on the antifungal activity and CHOS enhance the activity of commercially available fungicides. According to them, the mechanisms for the synergism in inhibition of fungal growth are not known, but most likely the synergism is due to the compounds' different modes of action. Therefore, synergistic effect of chitosan with BCAs and chemicals could be utilized for development of

safe and effective strategy for management of late blight of potato caused by *P. infestans*. To this end, two-fold and three-fold combinations of copper, chitosan and *Trichoderma* were designed and tested for late blight inhibition.

13.5 Combination of Copper/Chitosan and Copper/*Trichoderma* Applications Provide Protection Against Late Blight of Potato

Potato late blight, caused by *P. infestans*, can be managed with multiple applications of commercially available synthetic chemical fungicides, even in favourable environmental conditions. An extent of late blight suppression has been realized due to the development of new potato varieties, tolerant for disease and through cultural practices such as planting disease-free seed, volunteer control, cull pile and irrigation management (Dorn et al. 2007a, b; Nowicki et al. 2012). With the uncertainties that accompany epidemics, disease management measures that directly kill the pathogen are often essential. The organic grower, however, is restricted to cultural controls, the use of some copper-containing fungicides or other methods that utilize natural ingredients (Dorn et al. 2007a, b). Therefore, several alternative strategies including dual combination of copper and chitosan or copper and *Trichoderma* have been reported for the management of late blight of potato (Medeiros et al. 2010).

Hadwiger (2008) described a strategy for late blight management using lower levels of copper sulphate pentahydrate in combination with a chitosan sticker and complexing agent in the excised leaf greenhouse experiments. In exciced leaf assays, processed copper sulphate pentahydrate (CT-100) alone, chitosan alone and CT-100 + chitosan combination were compared with the two commercial fungicides, chlorothalonil (Bravo) and copper hydroxide (Kocide 2000). It revealed that combination of CT-100 + chitosan provided moderate control of late blight and protection agaianst copper-related leaf yellowing at approximately 40-fold lower copper levels than those recommended for a commercial fungicide.

In large-scale investigations, the use of BCAs often has to be combined with low doses of chemicals to attain a level of disease control equivalent to synthetic fungicides for several diseases (Droby et al. 2003). However, the existing literature reveals few references to the use of copper and *Trichoderma* dual combination for management of late blight disease caused by *P. infestans*.

The combination of *Trichoderma asperellum* and a chemical agent, copper, is intrinsically difficult due to the antimicrobial activities of the latter. Erayya et al. (2020) developed a dual combination product involving a lower dose of chemicals (copper) and biocontrol agent (*Trichoderma*) in which the preferred *Trichoderma* strain was copper tolerant. *Trichoderma asperellum* accumulated copper in/on the surface of cell wall as a mechanism of metal tolerance. *Trichoderma* sp. promises great potential as a natural copper removal agent as it is naturally tolerant to high concentrations of copper. The developed combination product was found effective in potato late blight disease management as obtained with higher doses of copper fungicides.

The literature suggests that dual combination of copper + chitosan and copper + *Trichoderma* was effective against reducing disease severity but lacks consistent field performance. Data is though available for commercial and experimental dual combination preparations for management of late blight disease. Nevertheless, innovative and superior control measures could be developed by designing a new generation of plant protectants, like combination products involving plant defence inducers along with threshold level of copper and BCAs.

13.6 Combination Product of Biopesticides as Alternative or Copper Reducing Strategy for Late Blight (*Phytophthora infestans*) Management

Due to environmental and health concerns, there is immediate interest in finding alternatives to chemical pesticides for management of plant pathogens that are not only safe but also highly efficacious with the objective of reducing the pesticide load below safe limits. One alternative approach could be strategic designing of a novel formulation involving lower doses of fungicide, plant strengtheners and biological plant protectant to manage this destructive disease. Such a combination could be an effective, low-cost, environment-friendly and consumer-safe plant disease management strategy for *P. infestans*. Very high multiplication rate of *P. infestans*, rapid establishment of infection and explosive disease development pose major barrier for its management with BCAs, and plant defence inducers, if applied alone. Therefore, the challenge is to design a copper-chitosan-*Trichoderma* combination for efficient and safe management of oomycete pathogen, *P. infestans*, and propose a process of eco-friendly formulation.

Accordingly, a triple combination natural product involving low dose (safe limit) of a fungicide, a biocontrol agent and a plant defence activator compound was conceptualized and developed. The first component of the combination includes copper hydroxide (CuOH), an effective and well-known fungicide against *P. infestans*. The second component includes copper-tolerant strain of *Trichoderma asperellum* with cellulase, β-1,3-glucanase and chitosanase activities. Thus, the copper-tolerant strain of *T. asperellum* theoretically has capability of decomposing chitosan into its oligomers to enhance its efficacy in combination. The third component, chitosan, a biopolymer and plant strengthener, is a natural substrate that is compatible for growth and sporulation of *Trichoderma* and/or stimulates the production of chitosanase. The specific concentration of all the three components in the combination product was as effective as double the dose of standard recommended fungicides to minimize the growth of *P. infestans* (Erayya 2014).

Copper fungicides are protectant/preventative products. The copper product must be applied onto the plant surface prior to appearance of the disease. Copper fungicides inhibit fungal spore germination and mycelial growth, and control is provided by the free copper ions (Cu^{2+}) released from the applied copper.

Chitosan is a polysaccharide consisting predominantly of repeating units of D-glucosamine and obtained by deacetylation of chitin. It is ensured that the level

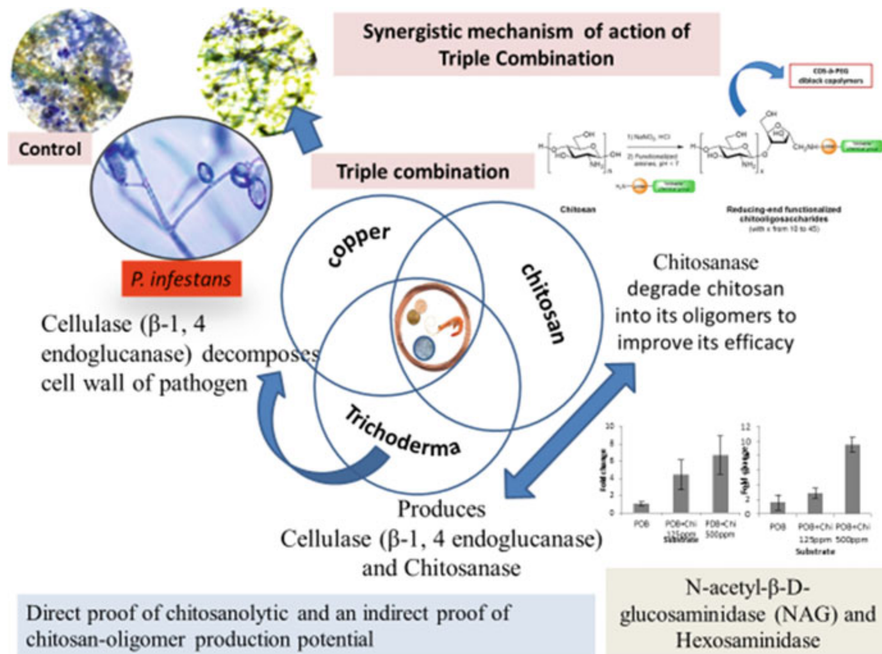

Fig. 13.1 Synergistic action of 'Cu-Chi-Tri' combination

of deacetylation is sufficient to render the chitosan water soluble at acidic pH or else liquid formulation of chitosan is utilized.

The BCA *Trichoderma asperellum* having copper tolerance and chitosanase activity could be applied as tuber treatment and/or foliar application. *Trichoderma asperellum* produced high concentration of chitosanase and cellulase. Chitosanase is an enzyme that selectively targets the chitosan in the 'Cu-Chi-Tri' combination and breaks chitosan into smaller units (oligomers) and enhances the efficacy of the triple combination in disease management, while cellulose is a fundamental component of fungal cell wall of oomycete pathogens (Mélida et al. 2013; Grenville-Briggs et al. 2008). Therefore, it could be inferred that *Trichoderma* plays a key role in the 'Cu-Chi-Tri' combination (Bhardwaj 2016). Once *T. asperellum* secretes chitosanase and cellulase, chitosanase utilizes/breaks chitosan into its oligomers and cellulase will degrade cellulose complex in its vicinity, which will thus be reduced to its simpler components, thereby lysing the cell wall and exposing its intracellular components to copper, leading to minimized growth and further spread of *P. infestans* (Bohra 2018). Based on the findings, a schematic diagram on the synergetic action of 'Cu-Chi-Tri' has been hypothesized (Fig. 13.1).

Efficacy of different copper compounds, namely, CuOH (technical grade), CuOH (Kocide) and copper sulphate pentahydrate ($CuSO_4.5H_2O$), in combination with chitosan and *T. asperellum* (*Tri*) to find out the most effective copper source in the combination for late blight disease management in potato has also been studied.

Among the three copper compounds, CuOH (TG) was found to be the most effective copper source in the combination (Erraya 2014; Rautella et al. 2018). Findings of Rautella et al. (2018) proved that the 'Cu-Chi-Tri' combination was similarly effective for management of late blight disease of tomato.

Chitosan/alginate microcapsules simultaneously loaded with copper cations and *Trichoderma viride* have also been developed (Marko et al. 2016). It has been reported that chitosan/alginate microcapsules could simultaneously incorporate *T. viride* spores and chemical bioactive agent without inhibiting their activities and can be suitable for plant nutrition and protection. Therefore, integration of induced resistance with the management of disease using chemical fungicides is a desirable component of integrated disease management.

13.7 Conclusion

Improved plant protection strategies are urgently needed that are less burdening to the environment and safer for the customer than existing chemical technologies, but that at the same time secure the same high quality and quantity of harvest known today. Biological treatments alone cannot fulfil these demands, but synergistic combinations of chemicals and biologics including biostimulants promise to allow significant reductions of chemical inputs. A synergistic combination of a biochemical biostimulant, further reinforced with a potential chemical plant protectant, justifies exploitation to promote growth, development and strengthening of plants to increase tolerance of abiotic and resistance against biotic stresses. Among the most widely used biostimulants are selected strains of the fungal genus *Trichoderma.* These biocontrol agents are difficult to combine with chemical plant protectants such as copper-based fungicides as these inhibit their growth. Previous attempts have shown that chitosan tolerance in *Trichoderma* can be increased, but not to the point that a combination with copper dosages required for effective plant protection would become possible. On the other hand, chitosan as a plant strengthener can lower the effective copper dosage, and *Trichoderma* are known to possess good chitosanolytic abilities so that they can be combined with potentially antimicrobial, plant strengthening chitosans. It has been shown that the synergistic combination of copper-chitosan-*Trichoderma* can give good plant protection against oomycete pathogen, *P. infestans*, at significantly lowered copper dosages. Thus, the 'Cu-Chi-Tri' combination promises sustainable, healthy and safe plant protection and necessitates development of a stable formulation product.

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14 Seaweed and Associated Products: Natural Biostimulant for Improvement of Plant Health

Jai Singh Patel and Arpan Mukherjee

Abstract

Seaweeds are macroalgae fit into the class Phyophyceae and best known as brown algae. They are mainly composed of polysaccharides such as laminarin, fucoidan, and alginates. Several products based on seaweeds are known to be useful for humans and plants. Extracts obtained from seaweeds contain several bioactive compounds. Such bioactive compounds induce resistance in plants against different biotic and abiotic stresses. Seaweed extracts can also contain countless plant-bioactive inorganic and organic compounds such as mannitol, polysaccharides, oligosaccharides, phytohormones (auxins, cytokinins, gibberellins, betaine), antioxidants, and vitamins. It also contains a low concentration of minerals (calcium, boron, zinc, potassium, phosphorus, magnesium, and several other trace elements). Seaweed extract can stimulate plant growth and enhance the rate of photosynthesis. Seaweed extracts boosted rates of seed germination, crop growth, yields, and shelf life of produce in post-harvest conditions. It can reduce the effect of diseases due to fungal, viral, and bacterial pathogens. The present chapter describes the impact of seaweed and their products in the agricultural system.

Keywords

Seaweed extract · Biostimulants · Plant defense · Plant growth promotion · Plant-microbe interactions

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14.1 Introduction

A biostimulant could be defined as any beneficial microorganism or any organic material having the ability to increase plant growth, enhance nutrient uptake, enhance abiotic and biotic stress tolerance, and increase crop yield. The knowledge about specific roles of biostimulants and the mechanism(s) behind its mode of action is the necessary requirement for industries based on the biostimulants. Several biostimulant products are available in the market. Based on their source, biostimulants were derived from seaweeds, bacteria, higher plants, fungi, humic acid, and several other industrially processed materials.

Phaeophycean seaweed, also known as brown seaweeds, are the biggest group having 2000 species. Their maximum biomass is available on the rocky shores of the temperate area of different countries. Brown seaweed-based products are mostly used (Blunden and Gordon 1986) in agriculture, and *Ascophyllum nodosum* (L.) Le Jolis is one of those that is thoroughly studied (Ugarte 2011). Several other brown seaweeds, including *Laminaria* spp., *Turbinaria* spp., *Fucus* spp., and *Sargassum* spp., were also known to be used as biofertilizer in agriculture (Hong et al. 2007). Plenty of reports are available regarding the role of seaweed-based biostimulants for crop protection and crop production in terms of elevated resistance against stresses, improved crop yield, early germination of the seeds, etc. (Beckett and van Staden 1989; Hankins and Hockey 1990; Norrie and Keathley 2005).

The use of brown seaweed-based products can enhance the cation exchange capacity of the soil. It can increase nutrient availability to the plants. It can be the source of nitrogen-based fertilizers as well as increase solubility of the nutrients available in soil. Many studies have shown the role of seaweed-based products for stimulation of root formation and growth (Pacholczak et al. 2016; Vernieri et al. 2006). Another study by Vernieri et al. (2006) showed enhanced root biomass of the plants grown in hydroponic system. Polysaccharide-rich extracts of seaweed have shown to have an enhancing effect on plant growth (Hernández-Herrera et al. 2016). Such activity of extracts suggests the role of oligosaccharides as signaling molecule for regulation of phytohormone-related genes in the treated plants. However, the polysaccharide-rich extracts that promoted root growth in mung bean plants also showed presence of synthetic hormones in the extract. A recent report suggested the role of *Ascophyllum nodosum* extract on the reduction of mycotoxin production in the *Fusarium* head blight infected wheat plants (Gunupuru et al. 2019).

14.2 Role of Seaweed as a Plant Growth Promoter

The emerging formulations based on the seaweed extract have the ability to improve plant growth and also enhance tolerance against abiotic stresses, including heat, drought, and salinity. Several plant metabolic pathways are targeted by the seaweed extracts to improve plant growth and tolerance against the abiotic stresses (Fig. 14.1). The use of seaweed in agriculture is a very ancient technology and still serving as organic fertilizers for the plants (Craigie 2011).

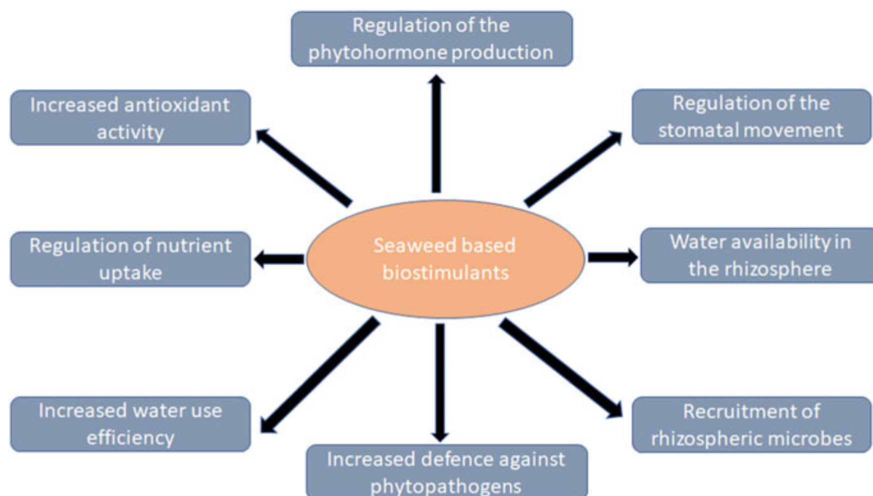

Fig. 14.1 Different physiological activities affected by the seaweed-based biostimulants

Presently more than 50 companies worldwide are producing seaweed extracts for promotion of plant growth, and these seaweed extracts are based on different seaweeds present in the sea. The products based on *Ascophyllum nodosum* getting the highest attention among them (Sharma et al. 2015). Several plant species have shown growth promotion under the application of the seaweed extract, but the mechanism behind such activity is not very well explored (Verkleij 1992; Battacharyya et al. 2015). The complex nature of the seaweed extracts makes their studies exceedingly challenging in determining the components of seaweeds that are responsible for such activities. The nature of commercial formulations available in the marketplace depends on the method of extraction used for isolation, such as water-based extractions, acid-based extraction, alkaline-based extraction, microwave-assisted extraction, ultrasound-assisted extraction, and enzyme-based extraction (Shukla et al. 2019) (Fig. 14.2).

Most of the commercial products are derived from red algae such as *Lithothamnium calcareum* as well as brown algae, including *Durvillaea potatorum* and *Ascophyllum nodosum* (Khan et al. 2009). Some of the products are listed in Table 14.1. Application of the seaweed extracts enhanced the seedling growth of lettuce (*Lactuca sativa* L.) (Moller and Smith 1998). The formulation based on *A. nodosum* has been reported to increase growth and accumulation of K^+ in almond plants (*Prunus dulcis*). The commercial products GroZyme and MegaFol showed a similar effect on foliar application and stimulated plant growth (Saa et al. 2015).

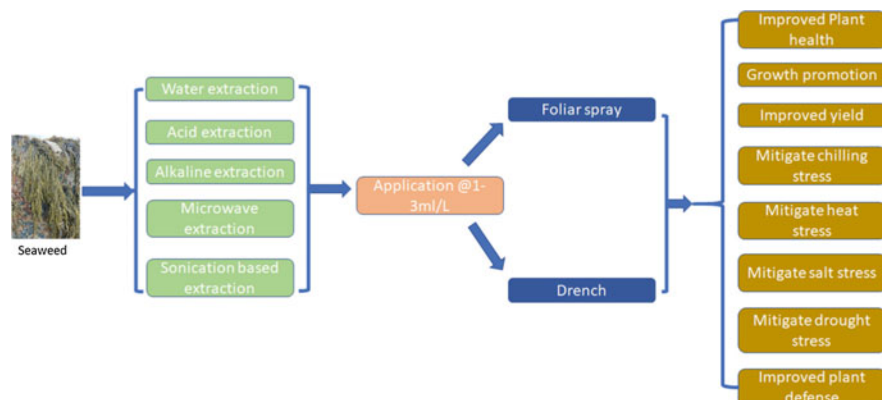

Fig. 14.2 Extraction processes, mode of application, and responses in the plants by seaweed-based biostimulants

14.3 Role of Seaweed Extract in Chilling Stress Mitigation

Production of several crops is going down due to the chilling stress. It can cause sterility in pollens and loss of grain setting in wheat (Chakrabarti et al. 2011). Seaweed extracts are also reported to develop tolerance in plants against the chilling stress. Several products have been tested for enhancement of cold tolerance in maize plants, and the extracts rich in Zn and Mn were able to enhance tolerance by increasing production of the reactive oxygen species (ROS) (Bradáčová et al. 2016). Nutrient deficiency stress via chilling stress can be overcome by the use of seaweed extract, which imposes its role in improvement of oxidative stress tolerance. The model plant *Arabidopsis thaliana* was also used for demonstration of the role of seaweed extract. Rayirath et al. (2009) suggested the role of *Ascophyllum nodosum* extract in *A. thaliana* against chilling stress. They used different organic fractions of the extract to search the component responsible for the activity. The authors have demonstrated that ethyl acetate fraction of seaweed extract was rich in the fatty acid content, which was responsible for increasing the tolerance against chilling stress. The treated plants with ethyl acetate fractions showed faster recovery of *A. thaliana* plants after chilling shock as well as increased expression of some key genes, including CBF3, COR15A, and RD29A. The extracts are responsible for the increment in total soluble sugar and proline content, which are critical compounds for the chilling stress (Nair et al. 2012). Not only chilling stress, seaweed extracts were also reported to mitigate heat stress (Zhang and Ervin 2008).

Table 14.1 Seaweed-based biostimulants and their functions

S. N.	Nature of processed product	Alga used	Function	References
1.	Extract	*Sargassum polycystum*	Provide resistance to leaf fall disease	Khompatara et al. (2019)
2.	Sulphated polysaccharide	*Acanthophora spicifera*	Defense responses against *Phytophthora palmivora*	Pettongkhao et al. (2019)
3.	A sulfated polysaccharide	*Sargassum vulgare*	Anticoagulant, antithrombotic, antioxidant, and anti-inflammatory effect	Dore et al. (2013)
4.	Extract	*Sargassum fusiforme*	Induced resistance in *Solanum lycopersicum*	Sbaihat et al. (2015)
5.	Lipophilic components	*Ascophyllum nodosum*	Enhance freezing tolerance in *Arabidopsis thaliana*	Rayirath et al. (2009)
6.	Extract	*Ascophyllum nodosum*	Enhance freezing tolerance in *Arabidopsis thaliana*	Nair et al. (2012)
7.	Extract	*Ascophyllum nodosum*	Drought tolerance in spinach	Xu and Leskovar (2015)
8.	Seaweed suspensions	*Ascophyllum nodosum and Laminaria hyperborea*	Enhances seedling growth in lettuce	Möller and Smith, (1998)
9.	Extract	*Sargassum muticum and Jania rubens*	Mitigate salinity stress in chickpea	Latef et al. (2017)
10.	Extract	*Ascophyllum nodosum*	Increases growth and yield of tomato	Ali et al. (2016)
11.	Extract	*Ulva intestinalis*	Regulation of hormone production in Arabidopsis	Ghaderiardakani et al. (2019)
12.	Extract	*Ascophyllum nodosum*	Clementine mandarin and Navelina orange	Fornes et al. (2002)
13.	Seaweed concentrate	*Ecklonia maxima*	Hormone regulation in tomato	Finnie and Van Staden (1985)
14.	Extract	*Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora*, and *Sargassum liebmannii*	Seed germination in tomato plant	Hernández-Herrera et al. (2014)
15.	Extract	*Gracilaria textorii* and *Hypnea musciformis*	Seed germination of some vegetable crops	Rao and Chatterjee (2014)

(continued)

Table 14.1 (continued)

S. N.	Nature of processed product	Alga used	Function	References
16.	Extract	*Sargassum myriocystum*	Stimulant of seedling growth of *Vigna mungo*	Kalaivanan and Venkatesalu (2012)
17.	Extract	*Sargassum wightii*	Increases growth of green gram	Kumar et al. (2012)
18.	Extract	*Ulva reticulata*	Growth of *Vigna mungo*	Selvam and Sivakumar (2013)
19.	Extract	*SUNRED*	Positive effect on the pigment characteristics of grape crop	Deng et al. (2019)
20.	Nano-size fertilizer	*Ascophyllum nodosum*	Vine growth, yield, berry quality of grapes	Sabir et al. (2014)
21.	Biochar of seaweed	*Saccharina, Undaria,* and *Sargassum*	Soil amelioration	Roberts et al. (2015)
22.	Extract	*Kelp*	Growth and development of *Brassica chinensis*	Zheng et al. (2016)
23.	Extract	*Gracilaria edulis* and *Sargassum wightii*	Plant growth of tomato	Vinoth et al. (2012)
24.	Extract	*Ascophyllum nodosum*	Growth of spinach	Fan et al. (2013)
25.	Extract	*Ascophyllum nodosum*	Growth promotion *Brassica napus*	Jannin et al. (2013)
26.	Extract	*Sargassum wightii* and *Caulerpa chemnitzia*	Growth of *Vigna sinensis*	Sivasankari et al. (2006)

14.4 Role of Seaweed Extract in Salinity Stress Mitigation

Salt stress is one of the significant threats to crops. Reports have shown that more than 20% of the croplands and around 50% of the irrigated lands are affected by salt stress, causing loss of production below the genetic capability of the crop plants (Ren et al. 2005). Algal extracts are also known to mitigate salinity stress, such as in turfgrass (Nabati et al. 1994). Several studies showed the role of seaweed extract for improvement of salinity tolerance in tomato, avocado, *Arabidopsis*, and passion fruit (Jithesh et al. 2019; Bonomelli et al. 2018; Jolinda et al. 2018; Di Stasio et al. 2018). Two different commercial extracts FiftyR and RygexR based on *A. nodosum* stimulated accumulation of antioxidants, essential amino acids, and minerals in tomato plants under salt stress conditions (Di Stasio et al. 2018). Application of extracts obtained from *A. nodosum* mitigates the salinity stress in avocado via

improvement in nutrient uptake and plant growth. There was a higher accumulation of Ca^{2+} and K^{+} in the seaweed extract-treated avocado plants compared to the control (Bonomelli et al. 2018). Seaweed extract based on *A. nodosum* was also found to improve growth of turfgrass under salt stress conditions (Elansary et al. 2017).

The organic fraction of *A. nodosum* extract especially the ethyl acetate extract induces salt stress tolerance in *Arabidopsis* plants. Global transcriptomics of the ethyl acetate fraction-treated plants revealed the pathways induced by the ethyl acetate fraction (Jithesh et al. 2019). Several genes related to salt stress were found to be influenced by two different seaweed extracts (Goñi et al. 2016). The mechanism includes decrease in water loss from cellular structures, accumulation of various ions, and protection of proteins from denaturation due to seaweed application (Wise and Tunnacliffe 2004; Goyal et al. 2005). Several genes related to flavonoid synthesis were shown to be induced by application of seaweed extract, which protects the cells from ROS-mediated oxidative damage under salt stress (Jithesh et al. 2019). Not only the regulatory genes but also expression of amino acids, carbohydrate synthesis, and sugar alcohol synthesis-related genes were also increased under the control of seaweed extracts (Elansary et al. 2017; Jithesh et al. 2019).

Accumulation of the stress amino acid proline can alleviate salt stress in different crop plants. The major mechanisms include enhancement of antioxidant activity and stabilization of intracellular organelles (Ashraf and Harris 2004; Ashraf and Foolad 2007). A recent study by Elansary et al. (2017) has shown enhanced synthesis of structural carbohydrate after application of seaweed extract on turfgrass. Research has also shown enhanced expression of genes related to sugar transport under the effect of seaweed extract in turfgrass.

14.5 Role of Seaweed Extract in Mitigation of Drought Stress

Drought is one of the major abiotic stress-causing losses of crop production. More than 40% of yield loss was observed in maize as well as more than 21% in the wheat crop due to 40% reduction in water availability (Daryanto et al. 2016). A yield loss ranging between 34 and 68% in cowpea was recorded due to drought stress (Farooq et al. 2017). The extract obtained from the *A. nodosum* has been reported to enhance drought tolerance in ornamental plants, including *Pittosporum eugenioides* and *Spiraea nipponica*. The major compounds, such as proline, phenolic compounds, and flavonoids, were increased after application of seaweed extract under drought stress (Elansary et al. 2016). Application of *A. nodosum*-based seaweed extract also enhances the fresh and dry weight in the leafy vegetable spinach under drought stress (Xu and Leskovar 2015). Isopropanol fraction of the seaweed extract was reported to increase the water potential and conductance of stomata in grape plants (*Vitis vinifera* L) and the K^{+} and Ca^{2+} fluxes under drought stress (Mancuso et al. 2006). Ionic and osmotic stresses can be overcome by the accumulation of K^{+}. Application of commercial extract of *A. nodosum* increases the water use efficiency in the orange

Table 14.2 Some seaweed biostimulant-based products and their producing companies

S. N.	Name of the product	Name of the seaweed used	Name of the company
1.	Aquasap (seaweed extract powder)	*Kappaphycus alvarezii*	SeaNutri LLC
2.	Kelpak (liquid seaweed concentrate)	*Ecklonia maxima*	Kelp Products International
3.	Asco-root (granular supplement with controlled release)	*Ascophyllum nodosum*	Organic Ocean Inc.
4.	Stimulagro (concentrated liquid extract of cold seaweed)	*Ascophyllum nodosum*	Organic Ocean Inc.
5.	Tonic (seaweed-based liquid fertilizer)	*Ascophyllum nodosum*	Organic Ocean Inc.
6.	Ecklomar (liquid extract)	*Ecklonia maximum*	Plymag
7.	AlgaFlex (liquid concentrated seaweed extract)	*Ascophyllum nodosum*	Biotechnica Services Ltd.
8.	Maxicrop liquid seaweed (liquid seaweed extract)	*Ascophyllum nodosum*	Maxicrop

tree *Citrus sinensis* under drought stress (Spann and Little 2011). The role of seaweed extract to increase water use efficiency under the drought stress could be a beneficial application for the drought stress-prone areas for cultivation of fruit trees (Table 14.2).

14.6 Role of Seaweed Extract in Plant Defense

Random change in the climatic conditions and unscientific way of agricultural practices have weakened the defense system and induced diseases in plants and thereby showed a negative effect directly on agricultural crops (Anderson et al. 2004; Ayliffe and Lagudah 2004). Infectious diseases in plants are caused by some biological agents including the family of bacteria, fungi, or viruses (Pieterse and Dicke 2007; Stadnik and Freitas 2014), and infectious diseases directly harm the plant health and crop productivity. To prevent pathogenic infection, plants have evolved inducible defense processes (Conrath et al. 2002; Wiesel et al. 2014), and it can be stimulated by any potential stimulants including seaweed extracts.

Till now, two types of disease defense mechanisms have been reported by scientists, viz., induced systemic resistance (ISR) and systemic acquired resistance (SAR). To protect plants from a wide range of pathogens, in induced systemic resistance (ISR), the defense responses are mediated through jasmonate (JA) and ethylene (ET), whereas salicylic acid (SA) is important to PR (pathogenesis-related) gene activation for systemic acquired resistance (SAR) mechanisms (Gaffney et al. 1993; van Loon et al. 1998). In plants, the elicitor molecules from pathogens are responsible for inducing the defense system (Conrath et al. 2002; Wiesel et al. 2014). Not only chitin, lipopolysaccharides, and flagella of microbes but also some

chemically synthesized components like 2,6-dichloro-isonicotinic acid, b-aminobutyric acid, chitosan, benzothiadiazole, and methyl jasmonate have the ability to induce plant defence mechanisms (SAR/ISR) by working as an elicitor, against a wide range of pathogens (Dixon 2001; Mercier et al. 2001; Bektas and Eulgem 2015; Iriti and Varoni 2015).

Over time, different seaweeds have evolved and equipped themselves with important defense mechanisms to protect themselves from their own pathogens (Potin et al. 1999; Shukla et al. 2016). Because of the presence of some important bioactive components such as carrageenans, fucans, ulvans, and fucose containing polymers (or laminarins) in the seaweeds, they showed resistance against a wide range of pathogens (Klarzynski et al. 2003; Sangha et al. 2010; Vera et al. 2011). These bioactive components of seaweeds work as elicitor molecules and play a role in inducing defense mechanisms against pathogens (Khan et al. 2009; Sharma et al. 2014; Shukla et al. 2016). These elicitors can act as pathogen-associated molecular patterns (PAMPs) (Sharma et al. 2014). PAMPs bind to the host pattern recognition receptors (PRRs), which are transmembrane proteins, and protect the plants, through induction of defense mechanisms ISR and SAR via mediation of a systemic signal (Eckardt 2008; Zipfel 2009). The primed plants showed higher defense response than the non-primed plants during pathogen infections.

Bioactive components in *A. nodosum* extract (ANE) induce defense responses against different pathogens (Patier et al. 1995; Sharma et al. 2014). The report showed that commercial extract of *A. nodosum,* namely, Marmarine (IFTCTM, Amman, Jordan), induces defense in cucumber plants against *Phytophthora melonis* (Abkhoo and Sabbagh 2016). Application of the seaweed extract led to induce activation of some important disease resistance enzymes like polyphenol oxidase (PPO), peroxidase (PO), phenylalanine ammonia-lyase (PAL), lipoxygenase, and, β 1,3-glucanase. This report suggested the role of seaweed extracts in inducing different enzymes and genes that increase the resistance power in cucumber (Abkhoo and Sabbagh 2016). Another report by Panjehkeh and Abkhoo (2016) showed that application of *A. nodosum* extract known as Dalgin was able to induce resistance (ISR) against *Phytophthora capsici*, a potential disease-causing agent of tomato. Another application of *A. nodosum* extract StimplexR with chlorothalonil (fungicide) showed decrease in the disease-causing ability by fungal pathogens in cucumber plants by inducing some of the defense genes and enzymes (Jayaraman et al. 2011). Stella MarisR, another *A. nodosum*-derived product, was reported to induce plant immunity by boosting the concentration of ROS through the synthesis of hydrogen peroxide. Cook et al. (2018) reported that the immunity response gene *WRKY30* (early phase), *CYP71A12* (mid phage), and *PR-1* (late phage) were upregulated by the application of bioactive components. *A. thaliana* plants treated with 1 g/L of ANE showed resistance against necrotic fungal pathogen *Sclerotinia sclerotiorum* (Subramanian et al. 2011). The report showed that fungal pathogens *A. radicina* and *B. cinerea* did not show any significant disease progression in carrot plants when ANE extract was sprayed (Jayaraj et al. 2008). It was noticed that the ANE-primed carrot plants induced defense-related enzymes, including PO, PPO, PAL, β 1,3-glucanase, and chitinase, and also increased the transcript accumulation

of the PR-1, PR-5, NPR-1, LTP, chalcone syntheses, and PAL. Mukherjee and Patel (2019) reported that application of seaweeds on agriculture crops enhances plant growth with respect to seedling, root, and shoot development, improves nutrient uptake ability and fruit setting, boosts immunity against biotic and abiotic stresses, and also enhances soil fertility and health.

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15 Secondary Metabolites from Microbes for Plant Disease Management

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Abstract

Microorganisms inhabiting soil/plant systems produce antimicrobial compounds including secondary metabolites (SMs). They are low-molecular-weight structurally diverse and complex compounds that are not essential in their life unless they meet undesirable conditions. SMs such as antibiotics, toxins, ribosomal peptides (RPs), low-molecular-weight volatile organic compounds (VOCs), polyketides (PKs), non-ribosomal peptides (NRPs) and hybrids between PKs and NRPs have shown a diverse performance in antagonistic activity against plant pathogens. These compounds are involved immensely with combatting pathogens following interactions with pathogens inhabiting in soil/plant systems, particularly by developing a disease-suppressive soil. SMs produced by certain microorganisms induce plant defence reactions leading to a systemic resistance to pathogen infection. Bacterial endophytes, particularly inhabiting inside plant tissues, are also source of SMs, which may act as elicitors of plant defences. Biochemical techniques and genomic-based studies have uncovered that several genes encoding SMs associated with biocontrol activity are located in gene clusters. Despite the fact that metabolites have been developed as biopesticides, bio-weedicides, the in-cooperation of new techniques is important for finding new resources from disease suppressive soil, which do not represent when the cultures are tested in vitro. However, this review finds isolation of active compounds is one of the major challenges in biotechnology as many biosynthetic genes are not expressed under standard culture conditions, thus proposing a possible solution to overcome the issue.

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Keywords

Plant disease · Plant pathogens · Biocontrol agents · Secondary metabolites · Disease suppressive soil · Co-cultivation of microbes

15.1 Introduction

Plant diseases caused by pathogenic microorganisms such as bacteria, fungi and virus can be controlled so as to protect plants from being infected with the help of certain microorganisms inhabiting rhizosphere, endosphere and phyllosphere (Mathivanan et al. 2008; Ryu 2013). These microorganisms are different species from bacteria (Caulier et al. 2019), fungi (Gawai 2018) and actinomycetes (Chen et al. 2018b; Olanrewaju and Babalola 2019) and are considered as biological control agents. They owe to several mechanisms involving hyperparasitism, competition, production of cell wall degrading enzymes and induced resistance (Khokhar et al. 2012). The production of antimicrobial secondary metabolites (SMs) and other factors such as siderophores and microbial cyanide and lytic enzymes also play an important role in plant protection against pathogens (Keswani et al. 2020; Andrić et al. 2020; Cipollone et al. 2008). The involvement of SMs produced by microorganisms inhabiting soil/plant systems as to this phenomenal process has been reported (Raaijmakers et al. 2002).

SMs of microorganisms are low-molecular-weight, structurally diverse and complex compounds that are not essential in their life unless they meet undesirable conditions. It has been reported that soil/plant-associated bacteria, fungi and actinomycetes produced a plethora of antimicrobial SMs contributing plant protection. For instance, fungal species that have shown antagonism against plant pathogens produce an array of SMs such as antibiotics and toxins (Mathivanan et al. 2008; Pusztahelyi et al. 2015). *Bacillus* spp. have also been reported to produce a range of bioactive metabolites such as ribosomal peptides (RPs), volatile compounds, polyketides (PKs), non-ribosomal peptides (NRPs) and hybrids between PKs and NRPs (Caulier et al. 2019). Actinomycetes that represent high proportion of the soil microbial biomass have the capacity to produce an array of SMs with the function of antibacterial, antifungal, antibiotic, antiparasitic, insecticide and herbicide (Aggarwal et al. 2016).

Microorganisms producing SMs have been shown to be able to use as biocontrol agents because of the antimicrobial effects those bioactive compounds hold (Mathivanan et al. 2008; Sansinenea et al. 2016). There is a growing importance of performing genomic analysis, which enables researchers to identify biosynthetic gene clusters encoding SMs that are associated with biocontrol activity (Chowdhury et al. 2015). The expression of these genes in liquid cultures and the ability of harvesting secondary metabolites in sufficient quantities are imperative when it comes to their application in disease management in agriculture. Therefore, it is important to discover new methods of finding how to improve the yield of SMs in liquid cultures.

The aim of this book chapter is to summarize current knowledge about SMs produced by microorganisms and their application in plant disease management. In addition, we highlight setbacks, when it comes to the expression of SMs in liquid cultures that limit the yield, and possibilities of enhancing the expression of genes in a way to get the sufficient quantity.

15.2 Secondary Metabolites from Microbes and Plant Disease Suppression

Microorganisms are a gifted source of a spectrum of natural products, which immensely contribute to the existence of all life forms and the planet. Natural compounds of microorganisms include primary metabolites such as amino acids, enzymes, vitamins, organic acids and alcohol, which are useful for human and planetary health, agriculture, forestry, etc. They are used as nutritional supplements as well as in the production of industrial commodities through biotransformation. Whereas microorganisms produce some other compounds such as pigments, alkaloids, toxins, antibiotics, gibberellins, carotenoids, etc. with no exact function for their life and derived from the primary metabolism, which are known as SMs (Malik 1980).

The production of SMs is not widespread among microbes from several genera, and the formation is generally repressed during logarithmic growth and is derepressed during the stationary growth phases (Malik 1980). These bioactive compounds have been studied widely due to the ecological significance, the environmental health and agricultural purposes (Behie et al. 2017; Gloer 2007) and for the purpose of enhancing human and animal health (Singh et al. 2017b). Plant-associated microorganisms, especially endophytic and rhizosphere bacteria, actinomycetes and fungi, have been shown to produce a series of bioactive small molecule/natural products (Gunatilaka 2006; Singh et al. 2017a) and produce SMs for interacting with other soil microorganisms to suppress the growth of disease-causing micrograms (Meisner and de Boer 2018) subsequently develop the disease suppressive soil in ecosystems.

A range of SMs produced by plant-associated microorganisms have shown to be involved immensely with eradications of pathogens. The soil application of plant probiotic *Bacillus* spp. has resulted in early colonization on root system and elimination of the potential of pathogen colonization and infection triggering the induction of a range of beneficial SMs and disease resistance (Rahman et al. 2018). In some cases, low-molecular-weight volatile organic compounds (VOCs) among the range of microbial SMs have been uncovered (Bailly and Weisskopf 2017; Giorgio et al. 2015; Hua et al. 2014; Kanchiswamy et al. 2015). *Streptomyces* spp. have showed to produce compounds including morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5.alpha. 6.alpha)-($C_{23}H_{35}NO_3Si_2$), cyclononasiloxane, octadecamethyl-($C_{18}H_{54}O_9Si_9$) and benzoic acid, 2,5-bis (trimethylsiloxy) ($C_{16}H_{30}O_4Si_3$) with good antifungal activity against *Rhizoctonia solani* (Ahsan et al. 2017).

15.2.1 Fungal Secondary Metabolites

Fungi are found to be the most prolific producer of SM including non-ribosomal peptides (NRPs), peptaibols, polyketides, pyrones, siderophores and volatile and non-volatile terpenes (Mukherjee et al. 2012; Crutcher et al. 2013) as they are the fundamental element to the health and prosperity of every terrestrial ecosystem (Bills and Gloer 2016). Despite the fact that fungal SMs promote vitality and plant growth and enhance the resilience against abiotic stress factors, they are known to trigger plant-induced systemic resistance (ISR) for the protection of plants from pathogen infections (Mathivanan et al. 2008; O'Brien 2017). A record number of microparasitic fungal species were reported to produce a diverse range of SMs with biological activity. Hundreds of SMs produced by beneficial fungi have been isolated and characterized, especially from biocontrol strains of the genus, *Trichoderma*.

Trichoderma spp., the common rhizosphere inhabitants, have been widely studied due to their capacity to parasitize other fungi (mycoparasitism) and to compete with deleterious microorganisms in the soil/plant system. The production of SMs in *Trichoderma* spp., as tested in in vitro conditions, is strain dependent and varies on i) the compound considered, ii) the phytopathogen used for elicitation, iii) the viability of elicitors and iv) the balance between elicited biosynthesis and biotransformation rate, and also the biocontrol agent seems to modulate the production according to the presence or the absence of the target pathogen (Vinale et al. 2009). However, it has been reported that their culture filtrates comprise SMs with biocontrol potential against severe pathogens, when the culture filtrates were examined. 6-Pentyl-α-pyrone (6PP) has been found as the major compound that can protect pruning wounds of grapevines from trunk disease pathogens (Mutawila et al. 2016).

The analysis of culture filtrates of fungal strains is known to be effective in identifying and characterizing SMs produced against severe plant pathogens. For instance, the culture filtrate of *Clitocybe nuda* strain LA82 when tested against *Phytophthora* blight of pepper caused by *Phytophthora capsici* and the leaf spot on pepper caused by *Xanthomonas axonopodis* pv. *vesicatoria* has resulted in an inhibitory substance. This substance was stable at low and high pH and high temperature, has a molecular weight between 1000 and 500 and negatively charged and is a hydrophilic compound, but not a protein (Chen and Huang 2009). From a similar kind of study, a variety of antimicrobial SMs, including polyketides and alkanes, has also been found from a new *Trichoderma asperellum* strain, GDFS1009 (Wu et al. 2017), enabling researchers to pursue investigation into the genetic analysis of SMs and their widespread application in plant disease management.

As shown by genomic analysis, genes responsible for the biosynthesis of SMs that have antagonistic activity against microbial plant pathogens are often clustered in the genome (Fanelli et al. 2018). Ascomycetes have more genes in SMs coding gene clusters than basidiomycetes, archeo-ascomycetes and chytridiomycetes, whereas hemi-ascomycetes and zygomycetes have none (Pusztahelyi et al. 2015). Ascomycete genomes code for an average 16 polyketide synthases (PKS), ten

non-ribosomal protein synthases (NRPS), two tryptophan synthetases (TS) and two dimethylallyl tryptophan synthetases (DMATS) with crucial importance in SM synthesis (Pusztahelyi et al. 2016). Despite the fact that NRPS is vital for the synthesization of siderophores facilitating virulence in several fungi such as *Cochliobolus heterostrophus, C. miyabeanus, F. graminearum* and *A. brassicicola* (Oide et al. 2006), other types of SM biosynthetic gene clusters (BGC) including destruxin, NG39x and ferricrocin, together with putative helvolic acid and pseurotin and tropolone/citrinin-related compound clusters, have been reported to be involved with the suppression of plant pathogens (Sbaraini et al. 2016).

15.2.2 Bacterial Secondary Metabolites

The common knowledge is that a range of bacterial species such as *Pseudomonas* and *Bacillus* have been used as biological control agents in agriculture due to the production of an array of antimicrobial SMs with antagonistic potential against disease causing microbial pathogens. It has been reported that soil bacterium *Pseudomonas fluorescens* BBc6R8 produced three siderophores such as enantio-pyochelin, pyoverdine and biosurfactant viscosin, which are mainly responsible for the antagonistic activity of the bacterium under iron-limited conditions apart from stimulation of the growth of ectomycorrhizal fungus *Laccaria bicolor* S238N that kills fungal pathogen (Palin et al. 2016). Seventy-one (71) strains of *Bacillus* spp. isolated from different Mexican sites have shown antagonism against several phytopathogenic fungi, *Fusarium oxysporum*, *Fusarium equiseti*, *Fusarium avenaceum*, *Bipolaris* spp. and *Alternaria* spp., and a range of SMs leading to the antagonism, which trigger morphological changes on reproductive structures of pathogenic microbes (Sansinenea et al. 2016). SMs such as bacillomycin D can cause severe injury to both cell wall and cell membrane of fungal spores and hypha as observed in the killing of *Aspergillus flavus* (Gong et al. 2014). In addition, they have the ability to produce compounds that belong to multiple classes of antibiotics, which is why a large number of *Bacillus* strains have been developed for an effective control of a broad range of plant diseases (Shafi et al. 2017).

In terms of the function, bacterial SMs seemed to be volatile apart from being soluble (Tyc et al. 2017). VOCs produced by a range of bacterial species including many species of *Pseudomonas* and *Bacillus* have a significant potential of enhancing antagonistic activity against disease-causing microbial pathogens (Rajer et al. 2017; Raza et al. 2016; Tahir et al. 2017; Xie et al. 2018). However, their production seems to be dependent on the inoculation strategy and the size of the inoculum. For instance, *Bacillus* spp. produce four VOCs such as benzaldehyde, nonanal, benzothiazole and acetophenone, when tested against *Clavibacter michiganensis* ssp. *sepedonicus*, the causative agent of bacterial ring rot (Rajer et al. 2017), and benzaldehyde, 1,2-benzisothiazol-3(2 H)-one and 1,3-butadiene with a robust antagonistic activity, when tested against *Ralstonia solanacearum*, the causal organism of bacterial wilt disease (Tahir et al. 2017). *Pseudomonas* spp. also produce volatilome with a robust chemical resources that could help plant for efficient control of

pathogens. Besides HCN, NH_3 and H_2S, a blend of other potential VOCs majoring 1-undecene and dimethyl disulphide (DMDS) has also been found, when *Pseudomonas* spp. tested against *Phytophthora infestans* (Bailly and Weisskopf 2017).

Some BVCs, such as DMDS and 2-methylpentanoate, are highly toxic to plant pathogens (Ossowicki et al. 2017; Raza et al. 2016; Sharifi and Ryu 2018), and mechanism of disease suppression primarily seems to be ISR (Sharifi and Ryu 2018). VOCs alter the transcriptional expression levels of genes involved in induced systemic resistance (ISR) in plants, leading to an inhibition of disease suppression. For instance, acetoin, 2,3-butanediol and tridecane from *Paenibacillus polymyxa* induce ISR genes such as salicylic acid, jasmonic acid and ethylene signaling marker genes *PR1*, *ChiB* and *VSP2* in *Arabidopsis* plants (Lee et al. 2012). In addition, VOCs have a direct inhibitory effect on conidia germination and the growth of plant pathogens, such as *Botrytis cinerea* (Sharifi and Ryu 2016). Carvacrol and trans-2-hexenal have reported to be effective in hampering in vitro growth and germination of *Monilinia laxa*, the agent of brown rot of stone fruit (Neri et al. 2007).

Most SMs from bacterial species are also mainly produced by gene clusters such as NRPSs and PKSs**.** Genomic analysis *showed* 13 biosynthetic gene clusters (BGCs) encoding SMs associated with biocontrol activity were identified in *Bacillus* spp. (Chen et al. 2018a). BGCs in *Bacillus* spp. included five NRPS clusters encoding three lipopeptides (surfactin, iturin and fengycin), a siderophore (bacillibactin) and the antibiotic dipeptide bacilysin. Three PKS clusters were identified which encoded for the antibacterials: bacillaene, difficidin and macrolactin. In addition, a ribosomally originated biosynthetic cluster, which encodes antibiotic plantazolicin, has been found from *Bacillus amyloliquefaciens*. Genomic analysis coupled with LC-MS/MS has confirmed the presence of nine metabolites or their derivatives and eight completed genomes in *B. amyloliquefaciens* (Dunlap et al. 2013). Bioactive metabolites of microorganisms identified by biochemical techniques and genomic-based studies enable researchers a rapid identification of bioactive metabolites and assemble extra information for applying them in plant disease management.

15.2.3 Secondary Metabolites from Actinomycetes

Actinomycetes represent a part of microbiome in the soil and soil/plant systems. Their potential of using in plant protection has been studied widely (El-Tarabily and Sivasithamparam 2006; Goudjal et al. 2014; Sharma and Salwan 2018; Shrivastava and Kumar 2018) as they display several desirable characteristics such as production of SMs, including a range of antibiotics and anti-infection agents against several phytopathogenic fungi in nature, when it comes to the suppression of plant diseases (Chen et al. 2018b; Viaene et al. 2016). Some of the SMs from actinomycetes have been found as blasticidin S, kasugamycin, streptomycin, oxytetracycline, validamycin, polyoxins, mildiomycin, natamycin, etc. Several SMs produced by a range of species act as fungicide and bactericides (Aggarwal et al. 2016).

Among diverse range of actinobacteria, the genus *Streptomyces* belong to the rhizosphere microbial communities are efficiently colonize rhizosphere and plant tissues inside, act as a promising resource of SMs (Aggarwal et al. 2016; Olanrewaju and Babalola 2019; Vurukonda et al. 2018). These compounds have two inhibitory effects such as fungicidal and fungistatic. The production of growth inhibitory secondary metabolites such as antibiotics, toxins, biosurfactants, volatiles and others can suppress or kill microbial rivals through interference competition (Vurukonda et al. 2018). The liquid culture of *Streptomyces* strain TKA-5 when tested against *Phytophthora capsici*, phytophthora blight of bell pepper, and *Alternaria brassicicola*, black leaf spot of spoon cabbage, showed antagonism due to the low-molecular-weight, non-protein chemical constituents, which have been found as SMs (Ko et al. 2010). These compounds enable them to stimulate morphological deformations such as shrinkage, collapse and tortuosity and debilitate spore germination (Chen et al. 2018b) and inhibit protein biosynthesis in different ways, toxic to the nervous system making irreversible paralysis, disrupt neuronal activity and inhibit neurotransmission of pathogenic microorganisms (Aggarwal et al. 2016). They eventually contribute to develop a disease suppressive soil (Viaene et al. 2016).

Disease suppression in soil appeared to be due to the concerted activities of multiple microbial genera working together at specific sites or operating at different stages of the infection process of the pathogen. Next-generation sequencing and other 'omics' technologies have provided new insights into microbial and chemical ecology that the suppressive soils contain a valuable source of biocontrol agents, which do not represent when the cultures are tested in vitro (Gómez Expósito et al. 2017). Therefore, practical solutions are important for developing new technologies to isolate effective biocontrol compounds for formulations as fungicide and bactericides.

15.2.4 Use of Secondary Metabolites for Controlling Plant Diseases

Eco-friendly chemical resources could help select for efficient biocontrol strategies and lead to a greener chemical disease management in the field. Therefore, it is important to use cultures of microorganisms for controlling weeds, pest and pathogens as their metabolites such as antibiotics, volatile compounds, enzymes and other toxic substances are the key factors involved in biocontrol potential. These metabolites have been developed as biopesticides, bio-weedicides and biofertilizers. It has been found that metabolites from *Trichoderma* spp. were more effective as biopesticides and biofertilizers than using living microbes (Vinale et al. 2009). Singh et al. (2016) have also shown that SMs of *Trichoderma* and *Streptomyces* have more antagonistic actions than their live culture treatments. As such use of SMs provides significant beneficial impact over the live culture application on the management of diseases in crop plants. Table 15.1 lists secondary metabolites produced by microorganisms that have been used in current agricultural practices for the purposes of controlling plant diseases.

Table 15.1 The summary of the aforementioned secondary metabolites produced by microbes that have been used in plant disease management at present agriculture

Secondary metabolites	Source	Biological activity
6-Pentyl-α-pyrone (6PP)	Fungi	Protect pruning wounds of grapevines from trunk disease pathogens
Polyketides	Bacteria, fungi	Antibacterials, antifungals, antivirals and antiparasitics (Han et al. 2018)
Alkanase		
Bacillomycin D	Bacteria	Antifungal, antibacterial
Benzaldehyde, nonanal, benzothiazole and acetophenone	Bacteria (*Bacillus* spp.)	Antagonism against *Ralstonia solanacearum*
1-Undecene	Bacteria (*Pseudomonas* spp.)	Antibacteral against against *Phytophthora infestans*
DMDS and 2-methylpentanoate	Bacteria	Inducing ISR in plants
Carvacrol and trans-2-hexenal	Bacteria	Hampering conidia germination
Acetoin, 2,3-butanediol and tridecane	Bacteria (*Paenibacillus polymyxa)*	Induce ISR genes in plants
Blasticidin S	Actinomycetes (*Streptomyces* spp.)	Antifungal against *Pyricularia oryzae*
Kasugamycin	Actinomycetes (*Streptomyces* spp.)	Antifungal against *Phytophthora sojae*
Streptomycin	Actinomycetes (*Streptomyces* spp.)	Antibacterial and antifungal against *Xanthomonas oryzae*, *Xanthomonas citri* and *Pseudomonas tabaci*
Oxytetracycline	Actinomycetes (*Streptomyces* spp.)	
Validamycin	Actinomycetes (*Streptomyces* spp.)	Antifungal against *Rhizoctonia solani* and other *Rhizoctonia* in rice, potatoes, vegetables, strawberries, tobacco, ginger, cotton, rice, sugar beet, etc.
Polyoxins, mildiomycin, natamycin	Actinomycetes (*Streptomyces* spp.)	Antifungal, antibacterial

Identification of new molecular effectors from a range of metabolites supports biotechnological advancement of the use of such molecules in plant disease management. However, isolation of active compounds is one of the major challenges in biotechnology as many biosynthetic genes are not expressed under standard culture conditions, thus resulting in a limited diversity of microbial compounds that can be obtained through fermentation. Genetic analysis has shown that gene clusters

encoding SMs are not expressed when microbes cultured alone, but in co-culture with other microorganisms (Lugtenberg 2018; Wakefield et al. 2017). Among 114, 16 bacterial isolates have resulted in compounds with strong activity such as 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; 9,12-octadecadienoic acid (Z,Z)-, methyl ester; 9-octadecenoic acid, methyl ester, (E)-; and decanedioic acid, bis(2-ethylhexyl) ester, when cultivated together with other microbes (Mohamad et al. 2018), although broad antimicrobial activities have shown against common fungal pathogens. All in all, co-cultivation seems to be an effective strategy to produce bioactive metabolites, especially novel compounds from plant beneficial fungi (Vinale et al. 2017).

15.2.5 Conclusion and Future Perspectives

SMs from bacterial antagonists have served as important sources of antimicrobial agents, which are of great use in the field of medicine and agriculture. Although new scientific approaches such as metagenomics have deployed to find new genes, the success of achieving the production in vitro is not as simple as it seems, due to the debilitated performance of microbes isolated from their original source and in vitro cultures. However, co-cultivation has provided a possible method of yielding strong and effective SMs from microorganisms. Thus, cultivation of microbes isolated from disease suppressive soil together with other microbes might be the possible solution to optimize and enhance yield of target compounds in a way that can be applied to protect plant from being affected by several diseases.

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Beneficial Root Microbiota: Transmogrifiers of Secondary Metabolism in Plants 16

Akanksha Singh, Rupesh Chaubey, Stuti Srivastava, Sumit Kushwaha, and Rakesh Pandey

Abstract

All plants in the ecosystem are found in close association with complex group of microbes both belowground and aboveground surfaces. Reports suggest that the association can be harmful, neutral, or beneficial to the plants depending upon the category of colonizing microbes. It is among them that certain microorganisms bring about modification in the plant metabolome, maneuvering to modifications in the biosynthetic pathway of plant metabolites of known and unknown origin. Plant secondary metabolites are exceptional group of chemicals released as an end product of biosynthetic pathways which have numerous secondary roles in survival and growth of the plants. Among the multifarious roles played by the metabolites, some of the important traits include repulsion of pathogens and attraction of beneficial group of microbes. The present chapter thus summarizes the till-date understanding of the role of root microbiome on the secondary metabolic status of plants, how the remodeling affects the health and defense status of the concerned plants, and finally the knowledge hiatus that needs to be fulfilled for harnessing the full potential of microbes.

Keywords

Root microbiome · Secondary metabolites · Plant defense · Cross kingdom communication

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16.1 Introduction

Host-associated microbial populations are reported to be engaged in elementary roles like nutrition status, different developmental phases, and immunity of both animal and plant kingdom. The different factors which help in architecturing the host–microbiome interactions are inadequately understood, which hold an important place in evolutionary and ecological sciences (Fitzpatrick et al. 2018). Talking about the plants, the roots bring together two different microbial sections namely rhizosphere and the endosphere. The colonization at the rhizospheric surface by microbes can either be beneficial, neutral, or harmful associations, depending upon the relationship they share with the host plant. With the advancement in technologies, especially pertaining to sequencing, the picture of different root-associated microbiomes is getting clearer day by day (Rout and Southworth 2013). The most recent information which is coming out from the experimental evidences is that the role of microbiome differs not only with plant tissues but also with the change in environmental conditions too (Yu et al. 2019). The Next Generation Sequencing (NGS) data clearly demonstrates that amazing number of taxonomically dissimilar microbes colonize the plant system, whose density can be sometimes much higher than the plant cells figures (Mendes et al. 2013; Panke-Buisse et al. 2015). The colonization affects the plant system either directly or indirectly either by facilitating nutrient uptake, phytohormone production, induction of systemic resistance, formation of physical barriers, and changes in secondary metabolite status of concerned plants (Etalo et al. 2018). The most recent area of current research in plant–microbe interaction is changes in metabolomic status of plants leading to alteration in some key metabolites of agricultural and medical importance (Etalo et al. 2018). Hence, it is hypothesized that exploring plant–microbe communication will pave way for not only boosting production of metabolites of pharmaceutical importance but other unknown secondary metabolites too. The current chapter has thus been written with the aim to provide exhaustive information about the key players involved in alteration of secondary metabolites in plants with special emphasis on beneficial microbes, root exudates, and bioactive metabolites.

16.2 Root Microbes

Microorganisms have been defined as smallest organisms that cannot be seen with the naked eye and can only be seen with a special equipment called microscope. Among the diverse range of microbes, we in this chapter have specifically discussed about the microbes colonizing the root zone of plants. Microorganisms are mostly found as free-living microbes and when they stick around the plant roots and root hairs, they are called root microbes.

Root microbes are classified into two types:

1. **Beneficial microbes** are those microbes which work toward enhancing the yield and overall well-being of plants and which can easily perform plant growth promotion, for example *Pseudomonas*, *Bacillus*, etc.
2. **Harmful microbes** are the category of root microbes that inhibits the growth of plants by destroying the plant cells, making the plants nutrients deficient, and killing the beneficial microbes.

16.2.1 Beneficial Root Microbes

In the early 1904, Lorenz Hiltner observed and stated that there are numerous microorganisms which live in the soil near the rhizospheric region than the distant part of soil (Hiltner 1904). Soil is been widely accepted as the home for array of microbial species, fungi, invertebrates, archaea, and mostly bacteria (Tringe et al. 2005). Hiltner gave the term **Rhizosphere** for that region where microbial population was the highest near plant roots. It has also been derived that some region of soil which is conventionally benefitted by root secretion and associated with microbes of soil is referred to as root microbiome. Moreover, plant root system always expands through the soil and penetrates it, resulting in release of water-soluble materials such as amino acids, organic molecules, certain sugars, and carbohydrate derivatives which are essential for microorganism to survive.

Surprisingly, plant physiologists noted that soil plays a role in providing nutrients to plants, but they forgot to add that soil is a different complex ecological system having a huge species like protists, animals, bacteria, and fungi specially (Bonkowski et al. 2009; Müller et al. 2016). The microorganisms' living in soil are the basic invisible mangers of soil fertility, and it doesn't matter if the soil condition or crop species favour them or not, because it is the nature which promotes microbes to become root symbionts. These symbionts promote plant growth and increase yield by different actions like nutrient uptake and nitrogen metabolism resulting in nitrogen fixation, and these particular activities help plants to counter pests, diseases, and biotic and abiotic stresses (Fig. 16.1). Collectively, by the enhancement of plant capacity in photosynthesis and production of organic acids, plants derive their health and the microbes which helped throughout this process are referred as "beneficial root microbes."

16.2.1.1 Types of Beneficial Root Microbes

The beneficial root microbes have been basically categorized into five different types, namely actinomycetes, bacteria, fungi, protozoans, and nematodes. The detailed information about these microbes has been discussed in the following sections.

Bacteria

Bacteria are the smallest living organisms and major key player of soil in bringing together the simpler forms around the root system so that the plants can firmly take up all the nutrients important to their growth and development, for examples

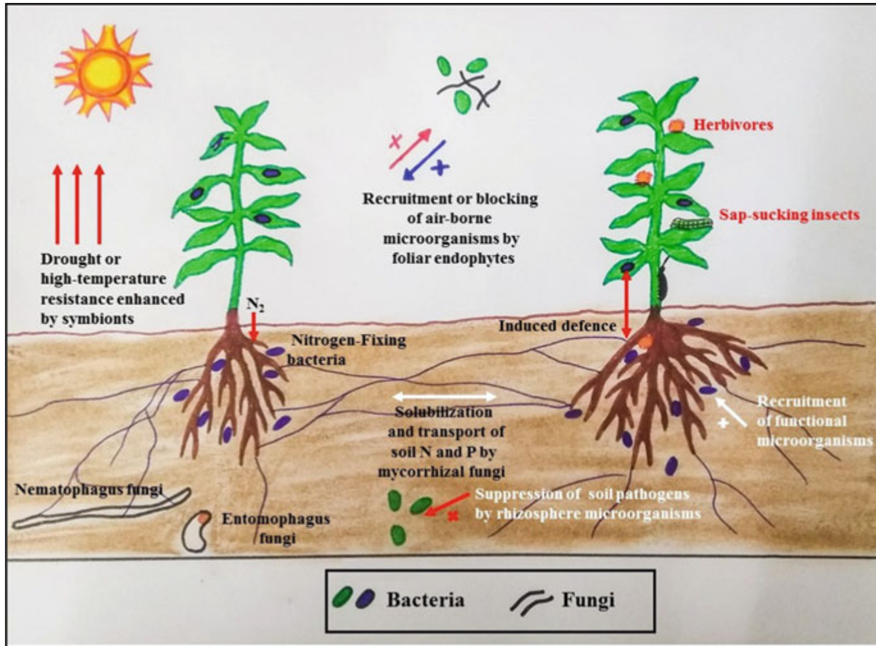

Fig. 16.1 Role of rhizospheric microbial community in mitigating biotic and abiotic stressors

macronutrients like nitrogen, phosphorus, potassium, etc. Phosphorus is usually not found in available form for the plants in soil, but some of the beneficial bacteria turns the nonavailable phosphorus into available form which a plant can easily utilize. In soil system there are huge number of bacteria which enhance plant growth and have thus been referred to as plant growth–promoting rhizobacteria (PGPR) (Bonkowski et al. 2009). Moreover, in obligate symbionts, PGPRs can easily interact with the host plants and enhance growth of the plant either by direct benefit via nitrogen fixation (Müller et al. 2016; Kloepper and Schroth 1987) or indirectly by secreting certain enzymes and hormones which can suppress other pathogens' activity (Soyano et al. 2014; Ferguson and Mathesius 2014). The root hierarchy is also dependent upon PGPRs, as the structural modification in root results in better conduction of molecules into plant parts which is inversely proportional to better crop yield (Pérez-Montaño et al. 2014; Lugtenberg and Kamilova 2009; Uga et al. 2013). The projectile PGPR activity and plant growth promotion attributes are also reviewed in some articles (Ogawa et al. 2014; Ning et al. 2014).

Example – Indole-3-acetic acid production by the *Rhizobium leguminosarum* has been discussed in literature for playing a key role in promoting certain effects on rice seedlings (Biswas et al. 2000). In the same way, *Azotobacter* has been reported to do the job for maize seedlings (Zahir et al. 2000).

Actinomycetes

Actinomycetes are spore-forming, gram-positive aerobic bacteria which form thread-like structures called filaments, and work in cycling or turning up the organic matters, mainly by decomposition of complex mixtures found from decomposed plants, animals, or fungal sheets over rocks. Somehow these enzymes and hormones also help in suppressing certain plant pathogens which pose threat to plants, for example *Streptomyces* sp. have been found responsible for nutrient uptake and plant growth in rice and chickpea plants (Gopalakrishnan et al. 2014, 2015). Likewise, *Frankia* has been found to be responsible for nitrogen fixation in Alnus plant (Simonet et al. 1990).

Fungi

Fungi are multicellular, eukaryotic, heterotrophic organisms that have absorptive mode of nutrition. They live in the root zone of plants and act like natural recycling bins, help in reabsorbing soil nutrients from dead organic matter, and redistributing them back to plants roots. In addition, they also help in making nutrients available to plants through formation of siderophores. For example, mycorrhizal association is a mutual relationship which exists between roots of plant and fungus for sharing the benefits. The association is usually two ways – ectomycorrhizal when the fungus resides outside of root, or endomycorrhizal when the fungus penetrates inside of the root. It is well reported in literature that most of the rhizospheric fungus produces metabolites for the inhibition of plant pathogens (Ali et al. 2015; Saraf et al. 2014).

Example – Plant defense mechanisms can directly or indirectly be controlled by arbuscular mycorrhizal fungi (AMF) (Di Benedetto et al. 2017). *Trichoderma harzianum* are involved in active colonization of tomato root and induced systemic resistance-like defense in Arabidopsis. (Engelberth et al. 2001). Likewise, *Trichoderma viride* has been found to be responsible for elicitation of jasmonic acid and salicylic acid biosynthesis in lima bean (Morán-Diez et al. 2009).

Protozoa

Protozoa are single-celled, microscopic, eukaryotic, and heterotrophic organisms (using organic carbon as a source of energy). They are non-filamentous and restricted to moist or aquatic habitats. Protozoans play important roles in the fertility of soils by eating soil bacteria and maintaining bacterial populations. Protozoans sometime help in promoting plant health by the mineralization of nutrients and alteration in the hierarchy or activity of plant root–associated families (Bonkowski 2004). It was also stated and reported that predation of some of the different plant pathogenic species has an inverse effect on the plant growth hormone production (Krome et al. 2010) or sometimes they support the beneficial microbes to survive (Jousset et al. 2010; Müller et al. 2013). Protozoans also excrete nitrogen in the form of ammonium and phosphorus as products of their metabolism, and it is because of this reason that the presence of protozoans in soil has been reported to enhance plant growth and development.

Example – *Acanthamoeba castellanii* grazing has been reported to maintain the bacterial population in the rhizospheric soil by consumption etc.

Nematodes

Nematodes are microscopic worms which live around or inside the plant and periodically rely and feed over bacteria, fungus, and other soil microbes. Nematodes can easily carry live microbes over their bodies and also inside their digestive systems, and by this activity wherever they go nematodes deliver microbes over the roots of plant or in soil. Few nematodes are also disease causing, while others feed over disease-causing organisms which can be identified as potential biocontrol agents.

Example – *Steinernema*, *Risbravis*, *Rhabditis*, etc., are the useful nematodes responsible for decomposing the organic matter and managing attack on insects and other pests.

16.3 How Useful Root Microbes Boost Crop Productivity?

Beneficial root microbes present in the rhizospheric soil near plant roots ameliorate plant productivity and its performance in a variety of ways like deterioration of pathogens, providing resistance against any infection, and help in plant growth promotion. The major mode action involves following steps:

16.3.1 Nutrient Availability

Rhizospheric microorganisms always take part in obtaining trace elements which are found in insoluble forms, where microbes turn this into soluble form and make them available to plants. By the use of certain molecules, like siderophore, iron chelation and conversion of complex to simpler form takes place (Aznar and Dellagi 2015). Most of the bacterial community works as key component to unlock the nutrients which are locked in the form of hydrocarbons essential for the plants. Some of the saprotrophs and fungi have been reported as nutrient extractors through solubilization or reabsorption processes, among which actinomycetes play a significant role in decaying organic matter to make it in available form (Aznar and Dellagi 2015).

16.3.2 Plant Growth Promotion

In a different manner we have seen PGPRs playing essential role in plant growth promotion where they produce metabolites which eventually trigger the release of plant hormones reported to play beneficial role for plants. Apart from working as PGPRs, some microbes work as bio-remediators. As a biocontrol trait, microbes effect plant pathogens through the different synthesis like regulation of ethylene level in plant, siderophore activity, acquired systemic resistance, antibiosis, quorum sensing, etc. (Babalola 2010; Olanrewaju et al. 2017). In addition, the beneficial microbes are reported to increase photosynthesis and production of hormones and enzymes as a result of improvement in crop growth. They also control various

insects and plant diseases as a consequence improvement in crop quality. The use of such kinds of microorganisms leads to reduction in the usage of chemical fertilizers.

16.4 Root Exudates: Role in Shaping Root Microbiome

In natural environment, plants health status mainly depends on complex and active microbial community present in the rhizospheric soil. In plants, root system is the essential part for nutrient and water conduction, which is inhabited and encircled by a major microbial community called root microbiota or rhizomicrobiome (Del Carmen Orozco-Mosqueda et al. 2018; Hacquard et al. 2015). Complex microbial community present in the root microbiome is referred to as plant's second genomic part which consists of total rhizosphere community's interactions present in relation to plant health (Berendsen et al. 2012). Crop growth and yield inside natural environment depends on microbial interactions, that is, bacteria and fungi, actinomycetes, etc. (Schmidt et al. 2016). Attachment of microbial diversities was preferred to be connected in two steps:

16.4.1 Rhizosphere

Rhizosphere as a term was first coined by Lorentz Hiltner (Hiltner 1904) and reconsidered by Pinton as the zone around the plant roots in the soil which is colonized by microbial community (Morgan et al. 2005; Pinton et al. 2007).

Example – Azotobacter, Nitrobacter, Proteobacteria, Rhizobacteria, Actinobacteria, Pseudomonas are some of the ruling populations of bacteria over rhizosphere (Sylvia and Prévost 2005).

16.4.2 Rhizoplane

Region of surface of the plant roots with epidermis and mucilage which is direct contact with the soil and colonized by microbial community.

Example – *Burkholderia*, *Acidobacterium*, *Dyella*, and *Edaphobacter* are the major genera abundant in the rhizoplane.

The soil–microbe interactions are usually specific and depend upon coevolutionary dilemma (Dobbelaere et al. 2003; Duffy et al. 2004); (Morgan et al. 2005). In the underground world, the specific plant–microbe interactions hold a very important place in various processes governing ecosystem, just like carbon metabolism, sequestration, and nutrient cycling (Singh et al. 2004).

For the export and secretion of molecules into the rhizospheric soil, plants use a hierarchical transport technique where plant roots along with root hairs and adventitious part release root exudates either by passive or active diffusion/secretion mechanism (Badri et al. 2009; Weston et al. 2012).

Table 16.1 Different types of root exudates released by the plants

Classes of compounds	Components identified
Amino acids	All 20 proteinic amino acids, homoserine, aminobutyric acid, mugineic acid, l-hydroxyproline
Lignins	Coumaric acid, sinapoyl aldehyde, benzoic acid, pyroglutamic acid phloroglucinol, gallic acid, nicotinic acid, chlorogenic acid, vanillin, catechol, sinapyl alcohol, quinic acid
Proteins and enzymes	Peroxidases, PR proteins, proteases, lipase, acid phosphatases, lectins, hydrolases
Phenolics and Coumarins	Umbelliferone, Caffeic acid, cinnamic acid, coumarin, ferulic acid, salicylic acid, syringic acid, vanillic acid
Indole compounds	Indole-3-acetic acid, brassilexin, sinalexin, methyl indole carboxylate, camalexin glucoside, brassitin
Flavonols	Strigolactone, kaempferol, quercitin, genistein, myricetin, naringin, rutin, naringenin, and their substitutes with sugars
Sugars	Galactose, mannitol, fructose, pentose, rhamnose, arabinose, raffinose, ribose, sucrose, xylose and glucose
Sterols	Stigmasterol, campestrol, sitosterol
Allomones	Juglone, 5,7,4′-trihydroxy-3′, 5′-dimethoxyflavone, sorgoleone, DIBOA DIMBOA
Anthocyanins	Pelargonidin, delphinidin, cyanidin and their substitutes with sugar molecules
Organic acids	Succinic acid, l-aspartic acid, l-glutamic acid, salicylic acid, oxalic acid, shikimic acid, chorismic acid, acetic acid, sinapic acid,, isocitric acid, citric acid, p-hydroxybenzoic acid, tartaric acid, gallic acid, malic acid, protocatechuic acid, p-coumaric acid, mugineic acid, piscidic acid
Aurones	Sinapoyl choline, benzyl aurones synapates
Glucosinolates	Cyclobrassinone, desulphoglucoalyssin, desulphoprogoitrin, desulphonapoleiferin, desulphoguconapin
Fatty acids	Palmitic acid, linoleic acid, stearic acid, oleic acid

Root exudates are usually referred to as a group of chemical molecules in rhizosphere which are secreted by plant root systems. They are a mixture of complex substances like sugars, organic acids, enzymes, amino acids, etc., which act as major source of organic carbon specifically obtained from rhizospheric soil (Hütsch et al. 2002; Nguyen 2003). Usually quality and quantity of root exudates depends upon plant species and is variable in different plants, individual plant's age, and some external factors like biotic and abiotic stresses. Knudson (1920) and Lyon and Wilson (1921) were the first who had provided indication regarding root exudation and microbe abundance in rhizosphere of the plants. Some of the important exudates usually found in the rhizosphere have been mentioned in Table 16.1.

16.5 Requirement of Root Exudates in Plant–Microbe Interactions

Phytochemicals secreted by plant roots mediate certain number of interactions like

- Plant–plant interaction
- Plant–microbe interaction
- Microbe–microbe interaction

Microorganisms live in the rhizospheric soil where they interact with roots and their components to enhance the plant health (Berendsen et al. 2012; Panke-Buisse et al. 2015). The interaction might be neutral in some ways and either advantageous or harmful in others (Mercado-Blanco and Bakker 2007; Raaijmakers et al. 2009). Most probably, depending on the environment, microbes also turn the table from pathogenesis to symbiotic association (Newton et al. 2010). In different examples, *Rhizobia* includes *Bradyrhizobium*, *Azorhizobium*, symbiotic nitrogen, and nitrogen-fixing bacteria like *Sinorhizobium* and *Mesorhizobium* (Davidson and Robson 1986; Zahran 1999). In nitrogen-limiting conditions, attraction and intimation of legume–rhizobia symbiosis result in secretion of flavones and flavonols by legumes (Coronado et al. 1995; Zhang et al. 2009). In the same way equal exchange of plant nutrients benefit both the partners like the mycorrhizal associations which is a common association found in alomost 80 percent of the plant species (Kiers et al. 2011).

16.6 Effect of Microbe–Microbe Interactions on the Soil Microbial Communities

For plants, rhizospheric zone is a kind of nutrient-rich site where the competition for food among microbes always takes place. Secondary metabolites produced by microbes are released in the environment to overcome other competitors which fight to occupy similar zone for establishing firmly itself outside or within the roots (Thomashow and Weller 1988; van Loon and Bakker 2005; Pierson and Pierson 2010; Kim et al. 2011). The metabolites released in environment consist of siderophore, lytic enzymes, toxic elements, and antibiotics (Bais et al. 2006). Some rhizospheric microbes hold a variety of genes for the production of siderophores and other antibiotics like *Bacillus amyloliquefaciens* (Chen et al. 2007) and few species of *Pseudomonas* (Paulsen et al. 2005). Antibiotics like 2,4-diacetylphloroglucinol (DAPG) and oomycin are also products of microbes (van Loon and Bakker 2005). The referred antibiotics play a significant role in restraining the pathogenic microbes (Aminov 2009; Pierson and Pierson 2010; Thomashow and Weller 1988; Kim et al. 2011).

Besides antibiotics, plant secondary metabolites also work toward altering signaling pathway and metabolic activity of plants (Přikryl et al. 1985; Brazelton et al. 2008; Costacurta and Vanderleyden 1995; Kim et al. 2011). These kinds of

microbial attributes sometime change the root exudates' composition, leading to the selective enhancement of any particular microbial partner in the rhizosphere (Přikryl et al. 1985; Bulgarelli et al. 2013). The whole scenario of communication between two bacterial communities results in release of signaling molecules which are relatively recognized by other communities via inter- and intra-species communication (An et al. 2014). In bacteria this scenario comprises of biofilm formation, motility, and cell adhesion (Sperandio et al. 2002; Chu et al. 2011); production of the virulence-associated factors; and cell proliferation. This kind of density-dependent stimulus and exchange of signals is referred to as quorum sensing (Fuqua et al. 1994; Miller and Bassler 2001; Atkinson and Williams 2009; An et al. 2014) (Yajima 2014).

In fungi, two important molecules namely farnesol and tyrosol have been reported for regulating quorum sensing–controlled traits like biofilm formation, resistance to drugs, and morphogenesis (Chen et al. 2007; Enjalbert and Whiteway 2005; Albuquerque and Casadevall 2012). Likewise, tryptophol has been reported to control morphogenetic behavior in *Saccharomyces cerevisiae* through both density-dependent approach as well via nutritional trigger (Chen and Fink 2006).

16.7 Coevolutionary Relationship of Root Exudates with the Rhizosphere

Microbial communities present in the soil are involved in multilevel intercommunication which are known to influence vital environmental activities, like biogeochemical cycling of nutrients, soil quality, and plant well-being (Barea et al. 2005; Giri 2005).

The age of the plants, crop species, and types of soil determine the variation in microbial communities present in the rhizospheric soil (Wieland et al. 2001; Buyer et al. 2002); (Kowalchuk et al. 2002). In some recent evidences it was observed that specific plant species cultivate their own soil fungal community and diversity composition, and this "culture" is mediated by root exudates (Broeckling et al. 2008).

Example – In native soil, when *Arabidopsis thaliana* and *Medicago truncatula* were grown at different places, it was observed that *Arabidopsis* and *Medicago* maintained its own fungal community in their resident soil. When the plants were grown in other soil different from the native soil that did not promote *Arabidopsis* or *Medicago* plants, the microbial communities in those soils decreased considerably. Similarly, when root exudates were added to the soil, the same response was observed, thus showing that plants secrete root exudates to drive these responses and this interaction has a coevolutionary component.

16.8 Bioactive Metabolites

Plants play a variety of roles either in metabolism or metabolites, which are required for the sustainability of plant system. These plant metabolites could be made up of proteins, lipids, carbohydrates, or nucleic acids which are then known as primary metabolites. Metabolites are primarily known as helping hand for plant system which directly intervenes in the growth and development (Ballhorn et al. 2009). The metabolites produced by plants have been broadly categorized into two groups namely:

16.8.1 Primary Metabolites

Primary metabolites are certain compounds which directly benefitted the plants for their overall growth. They have been classified as carbohydrates, lipids, proteins, etc., which are likely used by the plants directly for different works (Schafer and Wink 2009).

16.8.2 Secondary Metabolites

Plant secondary metabolites are those compounds which do not having any direct role in plant metabolism and are often useful in respect to defense-related properties. They are usually low molecular weight around 3000 dalton (Osbourn et al. 2003). The production and secretion of secondary metabolite varies from species to species and somehow difference between natural products and secondary metabolites is hard to define (Vasconsuelo and Boland 2007).

In so many different ways, secondary metabolites are involved in upregulation of primary metabolism and act as triggers for signaling any known process. Secondary metabolites often maintain the balance of plant molecules with the environment either via adaptation mechanism or by making a complementary framework to intricate fine balance (Osbourn et al. 2003; Berni et al. 2018; Grayson 1998).

16.9 Principal Groups of Secondary Metabolites

Plant secondary metabolites have been majorly categorized into four major classes (Goldberg 2003). These four categories include terpenoids, nitrogen-containing compounds, phenolics, and sulfur-containing compounds (GSH, defensins, and lectins) (Mazid et al. 2011).

16.9.1 Terpenes

Terpenoids are the on the whole most varied class of plant secondary metabolites as they have approximately 40,000 dissimilar compounds, and thus they stand out as the biggest class of important plant metabolites (Bohlmann and Keeling 2008).

16.9.2 Phenolics

Phenolics are molecules that have an aromatic ring bound with one or more hydroxyl groups (Nicholson and Hammerschmidt 1992). By the chemical formula and its structure, it differs from simple phenols like catechol to catechol melanins through a long chain polymer. Phenolic compounds are reported to guard plants from different herbivores and pathogens. Apart from protecting plants from above-mentioned stressors, phenolics also protect plants from UV radiation, heat shock, and frost situation (Parr and Bolwell 2000).

16.9.3 Alkaloids

Alkaloids are amino acids–derived nitrogen-containing compounds just like tyrosine and tryptophan. They also present in huge amount but take 20% of total metabolites (Hegnauer 1988). Alkaloids occupy a major share in drug industry and are being mainly used as narcotics or in pharmaceuticals (Hesse 2002; Yao et al. 2004). The most common alkaloids derived from plant sources are vincristine and vinblastine, morphine, and codeine (Crozier et al. 2006).

16.9.4 Sulfur-Containing Secondary Metabolites

Sulfur-containing metabolites are derived from two different ways; one group is formed from hydrolyzation of glucosinolates by myrosinase enzyme. Second group is made up of allin by alliinase enzyme found basically in onion and garlic. Both of these groups are in nature for a purpose which we always face off with and help in guarding plants from the herbivores (Ober et al. 2003).

16.10 Role of Rhizospheric Microbiome on Plant Growth Promotion

Microbial communities are well acclaimed for playing a crucial part in the overall development and growth of plants by manipulating diverse physiological processes. The shaping of rhizospheric microbiome is a mutual process which is largely influenced by the rhizodeposits (Sharma and Chauhan 2017). Recently, people have started focusing on studying the microbiome associated with host plants in

order to expand sustainable farming customs via the utilization of microbial biopesticides and biofertilizers. Within a given set of soil type, the indigenous plants restructure and reframe the native rhizospheric microbial community by applying a selective pressure. It is exhaustively reported in literature that within a given set of soil type, the indigenous plants put forth a selective pressure on this immense biodiversity pool, thereby reshaping the rhizospheric microbial community structure.

Manipulation of bacterial microbiome has attracted more attention of researchers in recent times than the other groups of organisms, as it has helped the scientists in altering numerous plant beneficial activities, namely enhancement in growth and yield, as well as suppression of phytopathogens with final effect on the usage of chemical fertilizers which is considerably reduced (Adesemoye and Kloepper 2009). Microorganisms living belowground are known to affect composition and total yield of natural plant communities directly and indirectly (Van Der Heijden et al. 2008; Turner et al. 2013). It is because of this reason that the soil microbial richness has been directly linked with the diversity and productivity aboveground plant (Lau and Lennon 2011; Wagg et al. 2011).

16.11 Role of Rhizospheric Microbiome on Plant Secondary Metabolite Status

The interconnection between plants and their microbial communities is active practice in which plants interact to their surrounding environment and accordingly respond to the changes (Chaparro et al. 2012). Microbes play important role in agriculture in order to maintain environmental equilibrium (Fig. 16.2). Both the shoot and root systems of plant are directly or indirectly contact with diverse group of microorganisms. Due to the presence of infinite number of microbes, various mechanisms occur around the plant root, and one of them is secretion by root exudate. The root exudation comprises the secretion of carbon-containing compounds that are primary and secondary metabolites products and many more molecules (Uren 2000).

Elicitors are chemical compounds for stress factors which when applied in minute quantity to a living being enhances the biosynthesis of metabolites, mainly secondary metabolites (Radman et al. 2003). In context to the plant system, elicitors play vital role in defense process against pathogens and environmental stress. The biotic elicitors include bacteria, fungi, and viruses whereas abiotic elicitors involve metal, ions, and inorganic molecules. Thus, PGPR can produce elicitors which in turn will originate the synthesis of secondary metabolites (Sekar and Kandavel 2010) [Table 16.2]. The herbaceous plant *Catharanthus roseus*, which is commonly called rose periwinkle, belonging to family Apocynaceae releases bioactive compound ajmalicine under drought stress (Jaleel et al. 2009). Likewise, in another study *Pseudomonas fluorescens*, a plant growth–promoting rhizobacteria was reported to increase the production of ajmalicine under drought stress. This bacterium also increased plant biomass and helped in protecting the plants against stress condition.

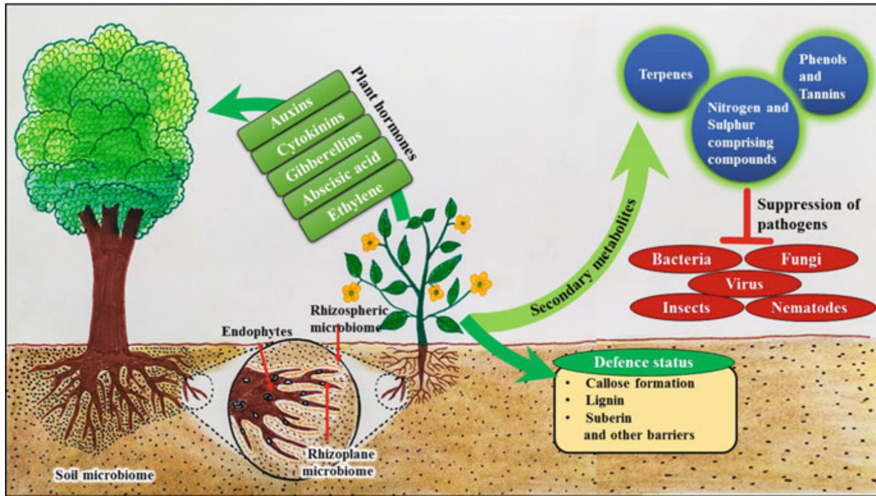

Fig. 16.2 Schematic representation of the role of rhizospheric microbiome on the growth, secondary metabolite, and defense status of host plants

C. roseus is also reported to secrete some metabolites like serpentine, catharanthine, tabersonine, and vindoline but among all of them ajmalicine content was found to be maximally increased (Jaleel et al. 2009).

The perennial plant *Crocus sativus*, commonly called saffron crocus, secretes crocetin, picrocrocin, and safranal compounds. In a study it was found that the contents were increased when plants were inoculated with *Bacillus subtilis* FZB24 (Sharaf-Eldin et al. 2008). Among all the compounds, crocetin was found to be increased maximally. *Trichoderma* belonging to fungal genera is usually present in almost all soil types (Hermosa et al. 2012). It has property to kill other harmful bacteria and fungi that act as biocontrol agent for the plant (Druzhinina et al. 2011). *Trichoderma* acts as a biotic elicitor for oleanolic acid which is secreted by *Calendula officinalis* plant. Oleanolic acid amount is intensified by application of *Trichoderma viride* (Wiktorowska et al. 2010).

Scopolia parviflora is a flowering plant belonging to family Solanaceae, which produces scopolamine compound whose concentration was found to be increased along with the amount of tropane alkaloids by different microbes such as *Bacillus cereus* and *Pseudomonas aeruginosa* (Jung et al. 2003). Tropane alkaloids concentration is high in roots as compared to stem and leaves. Tropane has cyclic amine group which has piperidine and pyrrolidine ring with single nitrogen atom and two carbon atoms (Hanuš et al. 2005). They are used as anesthetics, bronchodilators, and mydriatics (Grynkiewicz and Gadzikowska 2008).

Apart from PGPRs, endophytes are those bacterial or fungal microbes that live their entire life with living cells of plant without causing any disease to the host (Wilson 1995; Sturz et al. 2000). Nowadays endophytes have been considered as an important source for secondary metabolites which include phenols, alkaloids, and

Table 16.2 Effects of different beneficial microbes on the status of important secondary metabolites

S. No.	Plant name	Secondary metabolite	Microbes	Reference
1.	*Medicago sativa*	Luteolin	*Rhizobium meliloti*	Hartwig et al. (1990)
2.	*Capsicum annum*	Capsidiol	*Trichoderma viride*	Brooks et al. (1986)
3.	*Catharanthus roseus*	Ajmalicine	*Trichoderma viride*	Namdeo et al. (2002) and Namdeo (2004)
4.	*Catharanthus roseus*	Ajmalicine	*Pseudomonas fluorescens*	Bais et al. (2002)
5.	*Catharanthus roseus*	Serpentine	*Pseudomonas fluorescens*	Jaleel et al. (2009)
6.	*Salvia miltiorrhiza*	Tanshinone IIA	*Trichoderma atroviride*	Ming et al. (2013)
7.	*Gymnema sylvestre*	Gymnemic acid	*Saccharomyces cerevisiae*	Chodisetti et al. (2013)
8.	*Gymnema sylvestre*	Gymnemic acid	*Bacillus subtilis*	Chodisetti et al. (2013)
9.	*Gymnema sylvestre*	Gymnemic acid	*Escherichia coli*	Chodisetti et al. (2013)
10.	*Datura metel*	Atropine	*Bacillus cereus*	Shakeran et al. (2015)
11.	*Taverniera cuneifolia*	Glycyrrhizic acid	*Rhizobium leguminosarum*	Awad et al. (2014)
12.	*Vicia sativa*	7,30-Dihydroxy-40-methoxyflavone	*Rhizobium*	Zaat et al. (1989)
13.	*Pisum sativum*	Apigenin and eriodictyol	*Rhizobium*	Firmin et al. (1986)
14.	*Sesbania rostrata*	7,40-Dihydroxyflavaone	*Azorhizobium*	Messens et al. (1991)
15.	*Glycine max*	Daidzein and genistein	*Bradyrhizobium japonium*	Kosslak et al. (1987) and Bassam et al. (1988)
16.	*Trifolium repens*	7,40-dihydroxyflavone and geraldone	*Rhizobium*	Redmond et al. (1986)
17.	*Ocimum basilium*	Rosmaric acid	*Aspergillus niger*	Bais et al. (2002)
18.	*Glycine max*	(i) Iturine	*Bacillus subtilis*	Ohno et al. (1995)
19.	*Hyoscyamus niger* L.	(i) Hyoscyamine (ii) scopolamine	*Pseudomonas putida* and *Pseudomonas fluorescens*	Ghorbanpour et al. (2010)
20.	*Crocus sativus* L.	Picrocrocin, crocetin and safranal compounds	*Bacillus subtilis*	Sharaf-Eldin et al. (2008)
.21.	*Calendula officinalis* L.	Oleanolic acid	*Trichoderma viride*	Wiktorowska et al. (2010)

terpenoids products. For example, hypericin is a bioactive compound which was isolated from *Hypericum perforatum* and whose production was increased upon inoculation of *Thielavia subthermophila* (Kusari et al. 2008, 2009).

Plumbago rosea L., commonly called Indian leadwort, is classified under angiosperms. It is used for medicinal purposes like in curing of certain kinds of chronic diseases, skin diseases, and used as an anticancer plant (Parimala and Sachdanandam 1993). It releases useful metabolite compound plumbagin from its root and *Aspergillus niger*, *Rhizopus oryzae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* have been reported to be its elicitor. Among the above-mentioned genera, fungal elicitors enhanced the content of plumbagin, whereas bacteria elicitors were not so effective (Komaraiah et al. 2002) The maize crop (*Zea mays*) discharges a compound named benzoxazinoid whose amount changed by rhizobacterium *Pseudomonas putida* KT2440, which protects the plant from pathogenic microorganism (Neal et al. 2012). These compounds function naturally toward the protection of plants. In cell culture roots of *Taverniera cuneifolia* (shrub), glycyrrhizic acid content was intensified when treated with bacteria *Rhizobium leguminosarum* as compared to the control roots. Other bacterial origin elicitors observed in *Taverniera cuneifolia* are *B. aminovorans*, *B. cereus*, and *Agrobacterium rhizogenes* which were also found to increase the amount of glycyrrhizic acid. But when it is treated with *Agrobacterium tumefaciens*, no significant increase in glycyrrhizic acid was found. In another plant, namely *Hypericum perforatum* compound hypericin and pseudohypericin is released, whose concentration is reported to be increased by *Rhizobacterium* (Mañero et al. 2012).

Alfalfa (*Medicago sativa*) belonging to family Fabaceae is a medicinal plant, which is a rich source of vitamins A, B, and C (Rashmi and Sarkar 1997). Luteolin is a bioactive compound released by alfalfa plant whose production is enhanced by plant growth rhizobacteria *Rhizobium meliloti* (Peters et al. 1986). Likewise, in *Datura metel, Bacillus cereus* and *Staphylococcus aureus* were found to increase the content of atropine, a compound largely used for relieving pain (Shakeran et al. 2015).

16.12 Conclusion and Future Prospects

Owing to the presence of diverse variety and multidimensional role of secondary metabolites, we can assume that these organic compounds are of immense importance for the growth, development, defense, and survival of plants. Plants preferably produce these compounds when they encounter herbivores or pathogen attacks. In totality, these compounds are also produced when plants face challenges like abiotic stresses, that is, salinity, drought, UV radiations, heavy metals, and harsh climate. In addition to the above, the biotic elicitors, namely rhizospheric microbes many times positively change the status of plant secondary metabolites production. Additionally, being relatively an unexplored area, the rhizospheric microbiome offers a huge potential for not only manipulating the plant growth but also the secondary metabolite status of plants too.

Therefore, though significance of the microbiome present in the rhizosphere has been identified way back, but still tremendous efforts needs to be put in to explore the potential of organisms which might have good properties for our plants and surrounding environment. Pairing traditional techniques with high-end, next-generation sequencing techniques for identifying cues, exudates and other molecules will really help in understanding the complex underground communication existing between plants and microbes.

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17 Microbial Consortia for Plant Disease Management and Sustainable Productivity

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Abstract

Crop diseases take heavy toll on agriculture. The estimated annual loss due to various diseases ranged from 15% to 20% of the total production. Apart from the yield losses in commercial productions, the losses in storage and perishables are equally significant. Out of several management options of disease control in commercial production (row crops, vegetables, and horticultural), potential use of microbial consortia as a holistic approach for integrated management option has been explored. The various aspects of microbial consortia such as development of formulation, strain compatibility, mechanism of action, and delivery methods are discussed in this chapter.

Keywords

Plant growth–promoting fungi · Disease suppression · Plant growth promotion · Biological control

Agricultural practices are getting innovative with advancement in science and technology. Even though revolutionary development has occurred in pesticide industry with the advent of many new molecules, the risk of pesticide residues, resistance development, and environmental safety are still major concerns and have opened the doors for new eco-friendly, sustainable approaches like biological control and integrated disease management. The use of plant growth–promoting fungi (PGPF) occupies a small but growing niche in the development of organic agriculture. Soil is enriched with microscopic life forms including bacteria, fungi, actinomycetes, protozoa, and algae. The interaction between soil, plants, and microbes may be beneficial, harmful, or neutral. Plant growth–promoting fungi

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(PGPF) are a class of soil-borne filamentous fungi that have beneficial effects on plants without causing any disease. *Trichoderma, Aspergillus, Penicillium*, and some endophytes have been harnessed as PGPFs in agriculture.

PGPFs produce substances such as plant hormones (e.g., indole-3-acetic acid – IAA), which help plants to utilize organic matter through mineral solubilization (N, P, and Fe) and to suppress plant pathogens in the rhizosphere by various mechanisms, such as the production of hydrolytic enzymes, aggressive mycoparasitism, competition for saprophytic colonization, and the induction of plant systemic resistance. Colonization of the root of plants is one of the most important characteristics of PGPF that helps them to interact with plants to enhance growth and development apart from protection against phytopathogens (Hyakumachi 1994).

The presence of native population of microorganisms in rhizosphere soil offers strong competition to seed-inoculated rhizobia that does not allow the inoculum to form nodules that results in low or failure of inoculation response (Kumar and Chandra 2008). The major approaches for biological control of plant diseases have focused on (i) altering genetic make of rhizosphere components with an aim of disease suppression operable against more than one pathogens; (ii) favorable rhizospheric environment for biological control agent and inhibit other competitive microflora; and (iii) to develop microbial mixtures or consortia with superior bio-control activity (Janisiewicz 1988). Mixtures of two or more antagonists will increase the efficacy or decrease the variability associated with biocontrol treatments (Haggag and Nofal 2006).

A microbial consortium is a group of different species of microbes that act together as a community. For developing a consortium, one can choose microorganisms that are resistant to environmental shock, fast acting, synergistically active, producing natural enzymatic activity, easy to handle, having long shelf life, good sustainability, nonpathogenic, noncorrosive of consistent quality, and economical. Combinations of biocontrol strains are expected to result in a higher level of potential to suppress multiple plant diseases. Commonly, control is based on the use of single biocontrol agents. This strategy must be changed because from the ecological point of view the disease is part of a complex agroecosystem. As opined by Fravel (2007), a holistic view of this system can help take correct decisions about management. Therefore, a special approach for improving the PGPR efficiency is the use of mixtures containing different genera or species that presents additive or synergistic effects such as nitrogen-fixing bacteria and mycorrhiza helper bacteria (MHB). Another strategy is to use PGPR, mixed or alternated with fungicides, integrating biological and chemical methods. The mutual actions between AMF and *Bacillus* spp. led to benefits in terms of plant development in papaya infected with *Meloidogyne incognita*. In the absence of the pathogen, PGPRs enhance the mycorrhizal effect in those plants treated with *G. mosseae* (Vega et al. 2005). There are several reports indicating the combined influence of fluorescent pseudomonads and *Bacillus* spp. along with other BCAs in effective management of crop diseases (Srinivasan and Mathivanan 2011).

Individual application of *Rhizobium* sp. and PGPRs alone significantly increased only N while PSB increased N and P uptake in grain and straw significantly over the uninoculated control. However, their mixed application (i.e., *Rhizobium* sp. + PSB, *Rhizobium* sp. + LK-786 and *Rhizobium* sp. + LK-884) was comparable to their inoculation alone in N uptake by grain and N and P uptake by straw. P uptake by grain was significantly better with *Rhizobium* sp. + PSB than the *Rhizobium* sp. and PSB alone inoculation (Kumar and Chandra 2008).

In recent years, more emphasis has been laid on the combined use of biocontrol agents with different mechanisms of disease control to achieve more consistent results introduced biocontrol agents. Attempts were also made to use a consortium of biocontrol agents that enables persistent suppression of plant pathogens (Chaube and Sharma 2002). Antagonistic bacteria and fungi isolated from the rhizosphere soil were evaluated for control of *M. phaseolina* and *S. sclerotiorum* infections in *Glycine max*. In vitro compatibility of the identified biocontrol agents were exploited for effective management of soil borne diseases with an objective of evaluating potential of consortium biocontrol agents in suppression of soil-borne pathogens of *Glycine max* as well as in greenhouse studies. Jetiyanon and Kloepper (2002) discovered that the use of mixtures of PGPR strains with high potential for inducing systemic resistance against diseases of several different plant hosts in the greenhouse. Jetiyanon et al. (2003) confirmed these findings in the field by application of mixtures of two PGPR strains that more consistently protected several different crop species against multiple diseases in field tests in Thailand when compared to single strain. These experiments were conducted under the multi- or intercropping agricultural conditions prevalent in Thai agriculture. During both rainy and dry seasons, mixtures of *B. amyloliquefaciens* IN937a and *B. subtilis* IN937b significantly protected against all the tested diseases including southern blight of tomato (caused by *Sclerotium rolfsii* Sacc.), mosaic viral disease of cucumber (caused by CMV), and anthracnose of long cayenne pepper (caused by *Colletotrichum gloeosporioides* Penz.). Shelf life of the formulations helps in the development of commercial formulations of biocontrol agents. Formulations should support the viable nature of the product for the increased period of storage. Biocontrol product should have the minimum shelf life of 8–12 months for industrialization. Carrier material should not affect the viable nature of the biocontrol agent. Hence, there is a need for more concentrated research to enhance the shelf life of the formulation by developing superior strains that support the increased shelf life, or the organic formulations that support the maximum shelf life with low level of contaminants in order to make biocontrol as a commercial venture.

Cook et al. (1996) reported eight species of microorganisms registered by U.S. Environmental Protection Agency for commercial use against soil-borne plant pathogens in the United States. These include two fungi (*Gliocladium virens* G-21 and *Trichoderma harzianum* KRL-AG2), three Gram-negative bacteria (*Agrobacterium radiobacter* K84, *Pseudomonas fluorescens* EG1053, and *Burkholderia cepacia* type Wisconsin), and three Gram-positive bacteria (*Bacillus subtilis* GB03, *B. subtilis* MBI 600, and *Streptomyces griseovir-idis* K61). Other than *A. radiobacter* K84, all others are used to manage damping-off diseases and

improve stand establishment and seedling vigor. Thirty isolates of bacteria and six isolates of *Trichoderma* were isolated from fertile agricultural soil and evaluated for their antagonistic activity against phytopathogens like *Macrophomina phaseolina* and *Sclerotinia sclerotiorum*, under in vitro conditions. Different isolates showed varying degrees of antagonism. The three most antagonistic bacteria *Pseudomonas aeruginosa* (MBAA1), *Bacillus cereus* (MBAA2), and *Bacillus amyloliquefaciens* (MBAA3) and one fungi *Trichoderma citrinoviride* (MBAAT) were selected as the most effective isolates as biocontrol agents. The present study was undertaken to develop a plant growth–promoting microbial consortium to reduce the disease incidence in *Glycine max* both under in vitro and in vivo conditions. Biocontrol attributes such as ammonia, siderophore, enzymes like β-1,3 glucanase, chitinase, and cellulase were more in consortia when compared to single isolates. Plants treated with consortia + pathogen showed lower disease incidence in comparison to single antagonist + pathogen and pathogen-infested control ($p \leq 0.05$). Maximum disease suppression was noticed in potted plants treated with *S. sclerotiorum* + MBAA1 + MBAAT showing only 15.8% disease incidence when compared to *Sclerotinia*-infested control (97%) incidence. Seed bacterized with MBAA1 + MBAAT exhibited enhanced seed germination of *G. max* up to 68% along with subsequent increase in other plant growth parameters. Considerable increase in seedling vigor index (1863.2) and chlorophyll content (13.518 mg/g) was observed in seeds treated with MBAA1 + MBAAT in plants infected with *M. phaseolina* (Thakkar and Sharaf 2015).

Diverse mechanisms are involved in the suppression of plant pathogens more often indirectly connected with plant growth. Plant growth–promoting microorganisms (PGPM) and biological control agents (BCA) possess secondary beneficial effects that would increase their usefulness as bioinoculants, regardless of the need for their primary function. Indeed, PGPMs such as *Rhizobium* spp. can promote plant growth and productivity (primary effect) but have now been shown to also play a role in reducing disease (secondary effect). Conversely, BCAs such as *Trichoderma* spp. and *Pseudomonas* spp. not only suppress the disease (primary effect) but have recently demonstrated stimulation of plant growth (secondary effect) either in the presence or absence of a pathogen. Based on these beneficial plant–microbe interactions, it is possible to develop microbial inoculants for use in agriculture. Depending on their mode of action and effects, these products can be used as biofertilizers, plant strengtheners, phytostimulators, and biopesticides. The use of microorganisms and the exploitation of beneficial plant–microbe interactions offer promising and environmentally friendly strategies in conventional and organic agriculture worldwide. However, PGPF-inoculated crops make up only a small fraction of current agricultural practices due to lack of commercialized and effective products. For the extensive commercialization of PGPFs in future days, a number of issues need to be taken care of that include (i) development of efficient strains of PGPFs with effective biological activities; (ii) the release of genetically engineered strains to the environment with the assurance of environmental safety; (iii) a better understanding of the advantages and disadvantages of use of PGPFs; (iv) selection of PGPF strains that function optimally under all environmental conditions;

(v) development of more efficient means of applying PGPFs to plants in various conditions; and (vi) a better understanding of the potential interactions between PGPFs and other soil fungi. Efforts has to be channelized more toward understanding the diversity of PGPFs and use of more suitable strains pertaining to suitable ecology and agriculture ecosystem rather than a blind recommendation with less effective impact on plant growth and development.

Modern agriculture is facing new challenges in which ecological and molecular approaches are being integrated to achieve higher crop yields while minimizing negative impacts on the environment. In this context, enhancing plant growth and plant resistance by using beneficial microorganisms is currently considered as an important key strategy (Pineda et al. 2010). Approximately 300,000 plant species growing in unexplored area on the earth are host to one or more endophytes (Strobel and Daisy 2003; Strobel et al. 2004) and the presence of biodiverse endophytes in huge number plays an important role on ecosystems with greatest biodiversity. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others (Tan and Zou 2001).

Plants are associated with a diverse community of microorganisms. The microorganisms residing within the plants or endophytes are unique in their adaptations to specific chemical environment of host plant. Endophytes are microorganisms (bacteria, fungi, and unicellular eukaryotes) which can live at least part of their life cycle inter- or intra-cellularly inside of plants usually without inducing pathogenic symptoms. This can include competent, facultative, obligate, and opportunistic endophytes. Endophytes can have several functions, and these may change function during their lifecycle (Murphy et al. 2015).

Biological control is gaining momentum in the management of sunflower necrosis virus disease (SNVD) because at present no effective method is available. In glasshouse experiment-I, six different plant growth–promoting microbes (PGPMs) – *Streptomyces* sp. PM5, *Trichothecium roseum* MML005, *Bacillus licheniformis* MML2501, *Streptomyces fradiae* MML1042, *Pseudomonas aeruginosa* MML2212, and *Bacillus* sp. MML2551 – and 2% *Morinda pubescens* fruit extract applied individually (seed + foliar applications) along with sunflower necrosis virus (SNV) were evaluated in sunflower. Among the treatments, *B. licheniformis* (*Bl*), *Bacillus* sp. (*Bsp*), *P. aeruginosa* (*Pa*), and *S. fradiae* (*Sf*) effectively increased the plant growth and significantly increased the reduction of virus titre, ranging from 32.5% to 52.5%. In experiment-II, the above four effective PGPMs (*Bl*, *Bsp*, *Pa*, and *Sf*) were developed as consortia in all possible combinations in this study and were applied along with SNV against SNVD. All the consortial treatments significantly reduced SNVD in virus titre with disease reduction and concomitant increase in growth promotion when compared to control. In experiment-III, the best PGPM consortia (PGPMC) were applied as seed + soil inoculations along with SNV to study the induction of systemic resistance enzymes. The four culture consortium significantly reduced the SNVD symptoms and virus titer with a concomitant increase in plant growth promotion and ISR enzymes compared to control. In

experiment-IV, based on biocontrol efficacy and ISR against SNVD from the experiments I to III, the two more dominant PGPMC treatments were selected and evaluated against SNVD under field conditions. From these results, *Bl* + *Bsp* + *Pa* + *Sf* effectively reduced the SNVD and improved the plant growth and yield parameters with additional seed yield with income and benefit–cost ratio when compared to farmer's practice. In conclusion, PGPM (*Bl*, *Bsp*, *Pa* and *Sf*) was found to be very effective against SNVD under glasshouse and field conditions.

17.1 Research Efforts in Management of Crop Diseases by Exploitation of Microbial Consortia and ITK Measures

Regarding validation of Indigenous Technology Knowledge, UAS, Bengaluru, is the pioneer research institute in India due to its contributions in plant protection. Management of soil-borne plant pathogens is very difficult, especially in Fusarium group which is soil inhabitant that survives in soil for more than 50 years. The management through chemical method is often uneconomical.

The cow milk, curd, ghee, cow dung, and cow urine have been used individually used for curing many ailments as described in ancient text. It is know that cow ghee and curd contain certain living entities and antimicrobial substances. The Panchagavya is the product of five cow products such as milk, curd, ghee, dung, and urine. In traditional Hindu families, it is also taken as Panchamratha in little quantity for purification both external and internal environment of systems. The innovative research on the use of modified Panchagavya mixture (MPG-3) was carried out on three soil-borne diseases like Fusarium wilt of tomato and banana, and also foot rot of black pepper. The traditional Panchagavya was modified by adding yeast and common salt, and all the three formulations were tested. The components of three MPG-3 were most effective in managing all these plant diseases, which included 2 ml of ghee, 5 ml of curds, 5 ml of milk, 40 g dung, and 48 ml of urine mixed with 2 g yeast and 2 g salt for 100 ml preparation. These components were mixed by adding one after the other in plastic container and kept for fermentation for 7 to 10 days with closing of plastic container. The addition of salt is to reflect Jim Martin's living water promoting microbial activity which is further augmented with addition of yeast. The fermented preparation was diluted ten times with water and filtered through two layers of muslin cloth to obtain clear filtrate. The filtrate was used in different delivery methods of seedling dip for 30 min and soil drenching for the pre-infested soil with the pathogen in the investigations.

(i) **Management of Panama disease of banana**: In case of Panama disease of banana, MPG-3 was used at 10^1 dilution along with different bioagents like *Trichoderma viride* (0.25%), *Pseudomonas fluorescens* (1 h dip, 10^8 cells/ml), and *Bacillus subtilis* (1 h dip, 10^6 cells/ml). The MPG-3 gave better influence on plant height, number of leaves, maximum root length, and pseudo stem girth etc. There was reduction in Fusarium population in MPG-3, which provided encouraging results compared with seedling dip. The population of *Fusarium*

oxysporum f. sp. *cubense* declined significantly to 11.8 × 10^4 cfu/g after 150 days of planting. These results indicate the promise shown by MPG-3 in eco-friendly and cost-effective management of Fusarium wilt (Jahagirdar 1995; Jahagirdar et al. 2000).

(ii) **Management of foot rot of black pepper**: Developed and standardized effective IDM package: Soil application of *T. viride* (75 g/pt) + spraying with metalaxyl (1.25 g per L) + Akomin (4 ml per L) or MPG 3 (10^1) for the management of foot rot of black pepper (Jahagirdar 1995; Jahagirdar et al. 2000).

(iii) **Management of damping off of tomato in nursery and main field**: The research work carried out UAS, Bangalore, clearly demonstrated the role of MPG-3 as PGPR component and ISR activity against Fusarium wilt of tomato (Padmodaya 1994).

(iv) **Management of tobacco mosaic virus through organics**: Tobacco Mosaic Virus (TMV) is the major stumbling block for successful cultivation of bidi tobacco in Nipani area. Identification of resistant source against such systemic biotic infection is a challenging task for plant pathologists and plant breeders. In order to give a boost to ruling cultivators which are susceptible for TMV, application of Virosin @ 2% (27.7% disease incidence) followed by bougainvillea leaf extract @ 5% (30.2%) incidence and neem 1500 ppm(31.8%) incidence. Among ITK measures, Panchaghavya @ 5% (37.7%) followed by cow urine @ 10% (37.8%) incidence. The untreated check recorded maximum incidence of 56.5% incidence. There was no significant difference among the treatments with respect to growth parameters. However, higher plant height, leaf length, and leaf breadth were recorded in Virosin, neem 1500 ppm, and cow urine application indicating role-induced systemic resistance. Maximum cured leaf yield (1206 kg/ha) was recorded in cow urine @ 10% followed by Virosin @ 2% (1157 kg/ha). Among quality parameters nicotine percentage ranged from 2.66 to 4.16 with maximum (4.16) in neem leaf extract followed by 3.77% in buttermilk @ 5%.The reducing sugar ranged from 5.63% to 10.14% with maximum (10.14%) in neem @1500 ppm followed by 9.78% in cow urine @ 10%. The chloride percentage was within the limit of <1 except buttermilk (1.07%). Thus, the investigations opened a new window of opportunity in managing TMV infections through ITK measures enhancing both leaf yield and quality parameters in bidi tobacco (Jahagirdar et al. 2008).

(v) **Management of Asian soybean rust in India**: The Asian Soybean rust, *Phakopsora pachyrhizi* Syd, is an economically important disease which causes significant yield loss in India. Lack of resistant cultivars, growing concern over use of chemical pesticides, and increasing area under organic soybean cultivation has led to exploitation of Indigenous Technology Knowledge (ITK) in the management of Asian Soybean Rust. The pooled analysis over 2 years revealed that among the ITK measures application of cow urine @ 10% + *Pongamia pinnata* oil @ 0.5% recorded minimum Percent Disease Index (PDI) of 37.9 followed by cow urine @ 10% alone (40.3). The chemical elicitors like MnS04, Muti-k, or plant-based extracts like *A. vesica*. *Pongamia*

pinnata oil and bioagent like *Trichoderma harzianum* along with cow urine are being used in developing Integrated Disease Management strategies against Asian soybean rust in India which will help in reducing the chemical pesticides in long-term sustainable management. The present findings draw on the first line of research on large-scale application of Indigenous Technology Knowledge in suppressing rust and enhancing both yield and quality parameters (Jahagirdar et al. 2009).

(vi) **Development of bio-intensive integrated disease management strategies against soybean rust:** In Karnataka, area under soybean is increasing and the crop has attained its multifold dynamism due to its cultivation in both *Kharif* and summer seasons. This has mainly affected the epidemiology of rust in the region. The disease has been observed in most severe form in major soybean-growing areas in northern Karnataka districts. Till today there are no promising resistant cultivars against this disease. Under this background development of eco-friendly integrated management becomes the key factor for successful management of the disease in the region.

The results of 2 years' study (2009 and 2010) on Development of Biointensive Integrated Disease Management strategies against soybean rust revealed the significant superiority of seed treatment with *Trichoderma harzianum* @ 6 g/kg + spray with cow urine @10% + *T.harzianum* @ 0.5% recorded minimum (35.1) Percent Disease Index (PDI) followed by 37.4, 38.9 PDI in case of spray with cow urine @ 10% + potassium phosphonate @ 0.3% and neem oil @ 1%, respectively. However, minimum PDI was recorded in Hexaconazole @ 1 ml/l (30.5) which is statistically on par with each other. The maximum incidence of 87.8 PDI was recorded in untreated check. The maximum seed yield of 18.06q/ha was recorded in Hexaconazole@0.1% followed by ST with *Trichoderma harzianum* @6 g/kg + Spray with Cow urine @ 10% + *T. harzianum* @ 0.5% (17.15q/ha) which are statistically on par with each other. The minimum seed yield was recorded in untreated check (9.06q/ha). The role of biochemical parameters in triggering defense genes and increasing in seed yield apart from bringing down the disease pressure has been demonstrated successfully in the outcome of this project. Among the biointensive strategies, reducing sugars was maximum(0.737%) in seed treatment with *Trichoderma harzianum* @ 6 g/kg + spray with cow urine @ 10% + *T. harzianum* @ 0.5% followed by 0.707% in case of seed treatment with *Pseudomonas fluorescens* @ 10 g/kg + spray with cow urine @ 10%+ *Pusedomonas fluorescens* @ 0.5%.The minimum reducing sugars was recorded in untreated control (0.071%). With respect to nonreducing sugars, seed treatment with *Pseudomonas fluorescens* @ 10 g/kg + spray with cow urine @ 10% + *Pseudomonas fluorescens* @ 0.5% recorded maximum (10.53%) followed by 9.59% in case of *Trichoderma harzianum* @ 6 g/kg + spray with cow urine @10% + *T. harzianum* @ 0.5%. The minimum nonreducing sugars percentage was recorded in untreated control (1.22%). We also studied role of different enzymes in triggering the host defense by use of these ITK measures by employing Lowry's Method and Poly Acrylamide Gel Electrophoresis (PAGE) for assessing the total protein and estimation peroxidase, polyphenol

oxidase, and catalase activity. The peroxidase activity ranged between 25 and 70 Kda. The peroxidase activity was better in all the biointensive treatments, indicating triggering of host defense genes. The maximum peroxidase activity was recorded in cow urine @ 10% and neem oil @ 1%. We also studied the expression of polyphenol oxidase which is the key factor for upregulation of defense genes in Induced Systemic Resistance (ISR) against rust pathogen. The maximum polyphenol activity was recorded in cow urine @ 10% + neem oil @ 5%, cow urine @ 10% + *Trichoderma viride* @ 0.5%, and neem oil @ 1%. The polyphenol activity ranged between 55 and 110 Kda. There was no expression of catalase activity in any names of treatments. This signifies the absence of catalase pathway in ISR against soybean rust. The studies brought for the first time a new information on salicylic acid–based pathway in inducing defense in the soybean using ITK measures. This information will be a key factor in developing ISR elicitors against soybean rust. This helps to reduce pesticide application by the farmers for managing soybean rust and minimizes the cost of production and also helps plant growth promotion and ISR activity against soybean rust. The expression of these defense genes ultimately helped in realizing maximum seed yield on par with chemical control (Shamarao Jahagirdar et al. 2013).

Regular collection of samples from rust-infected areas only yielded uredospores and failed to get telial stage. The identification of telial stage still forms the basis for understanding epidemiology of the disease in the region.

17.1.1 Technology Adoption and Spread

Thus, seed treatment with *Trichoderma harzianum* @ 6 g/kg + spray with cow urine @ 10% + *T. harzianum* @ 0.5% or cow urine @ 10% + potassium phosphonate @ 0.3% *or* neem oil @ 1% be recommended for management soybean rust in Karnataka that helps to minimize the use of hexaconazole.

(vii) **Endophytes in management of soil-borne diseases of soybean**: The antagonistic effect of 30 bacterial endophytes of soybean collected from northern Karnataka and parts of Maharashtra against *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Fusarium oxysporum* were assayed *in vitro* through dual culture plate technique. The bacterial endophytes RB-KK-6 (40.78%), SB-BS-6 (50.08%), and LB-BU-1 (47.02%) were found effective against *S. rolfsii* and the isolates SB-DG-11 (47.41%) and LB-BiN-8 (41.22%) were effective against *R. bataticola.* The effective bacterial endophytes against *F. oxysporum* were RB-HS-1 (41.99%), SB-BiJ-9 (40.07%), LB-BU-1 (54.20%), and LB-BV-2 (51.64%). Based on molecular characterization, the effective bacterial endophytes were identified as *Acinetobacter* sp. (RB-HS-1), *Alcaligenes faecalis* (RB-KK-6), *Stenotrophomonas* sp. (SB-BiJ-9), *Bacillus pumilus* (SB-DG-11 & LB-BiN-8), *Paenalcaligenes* sp. (LB-BU-1), *Bacillus cereus* (SB-BS-6), and *Brevibacillus* sp. (LB-BV-2) (Brunda et al. 2018) (Fig. 17.1).

Fig. 17.1 Diversity analysis studies of endophytes of soybean

(viii) **Eco-friendly approaches in the integrated management of root knot nematode in bidi tobacco – 2003 to 2007**: Preamble: Different types of tobacco are being cultivated in India under different agro-climatic conditions. In India, various types of tobacco are being cultivated, and among these the Nipani area of Belgaum district of Karnataka is known for production of quality bidi tobacco. In this region, continuous cultivation of tobacco has led to building up populations of *Meloidogyne incognita* (Kofoid and White) Chitwood. The disease has become a constraint for tobacco cultivation in the area. It infects at any stage of the crop and causes considerable loss in quality and yield of tobacco. Bidi tobacco is bread and butter of Nipani farmers of northern Karnataka. Our 4-year research efforts finally gave solid recommendation as poultry manure (1 t/ha) mixed with Carbofuran 3G (5 kg/ ha) was the most effective, suitable, eco-friendly, and economically viable strategy for the management practice of root-knot disease of bidi tobacco which has reduced excess use of carbofuran in the area (Jahagirdar and Hundekar 2007a, b, 2008).

17.2 Classical Examples of Local Practices Adopted for Plant Disease Management Are as Follows

- Regulation of shade in the orchard for the management of coffee leaf rust and blister light of tea.
- Growing of windbreakers like silver oak, casuarina, jackfruit, etc. to avoid sun scorching of young shoots of plantation crops.
- Tying of areca nut seedlings with coconut and areca nut fronds to protect them from western sun scorching.
- Lime pasting on areca trees to avoid ill effects due to sun scorching.
- Watering nursery beds in early morning for higher seedling vigor and stand, particularly followed in chili and brinjal.
- Burning of leaf litter and farm waste to overcome certain soil-borne pathogens in the seed bed nursery.
- Raised beds, fields, and ridges used to manage some soil-borne pathogens. In Mexico, raised beds are called as Chinampas or floating garden and were used to control *Pythium*, *Phytophthora*, and other soil-borne pathogens.
- Collection and burning of plant residues and stubbles in the field to tackle the problem of soil-borne pathogens.
- Flooding with water to overcome the problems of soil-borne pathogens by creating anaerobic conditions. In our studies, flooding for 85 to 100 days brought down significantly the *Fusarium oxysporum* f. sp. *cubense* population causing panama disease of banana.
- Earthing up to overcome the problem of pythium damping off in nursery in brinjal and tomato.
- Kotte tying for areca bunches to overcome problem of koleroga of areca nut.

- Planting across the wind direction helps to manage some airborne diseases.
- Mixed cropping of jowar with tur to prevent the movement of mites which transmit sterility mosaic of pigeon pea and to minimize tur wilt.
- Manipulation of planting time/sowing to overcome problems of foliar diseases, for example, Tikka disease of groundnut and anthracnose of chili.
- Ploughing in summer to reduce the problem of nematode infestation and soil-borne pathogens.
- Crop rotation with legumes, cereals, and millets to overcome the problem of soil-borne plant pathogens.
- Mulching with green manure to overcome problem of soil-borne pathogens in paddy.
- Saltwater treatment to overcome the problem of seed-borne diseases, for example, Bunt and seed gall in wheat.
- Cultivation of covered beans and combination of mulch and beans effectively prevented bean blight in north Costa Rica by traditional farmers through a system called tapaga.
- Maize earheads benching in Mexico to manage seed-borne fungal pathogens.
- To tackle problem of stem bleeding in areca nut, tying of paddy thread prepared out of hay near the crown region of coconut and placement of 1 kg of salt (Jahagirdar et al. 2003a, b, c).

17.3 Role of Biological Control in Organic Agriculture

Application of bioagents in management of crop diseases is generally termed as biological control. Many workers have tried to define biological control. The most commonly accepted definition by Baker and Cook (1974) specified: "reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of environment, host or antagonist, or mass introduction of one or more antagonists." Further, Cook and Baker simplified biological control as the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man.

Mukhopadhyay (1987) underlined that "Biological control of soil borne plant pathogens by *Trichoderma* spp. and other bioagents as a vital area of plant pathological research all over the world these days." Biological plant protection is an integral component of ecofriendly management of plant diseases all over the globe. It is now widely accepted as a distinct possibility for the future and can successfully be exploited within the frame work of Integrated Pest/Disease Management System.

17.3.1 Why We Are Giving Importance to Biological Control of Plant Diseases?

Following are some of the points presently supporting management of plant diseases through bioagents.

1. They avoid environmental pollution of soil, air, and water unlike in chemical control.
2. They avoid the residual toxicity of crop products unlike in chemical control.
3. They avoid adverse effects on beneficial microorganisms including antagonist in the soil whereas chemical controls are lethal.
4. They are comparatively less expensive compared to chemical control.
5. There is no development of resistance by the pathogens unlike in chemical control.
6. Bioagents application is usually once and do not need repeated applications while chemicals have to be applied at regular intervals.
7. Bioagents are more effective especially for soil-borne diseases whereas fungicides are generally uneconomical and fail to reach target site.
8. Biological control is the only option to tackle problem of virus diseases in the absence of host plant resistance.
9. Biological control is risk-free management of plant diseases when compared to chemical control, that is, phytotoxicity and residue problems.
10. They have become an integral part of modern large-scale agriculture for sustainable productivity.

17.3.2 Advanced Approaches to Biological Control

The four fundamental approaches of biological control are as follows:

(a) Destruction of propagules or biomass of pathogen by hyper-parasitism or predation.
(b) Prevention of inoculum formation entering into disease-free areas.
(c) Reduction in pathogen virulence by competitive saprophytic ability.
(d) Reduction of vigor of virulence or pathogen by agents such as mycoviruses or hypo-virulence determinate.

Biological protection agents' infection is achieved by

(i) Protection of planting material
(ii) Protection of roots with biological seed treatment
(iii) Biological protection of foliage and flowers
(iv) Inoculation of pruning wounds with antagonists

17.3.3 Mechanisms of Biological Control

Antagonism includes antibiosis, competition, and mycoparasitism, and the mechanism of biological control of plant diseases operates through one or both or all of these together or singly. In addition, the mycorrhizae, plant growth–promoting rhizobacteria (PGPR), cross-protection, and induced resistance are also operating during the biological control process. In addition, non-pathogenic strains/avirulent strains, mycoviruses, and hypovirulences are being used in suppressing the plant pathogens either through ISR/SAR approaches, HR reaction, or RNAi-mediated resistance.

Antibiosis Antibiosis is the inhibition of pathogen by the metabolic (antibiotic) product or products of the antagonist. The antagonist releases antibiotics or other metabolic products (enzymes), which are harmful to the pathogen and inhibit its growth.

Competition It is the endeavor of two or more microorganisms to gain the measure each wants from supply of substrate in the specific form and under specific conditions in which that substrate supply is not sufficient for both. In essence the competition is for nutrients (high energy carbohydrates and nitrogen), and also for space and oxygen, but not for temperature, PH, and water potential. The antagonists grow very fast and utilize all the food and occupy the space, and thus make the pathogen weak. Heterotrophic rhizobacteria like fluorescent pseudomonas compete for iron with plant pathogens, very efficiently use the iron, and produce siderophores (microbial iron transport agents), which complex the iron and thus affect them adversely.

Mycoparasitism and Predation Mycoparasitism (= Hyperparasitism) is defined as parasitism of one fungus by another. Several necrotrophic mycoparasites have potential biocontrol agents. The mechanism of mycoparasitism includes different kinds of interactions like coiling of hyphae around the pathogen, penetration, production of haustoria, and lyses of hyphae. Recently, it is also postulated that necrotrophic parasites kill susceptible fungi by the action of toxins, antibiotics and or enzymes.

Induced Systemic Resistance In the later part of the 1990s, the research on plant growth–promoting rhizobacteria (PGPR) and induced systemic resistance (ISR) clearly gave a hint on the role of useful microbes in imparting resistance to plants for specific group of pathogen. Free-living root-colonizing bacteria (rhizobacteria) have been studied for the past century as possible inoculants for increasing plant productivity and controlling microbial pathogens. Soil or seed applications with PGPR have been used to enhance the growth of several crops (Glick 1995), as well as to suppress the growth of plant pathogens. PGPR that colonize root systems through seed applications and protect plants from foliar diseases include *Pseudomonas* spp., *Bacillus* spp., *Paenibacillus* spp., and *Serratia* sp. The mechanisms for

plant growth promotion and induced systemic resistance (ISR) by PGPR have been extensively studied in the past decade. There are several determinants for mechanisms of growth promotion that include bacterial synthesis of the plant hormones (indole-3-acetic acid (IAA), cytokinin, and gibberellins), breakdown of plant-produced ethylene by bacterial production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and increased mineral and N availability in the soil. Recently, the phenomenon that PGPR elicit plant defense has also been found to lead to a state of ISR in the treated plant. ISR occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens ISR is different from systemic acquired resistance (SAR) that triggers systemically plant defense response following hypersensitive response after inoculation of plant pathogens. Previous works demonstrated that several bacterial determinants such as siderophores, salicylic acid (SA), and lipopolysaccharides (LPS) contributed to ISR (Choong-Min Ryu et al. 2005).

17.3.4 Role of Biological Control in the Integrated Disease Management (IDM)

Biocontrol agents form an effective component in the integrated management of diseases. Under high disease pressure or pathogen population pressure, the biocontrol may be less effective and needs other practices also to completely manage the disease. Jahagirdar et al. (2001) reported the effectiveness of seed treatment with biocontrol agent if integrated with other management practices, that is, the use of moderately resistant cultivar + biocontrol + FYM helped in managing the disease. The effectiveness of biocontrol agents in other crops against soil-borne pathogens are presented in table for reference.

Success of Biological Agent Depends on the Following

1. Selection of virulent strain of antagonist.
2. Fast growing and highly sporulating on the mass culture media in case of facultative before applying.
3. Preventive application to provide enough time for interaction between antagonist and target pathogen.
4. Optimum soil temperature, moisture for establishment, and growth of antagonist.
5. For survival and multiplication of the antagonist sufficient organic matter (food base) should be present in the soil.
6. Regular assessment of population of the target pathogen and antagonist from time to time on selective media.
7. Ease in handling, production, storage of product and should be cheap and available.
8. Integrated strategy of biocontrol with tolerant/resistant varieties rather than chemical control alone.

Future Line of Research in Respect of Biological Control

1. Isolation and evaluation of native antagonists, their multiplication, and preparation of seed treatment formulations.
2. Mapping up of population dynamics of pathogen and biocontrol agents in various geographical areas of production systems.
3. Application biotechnological techniques, genetic engineering, protoplast fusion technique, etc., may be employed in developing efficient strains of antagonists which are site specific, area specific, and crop specific would further pave way for faster developments in biological control.

Inference

Scientists dealing with management of crop diseases are beginning to evince keen interest in indigenous technology knowledge. Recently this knowledge is being made available, and research endeavors are reoriented toward validation of indigenous methods encouraging Integrated Disease Management (IDM) practices. Identification of ideal bioagent for its per se performance under different regions in the backdrop of climate would surely lay the foundation for effective management of diseases without affecting natural ecosystem adversely.

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Microbial Biofilm: Formation, Quorum Sensing, and Its Applications in Plant Disease Management

18

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Abstract

In search of an eco-friendly plant disease management, a ray of hope for sustainability was created after the recognition of microbial strategy on plant surfaces was adapted under adverse environmental conditions in the early 1970s, which are the microbial aggregations termed as biofilms. The assemblage of microbes on plant surfaces are formed due to microbial adhesion, growth, and expansion process, which in turn depends on surface tension, texture, and wettability. The microbial cells in biofilm communicates by various signaling molecules in order to modulate their functional mechanism by controlled release of antibiotic and toxins and in regulation of gene expression through quorum sensing. The microbes that are capable of forming biofilms include various bacteria, yeast, fungi, and symbionts which are not only antagonistic to phytopathogens but also help in enhancement of plant growth and development by acting as a sink for nutrients as a function of site of colonization of plant parts.

Keywords

Biofilm · PGPR · Microbes · Quorum sensing · Biocontrol · Plant diseases

18.1 Introduction

Plants come across a number of pests and diseases, a scenario which can be compared to a battleground in which plants struggle against pathogens for their survival. In several strategies adopted to combat phytopathogens, using of synthetic chemicals became quite common. As most of them cause environmental threat due to their broad-spectrum activity, it is one of the major ecological challenges for plant

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pathologists as well as microbiologists in near future. Therefore, there is an urge to replace such environmental harmful chemicals with environment-friendly substitutes like biocontrol agents. These beneficial microorganisms are involved in plant disease control, which is considered as one of the most promising methods for rational, ecological, and eco-friendly crop management practices and also ensures reproducible performance in natural environments. When the combination of different microorganisms with different modes of action are imposed in both in vitro and in vivo, the efficacy of different biocontrol agents can be enhanced such that every component can be individually colonized without posing negative effect on development of the others. Therefore, mono strain of a biocontrol agent having several mechanisms to reduce disease incidence is a prerequisite in adopting any biocontrol agent against agricultural pathogens. Despite all the research work on the biological control of oomycetic soil-borne plant diseases, there are still no commercially thriving examples against such oomycetic diseases. There are several reasons for the lack of adoption of biocontrol management by the growers, which may be due to insufficient knowledge on the mechanism of pathogenesis and mode of action of biocontrol agents and plant pathogens. Biocontrol microorganisms do not generally perform well in uncontrolled conditions in soil to compete with chemical fungicides so, there is a need to understand the mechanism of biocontrol agents. Considering safety issues associated with the use of biocontrol agents, four possible adverse effects are generally identified such as dislocation of nontarget organisms, allergenic to animals and humans, toxicity and pathogenicity, and genetic stability (Cook et al. 1995). Except for allergenic activity, all the factors that enhance inoculant survival and efficacy also increase their reputed adverse effect, therefore a minimal potential biohazard is inherent to any employment of biocontrol agent (Migheli 2001). Furtherance, this chapter emphasizes the need of thorough risk evaluation for the safe use of any novel biocontrol agent.

Recently, the capacity to form biofilms on plant parts was recognized as a possible mechanism of biocontrol management (Scherm et al. 2001; Ortu et al. 2005). Generally, microorganisms exist in multicellular aggregates in natural environment which is usually described as biofilms in which the individual cells have intimate contact with other cells. Its formation is a major bacterial adaptive strategy to environment in aquatic and also on other solid surfaces. Cells adhere to each other's surface through a complex matrix medium comprising a variety of extracellular polymeric substances (EPS) including exopolysaccharides, proteins, and DNA.

The recognition of aggregated microbes surrounded by EPS adhering to the surfaces or located in tissues is not new to human beings, but a well-known phenomenon since Leeuwenhoek and Pasteur described it in environmental and technical microbiology. The concept of biofilms, however, was initiated in the early 1970s due to the observation of heaps of *Pseudomonas aeruginosa* in lung tissue and sputum in cystic fibrosis–infected patients. The term "biofilm" was introduced into medical industry by J. W. Costerton in Costerton 1995. This chapter emphasizes the importance of biofilm formation in plant disease control. Microbes have many benefits of biofilm, for example, it acts as a sink for the nutrients in the

rhizosphere, aids bacteria to survive under unfavorable conditions, etc. Besides that, it helps in exchanging the genetic material.

18.2 Biofilm: Definition and Concept

Biofilms have great realistic importance in agricultural, medical, and industrial sectors and its formation plays a predominant role in microbial lifestyle. Biofilm is defined as “Highly structured and surface attached closed communities of the cells enclosed within a self-produced extracellular polymeric matrix substance” (Branda et al. 2005). Bacterial cells produce a mixture of extracellular polymeric substances (EPS) as well as different exopolysaccharides, DNA, and proteins while attached on the surface, and they have distinct physiological construction cells within it that vary from each other, and up- and downregulation of genes also may vary from cell to cell (Ramey et al. 2004). A biocontrol bacterium can affect plant growth by various mechanisms (Glick et al. 1999; Timmusk and Wagner 1999; Timmusk et al. 2005; Perneel et al. 2007; Rezzonico et al. 2007; Tran et al. 2007). Plant root exudates and root electrical signals selectively influence bacterial colonization and biofilm formation (West et al. 2002; Bergsma-Vlami et al. 2005; Kiely 2006; Van Loon 2007). The colonization rarely occurs as individual cells. Complex multicellular communities such as biofilms and fruiting bodies are commonly coexisting forms in nature (Davey and O’Toole 2000; Palkov’a and V’achov’a 2006; Ngo Thi and Naumann 2007). Biofilms are formed due to cellular recognition of specific or nonspecific attachment sites on the surface, nutritional signal, or by exposure of planktonic cells to sub-inhibitory concentrations of antibiotics (Watrick and Kolter 2000).

The microbial biofilms were protected by EPS surrounding them as they consist of carbohydrates, nucleic acid, proteins, and various other substances and also have structural and functional role for biofilm communities under diverse conditions. As extracellular polymeric matrix substance acts as an anion exchanger, there is a massive restriction of entry for foreign microbes and other compounds into the biofilm. It also protects the bacteria from various environmental stresses like desiccation, osmotic shock, pH change, and UV radiation and acts as a sequester for metal ions and different toxins. Microbes in biofilm formation work in a syntrophic manner, and because of multispecies approach of biofilm lead to the effective nutrient ability by syntrophism and anaerobic degradation of compounds (Rafique et al. 2015).

In order to cope up with altering environmental circumstances, microbes in the biofilm carry out exchange of genetic material. This results in genetic diversity in which microbial communities obtain new genetic material, followed by transcribing it to genes. Biofilm has the potential to develop a barrier of EPS against antimicrobial diffusion molecules (Rafique et al. 2015).

The mechanism initially reported by Thomashow’s group (Weller and Thomashow 1994) has gained less attention due to difficulties in studying natural systems. However, biofilms could have the potential find for combating against under natural conditions.

18.2.1 Identification of Biofilm

Biofilm configurations may range in complexity from flat, relatively featureless films to tightly clustered aggregates to complex heterogeneous cellular arrangements such as towers and streamers. The biofilm forming cells responds to waste and nutrient product diffusion gradients and modulate their metabolism which depends on their site of colonization within the biofilm and thus engage in cell–cell communication (Ramey-Hartung et al. 2005).

The biopolymers in biofilm were purified with several precipitation steps using ethanol and cetyltrimethylammonium bromide and the carbohydrates were analyzed using various color reactions, infrared spectroscopy, and high performance liquid chromatography which revealed that the biopolymer is a homo polysaccharide and it consists of various sugars such as glucose, galactose, mannose, and xylose, and such secretion is important in *P. polymyxa* biofilm development (Haggag 2007).

Infrared microspectroscopy assays were used for the characterization and detection of antibiotics in addition to the development of major biofilm-forming metabolites. Furthermore, atomic force microscopy assays are being used for understanding of physical properties and persistence of biofilm on solid surfaces which are important for agricultural applications (Fig. 18.1) (Haggag 2007).

18.2.1.1 Sites of Biofilm Formation on Plants

The activity and formation of biofilm mainly depends on the microenvironment of the plant parts which differs in saturation levels, nutrient availability, and surface chemistries (Table 18.1).

The gram-positive bacteria – *Bacillus* spp., *Listeria monocytogenes*, *Staphylococcus* spp., *lactic acid bacteria*, and gram-negative bacteria – *E. coli* and *P. aeruginosa* also forms biofilms. Some nitrogen-fixing symbionts form biofilms on legume roots and other inert surfaces, for example *R. leguminosarum* and *Sinorhizobium meliloti*.

18.2.1.2 Agents and Factors Involved in the Formation of Biofilms

Microbial biofilm formation and its growth are impacted by several aspects such as nature of fabricated material, texture, surface tension, and wettability (Rafique et al. 2015).

Rough surfaces offer more chances for the attachment of microbe to form biofilm and also influenced by the nutrients release and exudation at different sites and hydration levels on various sites of colonization. Also, it was observed that in moist and nutrient-rich sites the bacteria can grow into aggregates and biofilms (Rafique et al. 2015).

One of the agents involved in biofilm formation are certain proteins present on outer membrane on the surface. In recent reports, a cell surface protein called Lap A

Table 18.1 Site of colonization of biofilms

S. No	Site of colonization	Bacteria
1)	Aerial tissue	*Erwinia amylovora* (Monier and Lindow 2003)*, E. chrysanthemi, P. fluorescens, P. syringae pv. syringae* (Rojas et al. 2002)
2)	Vascular tissues	*Clavibacter michiganensis subsp. sepedonicus* (Marques et al. 2003), *Pantoea stewartii subsp. stewartii* (Leigh and Coplin 1992), *Ralstonia solanacearum*, *Spiroplasma* spp. (Kang et al. 2002), *Xanthomonas campestris pv. campestris, Xylella fastidiosa* (Purcell and Hopkins 1996; Newman et al. 2003, 2004)
3)	Roots	*Agrobacterium tumefaciens* (Gage 2004), *Azospirillum brasilense* (Burdman et al. 2000)*, Bacillus cereus*, *P. aeruginosa, P. fluorescens, P. putida, Rhizobium* spp., *R. leguminosarum*, *Biovar trifolii* (Espinosa-Urgel et al. 2002; Williams et al. 2008)
4)	Seeds and sprouts	*Escherichia coli* (Fett and Cooke 2003), *P. fluorescens, P. putida, Salmonella* (Espinosa-Urgel et al. 2002)

(Large adhesion protein A) of 900-kDa is identified to affect colonization. Similarly, in *P. putida*, homologues to Lap A, KT2440 are reported to involve in the seed adhesion and competitive root colonization (Rafique et al. 2015).

In *A. tumefaciens* transcription factor SinR was reported to be a regulator in biofilm formation which is an oxygen-responsive regulator of fumarate and nitrate reductase (FNR) family of proteins (Rafique et al. 2015).

18.2.2 Stages of Biofilm Formation

Microbial biofilm formation includes five different stages (Monroe 2007) during 12-day incubation period. Spectroscopic and microscopic equipment can be used for the identification of different developing stages of bacterial biofilms (Fig. 18.2). The biofilm-forming microbes follow the principle of Brownian motion, which attaches to the surface and readily removes from the surface which follows mild rinsing (Rafique et al. 2015).

In biofilm formation, initiation stage is slow and persists for short period of time, which is induced by environmental signals. This stage is reversible, and there is a chance of detachment of cells. Their individuals exhibit logarithmic growth rate followed by irreversible growth stage. The biofilm formation cells induce chemical signals to communicate with adjacent cells. Motility is decreased, cell aggregates are formed, and cell aggregates become progressively layered (Monroe 2007). The genetic mechanism for EPS production is activated that can able to trap nutrients and planktonic bacteria. In maturation stage, cells are progressively layered and attain a thickness of >10 μm. Followed by dispersion happens either by shedding of daughter cells from actively growing cells and depletion of nutrient levels or quorum sensing or by detachment of biofilm aggregates by physical forces (Monroe 2007).

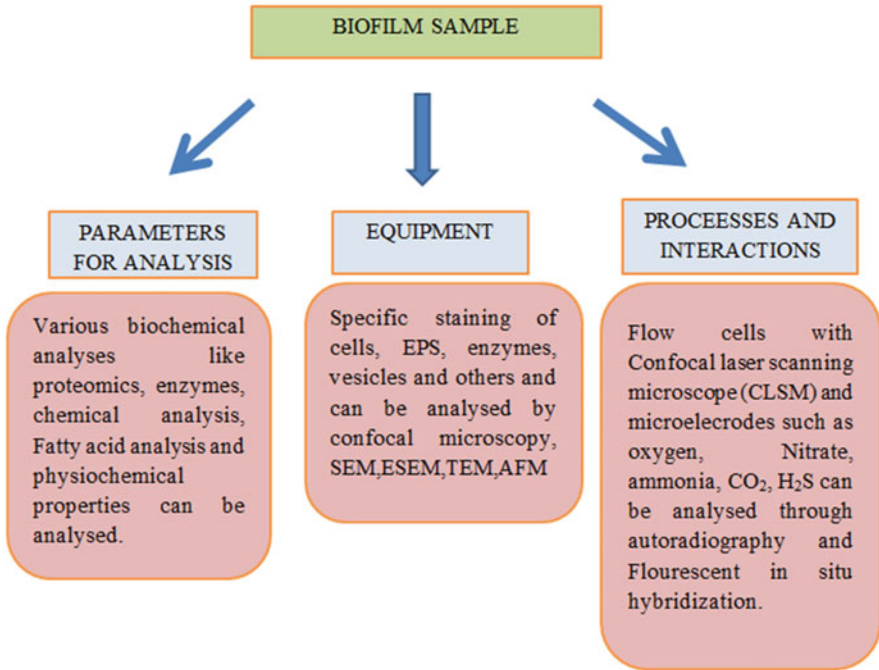

Fig. 18.1 Biofilm analysis

18.2.2.1 Composition of a Biofilm

Biofilm is composed of many substances of which the function of exopolysaccharides, proteins, extracellular DNA (e-DNA), lipid components of biofilm matrix were widely studied.

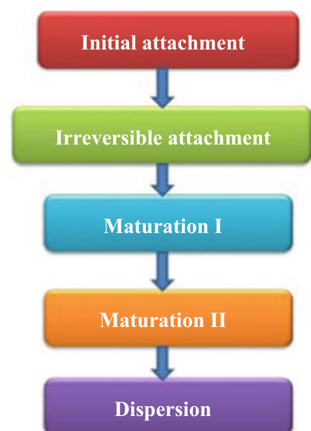

Fig. 18.2 Flow chart representing five stages of biofilm formation

Though the function and chemical composition of each exopolysaccharides varies with different species, most of them are polyanionic molecules due to presence of uronic acids and sugar having substituents such as pyruvate, sulfate, or phosphate. EPS regulates the carbon source (Amellal et al. 1998) and stabilizes the biofilm structure by enhancing the water retention in microbial environment (Bogino et al. 2013).

Proteins are major constituents in biofilm matrix as they perform as extracellular enzymes and they have a role in degradation and recycling of biopolymers as well; thus they enable the nutrients to retain in the matrix. Proteins help in shaping and dispersion of cells from the biofilm structure by modifying other exopolymers. Some of the proteins in the biofilm matrix have structural functions, for example, lectins that bind bacterial cells to polymeric matrix (Bogino et al. 2013). Glucan-binding proteins in *Streptococcus mutans*, LecA and LecB in *P. aeruginosa*, Lectins in *A. brasilense* in *P. aeruginosa*, a large quantity of matrix proteins was found in outer membrane vesicles, a typical biofilm component in this species. Amyloids are another type of matrix protein which is common with extracellular adhesin function (Bogino et al. 2013).

Extracellular DNA (e-DNA) plays an important role for exchange of genetic material as a part of evolution process. In some gram-positive bacteria, e-DNA is found to be involved in adhesion to hydrophobic surfaces (Bogino et al. 2013).

In biofilms, generally lipids act as biosurfactants with some functions such as surface activity, dispersal, and bioavailability of hydrophobic substances, antibacterial or antifungal properties, and bacterial attachment and detachment (Bogino et al. 2013). Lipopolysaccharides from bacterial outer membranes have been reported to be involve in the induction of induce systemic reaction (Peer and Schippers 1992).

18.2.3 Quorum Sensing

Biofilm cells communicate with each other to modulate/transform their metabolic functions through a mechanism known as quorum sensing. Greenberg introduced the term "quorum sensing." In biofilm formation, the contribution of this mechanism is not clearly understood as it is distinct among various bacterial species. Quorum sensing (QS) is a density-dependent cell signaling mechanism and is said to be involved in the biofilm formation, bacterial pathogenicity, and virulence. It can be defined as cell–cell interactions which are mediated by diffusible chemical signal molecules called autoinducers (Als) (Hooshangi and Bentley 2008). A high population density provides us with a chance to perform certain processes that single cells cannot carry out efficiently (Danhorn and Fuqua 2007; An et al. 2006). They can intercommunicate with each other by quorum sensing and can able to confuse the pathogenic bacteria.

The quorum sensing response can be observed after the phosphorylation of a response regulator protein. In yeast strains, bicarbonate, acetaldehyde, and ammonia are known as cell-to-cell signaling molecules (Rutherford et al. 2011; Hooshangi and

Bentley 2008). Besides, farnesol, which is found in the biofilm matrix produced by *P. aeruginosa*, is another signal molecule that provides the inhibition of *Candida albicans*. The mechanism of quorum sensing was first discovered in bacterial cells. The fungal quorum sensing mechanism has been studied in recent years (Rutherford et al. 2011 and Hooshangi and Bentley 2008).

There are three classes of signaling molecules associated with QS in bacteria (Hooshangi and Bentley 2008):

1. Oligopeptides class of signaling molecules used by gram-positive bacteria as a means of communication (Danhorn and Fuqua 2007).
2. Acyl homoserine lactones (AHLs) class of signaling molecules used by species-specific gram-negative bacteria as a means of communication (Danhorn and Fuqua 2007).
3. Autoinducers-2 (AI-2).

18.2.3.1 QS-Signaling Pathways

The signal molecules are produced and released into the medium by microorganisms and the extracellular concentration reaches a threshold level (Hooshangi and Bentley 2008). Then the signal molecules lead the changes in the behavior of the microbial population due to activation of transcriptional regulator and the gene expression has been carried out by the regulators (Hooshangi and Bentley 2008). The expression of quorum sensing molecules varies in accordance with microbial diversity.

In gram-negative bacteria, the autoinducer system is synthesized by homoserine lactones (HSL), which are fatty acid derivatives and synthesized by LuxI and LuxR homologues and the complex of quorum sensing mechanism is called LuxI/LuxR systems (Rutherford et al. 2011). In gram-positive bacteria, the autoinducers are amino acids and secreted short peptides, unlike the system observed in gram-negative bacteria for quorum sensing (Rutherford et al. 2011).

In general, the signal molecules found in the filamentous fungi such as *Aspergillus* and *Penicillium* species are secondary metabolites. Quorum sensing can be also observed in the different genus of microorganisms such as bacteria–fungi and yeast–fungi interactions (Annous et al. 2009).

18.2.4 Advantages of Biofilms to Microbes

(1) Bacterial biofilms which are formed on plant roots not only protect the sites of colonization, but also acts as sink for the nutrients in the rhizosphere, hence reducing the root exudates nutritional elements availability for pathogen stimulation or their colonization on the root (Weller and Thomashow 1994).
(2) Biofilm assists bacteria to survive under unfavorable environment and nutritional conditions.
(3) It gives resistance to biofilm agents. It increases local nutrients concentration and thus helps in the plant growth and development.
(4) It gives an opportunity for exchange of genetic material.

(5) It has the ability to intercommunicate between bacteria population of same and or different species.
(6) It produces growth factors across species boundaries.

18.2.5 Role of Microbial Biofilm in Biocontrol of Plant Diseases

The peanut seeds that are pretreated with the exopolysaccharide-producing strains of *Paenibacillus polymyxa* (B5 and B6) showed inhibitory effect against *A. niger*, which can cause crown rot disease than untreated seeds due to combination of antibiosis, induction of resistance, and exopolysaccharides production at rhizoplane of peanut plants. The activity of plant defense enzymes 1, 3-glucanase and chitinase were significantly stimulated in treated roots which are positively correlated with resistance to pathogens (Timmusk and Wagner 1999). The resistance was triggered against *A. niger* by the peanut plants sown from previously treated seeds with *P. polymyxa* strains (Haggag 2007).

A biofilm-producing strain of a yeast *Pichia fermentans* was found to have dual nature which controls brown rot disease on apple caused by a phytopathogenic isolate of *Monilinia fructicola*, in its yeast-like shape. But when the same strain applied to peach fruit, it showed unexpected pathogenic behavior due to its transition from budding growth to pseudohyphal growth even in the absence of *M. fructicola*, suggesting that the pseudohyphal growth plays a major role in the expression of potential pathogenicity of *P. fermentans.* Also showed that the biocontrol exerted by this strain should not depend upon the production of toxic metabolites (Giobbe et al. 2007).

The antagonistic properties of rhizobacterium, *P. polymyxa* strains (B2, B5, and B6) toward *Phytophthora palmivora* and *Pythium aphanidermatum* on *Arabidopsis thaliana* was studied in liquid assays and soil assays. In liquid assays, when *A. thaliana* was pretreated with bacterial strains, all the strains of *P. polymyxa* (B2, B5, and B6) reduced the zoospore colonization of *P. palmivora* and *P. aphanidermatum*. B2 and B5 isolates produced significant protection toward both the pathogens by producing highest amount of mycoidal substances and high rate of survivability of *A. thaliana* plants. The latter assay showed the incompetence of *P. polymyxa* strains to reduce the zoospore colonization of *P. aphanidermatum* compared to the liquid assay. Among all the isolates of *P. polymyxa,* B6 was less potent in reducing the colonization of oomycete plant pathogens in both the assays (Timmusk et al. 2005).

In the enlightenment process of benefits of PGPR in management of plant disease, *Bacillus* lipopeptides (surfactins, iturins, and fengycins) were studied for their antagonistic activity for a wide range of phytopathogens, and further in-depth studies have shed light on the fact that these lipopeptides can also influence ecological fitness of the producing strain colonization by stimulating host defense mechanisms (Bais et al. 2004). When *B. subtilis* strain 6051, a wild-type, was treated to *Arabidopsis* root surfaces against *P. syringae* with confocal scanning laser microscopy, it revealed the biofilm formation process includes the surfactin, a lipopeptide

antimicrobial agent secretion. The mutant strain, M1 (deletion of surfactin synthase gene) of *B. subtilis* was found ineffective as a biocontrol agent against *P. syringae* in both infectivity and in biofilm formation on either roots or on inert surfaces (Bais et al. 2004).

18.2.6 Summary of the Chapter

The colonization of biocontrol agents on the plant surfaces plays a crucial role in plant disease control. Generally, bacteria persist in natural environment by forming biofilms (Davey and O'Toole 2000). The beneficial rhizobacterium is *B. subtilis*, which is ubiquitous in soil, promotes plant growth, protects against fungal pathogen, and plays a vital role in the degradation of organic polymers in the soil (Emmert and Handelsman 1999). On an average, it contributes 4–5% of its genome to antibiotic synthesis and capable of producing more than 24 antimicrobial compounds which are structurally diverse (Stein 2005). *Bacillus subtilis* forms adhering biofilms on inert surfaces under the variety of transcriptional factors (Hamon and Lazazzera 2001). Biocontrol ability of a wildtype *B. subtilis* strain 6051 against *P. syringae* was demonstrated by using an infection model (Kinsinger et al. 2003). Root-associated *Pseudomonas* sp. act as biocontrol agents and also promote plant growth. *Pseudomonas putida* responds rapidly to root exudates in the soil converging at root sites and establish stable biofilms (Espinosa-Urgel et al. 2002). Some microorganisms inhabit leaf surfaces and forms biofilms. For instance, *Burkholderia* sp., FP62 is a biocontrol agent of *B. cinerea* in geranium, forms biofilms in the phyllosphere (Haggag 2007).

18.3 Conclusion

The need to fulfill the food, health, and high-yielding plants for growing population, the use of microbes in plant disease management offers an attractive alternative to the use of the synthetic chemicals and could be a hope for food security problem. The microbial strain used in biocontrol management suppresses the pathogens without leaving residues which could be an eco-friendly approach. Biofilm formation by PGPR could serve as a novel model system which serves to study and understand the microbial colonization on various sites of plants. However, as a coin has both sides, both beneficial and harmful microbes exist on the plant surfaces, but it depends on the extent to which a beneficial microbes compete with pathogens and aids in plant growth promotion by reducing disease incidence.

There is a need to explore many other beneficial microorganisms and employ in sustainable plant disease control for healthy farming and nation and research findings are insufficient in understanding the intimacy of different genus of microorganisms such as bacteria–fungi and yeast–fungi interaction. Need to emphasize the mechanism of beneficial microbes completely before their release as biocontrol agents is also important. Recently, the isolates of *P. fermentans* were found

in the blood stream infections (Pfaller and Diekema 2004) and caused polyarthritis in a patient suffering from alcoholism (Trowbridge et al. 1999).

The significance and impact of the chapter highlights the need to consider the potential of biofilm formation for biocontrol assays in natural conditions and reveal the need in advancement of research in this area for management of plant diseases in a sustainable approach.

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Role of Endophytes in Plant Disease Management 19

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Abstract

Sustainable agriculture and agri-food production can only be preserved for our next generation by protecting different natural resources. So a thrust interest has been developed to exploit internal colonisation of healthy plants that termed as endophytes to execute in a systemic way against plant disease management. The matter in this chapter is to have an impact on economic and environment by limiting substantial side effects of abiotic and biotic factors by immediately protecting them by living organisms, i.e. endophytes within the plant tissues. The future implication of combinations of endophyte with commercial pesticide both as seed and seedling treatment could have a synergistic effect against multiple disease resistance under changing climate scenario.

Keywords

Endophytes · Sustainable agriculture · Colonisation · Disease resistance

19.1 Introduction

In the twentieth century, agricultural intensification has been achieved with the help of advanced farm machineries, intensive tillage, high-yielding varieties and heavy doses of fertilizers and pesticides (Foley et al. 2005). These practices, however, had a detrimental effect on the soil as well as human health, with a reduction in soil fertility, high water needs and increased resurgence and resistance to pest and diseases. Therefore, alternative environmentally benign approaches need to be utilised to maintain sustainable agricultural production while overcoming threats that lead to various abiotic stresses, such as soil salinity, temperature extremes or

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drought, as well as biotic stresses caused by pests and plant pathogens. So, the use of microorganisms has come up as an essential alternative for improved plant performance in integrated plant disease management (reviewed by Singh et al. 2011; Jha et al. 2013). In this context, the plant endophytes are being extensively studied in the recent years for their optimum utilisation in managing plant diseases while enhancing overall soil and plant health.

The word endophyte, first introduced by Anton de Bary (1866), is derived from two Greek words, 'endon' meaning within and 'phyton' meaning plant, i.e. the word endophyte literally means 'in the plant'. Endophytes are microorganisms which colonise healthy plant tissues intracellularly and/or intercellularly but do not cause any apparent symptom of the disease. Endophytes were reported as early as 1904 but did not receive much attention till the recent discovery of its beneficial role in pharmaceutical and ecological aspects. It is now reported that endophytes are not only instrumental in direct inhibition of plant pathogens by the production of antibiotics and various enzymes but are also responsible for improving plant physiology, production of beneficial secondary metabolites, induction of plant resistance, improving soil fertility and phytoremediation.

As per Sturz and other associates (2000), endophytic bacteria can be isolated from a large diversity of plants, not a single plant is devoid of endophytes. However, there are only a few differences exists between endophytic microbes and other microbiomes present in the rhizospheric soil (Hallmann et al. 1997; Rosenblueth and Martinez-Romero 2004). But among them, those which are most beneficial still have controversies around world. As both types of microbiomes are present together in plant and soil rhizosphere, the more advantageous ones are very hard to detect. But through many researches, it has been proved that endophytic population in plants is conditioned by different biotic and abiotic factors, but endophytes perform better compared to rhizospheric bacteria against biotic and abiotic stresses (Hallmann et al. 1997).

In this review, we can address together in a same frame about types, ecology, colonisation, mode of action, stress tolerance and recent formulations of different endophytes that would be useful for agricultural sectors, for future research as well as for commercial purposes.

19.2 Origin

The history of endophytes is very ancient. Studies on fossil record prove that endophytes had close relationship with terrestrial land plants for >400 million years ago (Krings et al. 2007). Now endophytes are known to be present in all types of plant habitats,such as fern, lichen, mosses, shrub and grasses, along with deciduous and coniferous trees (Sun and Guo 2012). Thus, endophytes have become an integral part of many ecosystems. Historically, endophytes started gaining importance when toxicosis, caused by *Neotyphodium coenophialum* (family *Clavicipitaceae*), was observed in cattle eating the grass, *Festuca arundinacea*. The first report on the isolation of endophytic fungi was from the plants belonging

to the families Araceae, Bromeliaceae and Orchidaceae by Petrini and Dreyfuss (1981). The abundance of endophytic fungus is variable from host to host, but at least one species of endophytic fungus has been found in whichever plant was taken for investigation (Faeth and Fagan 2002).

The hypothesis behind the bacterial endophytes is that they may originate from the microflora belonging to the rhizosphere (root zone) and phyllosphere (aboveground portion) (Sturz and Nowak 2000). In a nutshell, the bacterial endophytes are special types of bacteria that were isolated from the internal parts of the plant such as xylem tissues and colonise there but without having any harmful effect on the host plant (Schulz and Boyle 2006).

19.3 Types of Endophytes

Endophytes are microorganisms that are associated with plants in various forms, including bacteria (actinomycetes or mycoplasma) or fungi, which are colonised inside the plant tissues intracellularly and/or intercellularly. Among the endophytes the large population is shared by fungi alone, and others are bacteria and actinomycetes. Endophytic fungi may be present in almost all parts of plants without expressing any symptom. The interesting fact about endophytes is that a single species of endophytes can occur in association with many plant species, while many species of endophytes can exist in the same host plant species as latent or with the interaction with other endophytes present in the same plant (Zabalgogeazcoa 2008).

Among bacteria, more than 200 genera from 16 phyla have been reported to be associated with endophytes, most of them belonging to the phyla *Actinobacteria*, *Proteobacteria* and *Firmicutes* (Golinska et al. 2015). Bacterial genera, such as *Achromobacter, Acinetobacter, Agrobacterium, Bacillus, Brevibacterium, Microbacterium, Pseudomonas, Xanthomonas*, etc., have been reported as endophytes (Sun et al. 2013). Bacterial endophytes have also been reported to produce bioactive metabolites such as antimicrobial and antifungal compounds including ecomycins and fusaricidins, respectively. Table 19.1 outlines the management of biotic and/or abiotic stresses by prokaryotic endophytes on host plant (Figs. 19.1 and 19.2).

Actinomycetes, belonging to the phylum *Actinobacteria*, are prokaryotic microorganisms that possess mycelium and have the ability to form spores (Chaudhary et al. 2013; Barka et al. 2016). Endophytic actinomycetes have been reported to produce different various chemical compounds that have important medicinal properties (Gayathri and Muralikrishnan 2013; Singh and Dubey 2015). Among endophytic actinomycetes, *Streptomyces* is a dominant genus, which produces approximately 76% of the antimicrobial and anticancer compounds that have been reported so far (Berdy 2012). Antimicrobial compounds of biological interest, such as coronamycin, naphthomycin (A and K), munumbicins (A and B), clethramycin, cedarmycin (A and B), kakadumycins and saadamycin, have been isolated from *Streptomyces*. Paclitaxel extracted from *Kitasatospora* sp. has been

Table 19.1 Role of bacterial endophytes in management of biotic and/or abiotic stresses in host plants

Name	Host	Pathogen/ abiotic stress	Changes in plants	References
Burkholderia phytofirmans	Grapevine plants	Cold tolerance	Altering photosynthetic activity and metabolism of carbohydrates	Ait et al. (2006), Fernandez et al. (2012)
Pseudomonas pseudoalcaligenes	Rice	Salinity stress tolerance	Accumulation of higher concentrations of glycine betaine-like compounds	Jha et al. (2011)
Azospirillum spp.	Maize plants	Water stress tolerance	Accumulation of the abscisic acid (ABA)	Tuteja (2007)
Achromobacter xylosoxidans AUM54	*Catharanthus roseus*	Salinity stress tolerance	Production of ACC deaminase and reduction of ethylene levels	Karthikeyan et al. (2012)
Pseudomonas indica	*Oryza sativa*	Salinity stress	Increased glycerol concentration	Jogawat et al. (2016)
Paecilomyces formosus LHL10	*Arabidopsis thaliana*	Cold tolerance	Accumulation of pigments and induced cold response pathway	Su et al. (2015)
Pseudomonas indica	*Capsicum annum*	Osmotic stress	Gene encodes the enzyme ACC oxidase	Sziderics et al. (2007)
Pseudomonas fluorescens strain 89B-61	Cucumber	*Colletotrichum lagenarium*	Elicitation of ISR	Wei et al. (1991), Kloepper and Ryu (2006)
Pseudomonas syringae pv. syringae	Rice	*Pyricularia oryzae*	Elicitation of ISR	Smith and Métraux (1991)
Curtobacterium flaccumfaciens	Citrus	*Xylella fastidiosa*	Elicitation of ISR	Araujo et al. (2002)

observed to inhibit food-borne microbes (Zhao et al. 2011; Gangwar et al. 2014; Golinska et al. 2015).

Fungi are a group of heterotrophic organisms which are often associated in a symbiotic relationship with a large number of autotrophic organisms (Saar et al. 2001). Table 19.2 outlines the management of biotic and/or abiotic stresses by fungal endophytes on host plant.

Generally, fungal endophytes are categorised into two broad categories, i.e. clavicipitaceous (C) and non-clavicipitaceous (NC), based on the evolutionary relatedness, taxonomy, host plant range and ecological function. C endophytes are

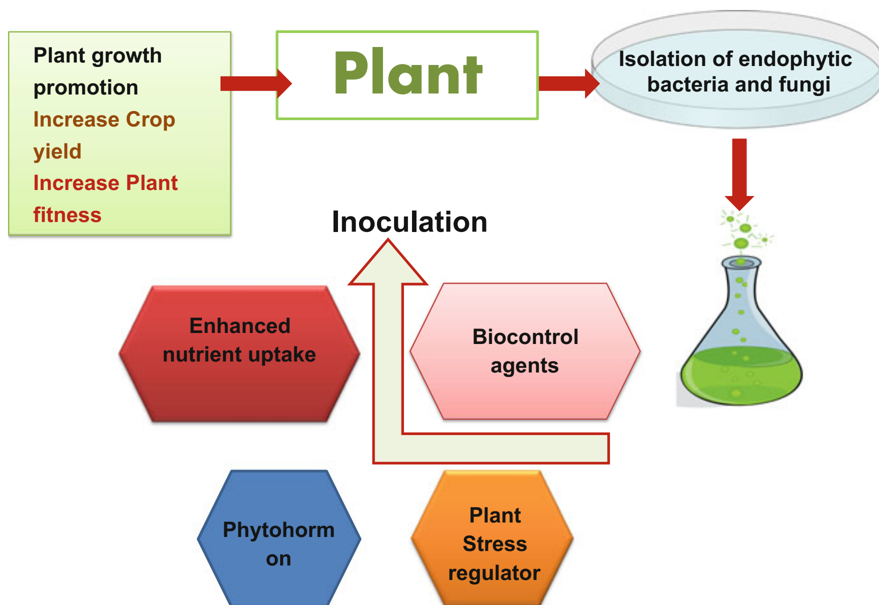

Fig. 19.1 Schematic representation of role of endophytes on plant health

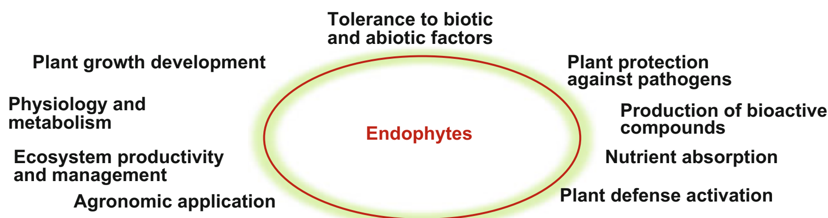

Fig. 19.2 Direct and indirect role of endophytes within the plant system

phylogenetically related to ascomycete fungi, and mainly they harbour the Poaceae family. Till now seven genera of C endophytes have been identified from the grasses of Poaceae family. Among them, the *Neotyphodium* genus (teleomorph *Epichloë*), harbouring cool season C3 grasses, is the most important and extensively studied. *Neotyphodium* species have also been found in cereals (Marshall et al. 1999). C endophytes mainly occurred in the shoots and caused perennial systemic intercellular infection without expressing any symptom and remained endophytic for the entire life cycle of the host (Rodriguez et al. 2009). However, they often vertically transmitted to the next generation plant from inflorescence and seeds (Saikkonen et al. 2010). Up to 20–30% of grass species worldwide are harboured by different C endophytes, including *Neotyphodium*. On the other hand, NC endophytes have been identified from almost all terrestrial plants especially ferns, conifers and angiosperms (Rodriguez et al. 2009). The basic differences between the NC and C endophytes are

Table 19.2 Role of fungal endophytes in management of biotic and/or abiotic stresses in host plants

Name	Host	Stress	References
Fusarium culmorum	*Leymus mollis*	Salt tolerance	Rodriguez et al. (2008)
Curvularia protuberata	*Dichanthelium lanuginosum*	Heat tolerance	Redman et al. (2002)
Piriformospora indica	*Brassica campestris* ssp. *chinensis*	Drought tolerance	Sun et al. (2010)
Chaetomium and Phoma	Wheat	*Puccinia* and *Pyrenophora* spp.	Dingle and McGee (2003), Istifadah and McGee (2006)
Acremonium strictum	*Dactylis glomerata* L.	*Helminthosporium solani* Durieu and Mont	Rivera Varas et al. (2007)
Piriformospora indica	Barley	*Blumeria graminis*	Waller et al. (2005)
Neotyphodium sp.	*Lolium pratense*	Barley yellow dwarf virus (BYDV)	Lehtonen et al. (2006)
Curvularia sp.	*Dichanthelium lanuginosum*	Tolerance to high soil temperatures	Márquez et al. (2007)
Trichoderma harzianum TH-56	*Oryza sativa*	Drought stress	Pandey et al. (2016)
Fusarium equiseti	Barley	*Gaeumannomyces graminis var. tritici*	Macia-Vicente et al. (2009)
Chaetomium sp.	Wheat	*Puccinia recondita*	Dingle and McGee (2003), Mahapatra et al. (2020)
Piriformospora indica	Wheat	*Pseudocercosporella herpotrichoides*	Serfling et al. (2007)
Beauveria bassiana strain 11-98	Cotton	*Rhizoctonia solani*	Griffin (2007)
Beauveria bassiana	Tomato	*Pythium myriotylum*	Griffin (2007)
L. lecanii (Zimm.) Zare and W. Gams	Coffee	*Hemileia vastatrix* Berk. and Broome	Vandermeer et al. (2009)
Chaetomium globosum	Wheat	*Puccinia tritici repentis*	Istifadah and McGee (2006), Mahapatra et al. (2020)
Cordana sp.	Wild banana	*Colletotrichum* sp.	Nuangmek et al. (2008)
Fusarium verticillioides	Maize	*Ustilago maydis*	Lee et al. (2009)

that NC endophytes may not harbour host plant for the entire life cycle of the host and that the endophyte itself may not remain endophyte for its own life cycle (Rodriguez et al. 2009).

In the recent years, another new class of root-inhibiting fungi, i.e. dark septate endophytes (DSE), has been identified, which belongs to heterogeneous ascomycete fungi. It is characterised by dark-pigmented septate hyphae, which generally

colonises in the root tissue both intercellularly and intracellularly (Jumpponen and Trappe 1998). *Exophiala pisciphila* isolated from maize and *Harpophora oryzae* (Yuan et al. 2010) isolated from wild rice in China are some examples of DSE.

Endophytic fungi are reported to produce some of the popularly used antibiotics and anticancer drugs. The *Penicillium* sp. produces penicillenols, which are cytotoxic to cell lines. Taxol is an effective and a very popular anticancer drug that is extracted from endophytic fungi, *Taxomyces andreanae*. Many other antibacterial and antifungal compounds, such as sordaricin (*Fusarium* sp.), jesterone (*Pestalotiopsis jesteri*), clavatol (*Torreya mairei*), javanicin (*Chloridium* sp.), etc., are reported to be highly effective against a large number of food-borne infectious microorganisms (Jalgaonwala et al. 2011). Pestacin, isolated from *P. microspora*, has excellent antioxidant properties.

19.4 Colonisation

The nature of colonisation in the internal tissue has made the endophytes a valuable tool for crop improvement performance in agriculture (Azevedo et al. 2000). Endophytes, both prokaryotic and eukaryotic, often colonise the root tissues systemically in both inter- and intracellular manner. Bacterial endophytes enter into their host via stomata (Roos and Hattingh 1983), nectarthodes (Rosen 1936), lenticels, germinating radicles (Gagné et al. 1987), broken trichomes, wounds (Daft and Leben 1972), foliar damage from windblown soil particles, rain or hail and undifferentiated meristematic root tissue. Colonisation by bacterial endophytes is primarily intercellular (Hallmann et al. 1997), though some of them, such as *Azoarcus* spp., have been found to colonise the root tissues intracellularly (Hurek et al. 1994). They are widely distributed in the vascular bundles which aid in their distribution throughout the plant (Kobayashi and Palumbo 2000), fungal endophytes colonise asymptomatically in both inter- and intracellular manner (Barrow 2003).

Endophytic microorganisms may not be endophytic for their entire life cycle, thereby encompassing not only symbiotic and communalistic species but also latent pathogens and saprotrophs. Generally, nitrogen-fixing bacteria (rhizobia) produce morphological changes in the roots by forming root nodules; otherwise, endophytes remain silent without any morphological change in the system (Malfanova et al. 2013).

19.5 Mode of Action

19.5.1 Direct Inhibition of Plant Pathogens

Endophytes have been studied extensively in the recent years for their ability to protect plants from various diseases as well as reduce the damage caused by plant (Mejia et al. 2008). By producing antibiotics, competition and secreting lytic

Table 19.3 Bioactive compounds produced by endophytes

Fungal endophyte	Host	Antibiotic	Target pathogens	References
Periconia sp.	*Taxus cuspidata*	Fusicoccane diterpenes	*Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhimurium*	Kim et al. (2004)
Acremonium zeae	Maize	Pyrrocidines A, B	*Aspergillus flavus, Fusarium verticillioides*	Wicklow et al. (2005)
Muscodor albus	Tropical tree	Tetrahydofuran, 2-methyl furan, 2-butanone, aciphyllene	*Stachybotrys chartarum*	Atmosukarto et al. (2005)
Phomopsis cassiae	*Cassia spectabilis*	Cadinane sesquiterpenes	*Cladosporium sphaerospermum, Cladosporium cladosporioides*	Silva et al. (2006)
Verticillium sp.	*Rehmannia glutinosa*	Massariphenone, ergosterol peroxide	*Pyricularia oryzae* P-2b	You et al. (2009)
Colletotrichum dematium	*Pteromischum* sp.	Collutellin	*Pythium ultimum, Sclerotinia sclerotiorum*	Ren et al. (2008)
Streptomyces sp.	*Oryza sativa*	Efomycins M and G, oxohygrolidin, abierixin and 29-O-methylabierixin	*Plasmodium falciparum*	Supong et al. (2016)
Aspergillus terreus	*Carthamus lanatus*	Aspernolide F	*Candida neoformans*	Ibrahim et al. (2015)

enzymes, endophytes are able to suppress the plant pathogens directly. However, these interactions are very complex and specific to species-species antagonism.

Antibiosis Many endophytes produce antifungal and antibacterial secondary metabolites, which inhibit the growth and development of other microorganisms including plant pathogens (Gunatilaka 2006). Antibiotics produced by a single endophyte may be of various chemical compositions such as alkaloids, terpenoids, polypeptides and aromatic compounds, which are harmful to the plant pathogens. *Chaetomium* and *Phoma*, after inoculation in wheat, reduced the severity of foliar disease caused by *Puccinia* and *Pyrenophora* spp. However, most interestingly, same result has been observed when the filtrate of above-mentioned endophytes' culture was applied in the wheat plants (Istifadah and McGee 2006). *Phomopsis*

cassiae, an endophytic fungus isolated from *Cassia spectabilis*, has been shown to produce 3, 11, 12-trihydroxycadalene, a cadinane sesquiterpene, which has been proved to be the most active antifungal compound against *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* (Silva et al. 2006). Table 19.3 enlists some antibiotics that are produced by endophytic fungi.

Alkaloids too have a strong ability to suppress microorganisms. Endophytic *Alternaria* spp. produces altersetin, an alkaloid, which has shown antibacterial activity against large number of pathogenic gram-positive bacteria (Hellwig et al. 2002). Volatile organic compounds (VOCs) are also considered to be effective in antibiosis. An endophytic fungus, *Muscodor albus*, has been reported to produce many volatile organic compounds including 2-methyl furan, tetrahydofuran, aciphyllene and 2-butanone, which possess antibiotic activities (Atmosukarto et al. 2005). In vitro, it was observed that endophytic fungi isolated from *Artemisia annua* produced EtOAc and n-butanol, which acted as antifungal compounds, inhibiting growth of a number of phytopathogens (Liu et al. 2001).

Lytic enzymes secreted from endophytes: Endophytes produce a wide array of enzymes that aid in the hydrolysis of polymeric compounds including DNA, protein, lignin, cellulose, chitin and hemi-cellulose (Tripathi et al. 2008). During colonisation, endophytes secrete these enzymes to break down the plant cell wall. At the same time, these enzymes possess the ability of hydrolysing the cell walls of fungi and oomycetes. The enzymes include β-1,3glucanases, cellulases and chitinases. It has been reported that β-1, 3-glucanase genes in *Lysobacter enzymogenes* strain C3 helped in biological control activity towards *Pythium* damping-off of sugar beet. *Streptomyces* has also exhibited antagonism to cacao witches' broom in vitro with the production of lytic enzymes (Macagnan et al. 2008). The biocontrol fungus *Talaromyces flavus* Tf1 (*Ascomycota*: *Eurotiales*) produces the enzyme glucose oxidase, whose reaction product, hydrogen peroxide, kills microsclerotia of phytopathogenic *Verticillium* (Fravel 1988).

Mycoparasitism Some endophytes may be mycoparasites. *Acremonium strictum* W. Gams is an endophyte which has been frequently isolated from *Dactylis glomerata* L. and other grasses (Sánchez Márquez et al. 2007); recently it has been shown that this fungus is a mycoparasite of *Helminthosporium solani* Durieu and Mont., a potato pathogen (Rivera Varas et al. 2007). A significant increase in resistance to dollar spot disease, caused by *Sclerotinia homoeocarpa* F.T. Benn., has been observed in *Festuca rubra* L. cultivars infected by *Epichloë festucae*. In the case of viruses, the incidence of Barley yellow dwarf virus (BYDV) was lower in *Lolium pratense* infected by *Neotyphodium* than in endophyte-free plants. Since BYDV is transmitted by means of aphid vectors, toxic fungal alkaloids may be the reason for this effect; in fact, aphid reproduction was lower in endophyte-infected plants than in those free of endophyte. An endophyte of the plant *Dichanthelium lanuginosum* (Elliott) Gould was found to confer tolerance to high soil temperatures to the plant. *Epichloë festucae* virus 1 (EfV1) is another virus, which

asymptomatically infects the grass endophyte *Epichloë festucae*; in this case, it is not known if the presence of the virus in the endophyte affects the plant host (Romo et al. 2007).

19.5.2 Indirect Effects to Enhance Plant Resistance

Plants experience a large number of stresses in the form of pathogens, pest, drought, salinity, cold, etc. Morphological and biochemical changes such as lignifications, cellular necrosis, hypersensitive response and phytoalexin production help in responding to those stresses in an efficient manner. Since endophytes may evolve from plant pathogens, they help in triggering plant defences against pathogens prior to attack. The defence is mainly increased by resistance enhancement and production of secondary metabolites.

19.5.2.1 Induction of Plant Resistance

Over the past two decades, researchers have extensively studied the responses exhibited by plants when subjected to various biotic and abiotic stresses. In this context, systemic acquired resistance (SAR) and induced systemic resistance (ISR) have been widely studied. SAR is a pathogen-induced response, mediated by salicylic acid and the accumulation of pathogenesis-related (PR) proteins, while ISR is induced by some non-pathogenic rhizobacteria and is mediated by jasmonic acid (Vallad and Goodman 2004; Tripathi et al. 2008). In some cases, ISR may also be associated with expression of pathogenesis-related genes. This is seen in fungal endophyte, *Fusarium solani* isolated from root tissues of tomato, which elicits induced systemic resistance against the tomato foliar pathogen *Septoria lycopersici* by triggering PR genes, PR5 and PR7, expression in roots (Kavroulakis et al. 2007). Also, plants colonised by endophytes have the ability to initiate defence response more rapidly as compared to non-colonised plants. This was evident when cucumber (*Cucumis sativus*) and watermelon (*Citrullus lanatus*) previously exposed to a non-pathogenic mutant of *Colletotrichum magna* exhibited increasingly higher levels of peroxidase activity lignin deposition, and phenylalanine ammonia lyase activity that resulted in the protection against disease caused by *Colletotrichum orbiculare* and *Fusarium oxysporum* (Redman et al. 1999).

Endophyte *Neotyphodium lolii* was reported to enhance superoxide dismutase (SOD) and peroxidase (POD) activities of host that resulted in reduced lesions on infected leaves of the host (Tian et al. 2008). Thus, it can be deduced that the reactive oxygen species (ROS) damage induced by pathogen infection is actively controlled by antioxidative systems including SOD and POD, induced by the endophytes.

19.5.2.2 Stimulation of Plant Secondary Metabolites

Plant secondary metabolites do not play an important role in basic life functions but are responsible for the adaptation of plants to their environment (Bourgaud et al. 2001). Among these compounds, plants produce phytoalexins (Smith 1996), which may contain terpenoid, flavonoid, etc. Studies have shown that endophytic fungi

Fusarium spp. E4 and E5 help in promoting the growth of *Euphorbia pekinensis* as well as increasing its terpenoid content (Yong et al. 2009). Further research has revealed that *Fusarium* E5 elicitor could induce terpene production in *E. pekinensis* cell suspension cultures and as well as increased production of defence-related enzymes including phenylalanine ammonia lyase, peroxidase and catalase (data have not been published).

The mechanism of endophyte elicitor induced plant secondary metabolite resistance is similar to the stimulation of plant resistance. Fungal endophytes stimulate the secretion of hydrolase of plant cell to limit the growth of fungi, which results in the production of endophyte fragments that act as elicitors. These elicitors stimulate plant defence and plant secondary metabolites, which help in effective suppression of the attack by the pathogen.

19.5.2.3 Promotion of Plant Growth and Physiology

Endophytes help in the plant defence activities by enhancing the plant growth and controlling the plant physiology (Giménez et al. 2007). An increase in plant growth results in higher plant vigour that provides potential protection to pathogen challenge (Kuldau and Bacon 2008). Studies conducted in the last two decades have revealed that plants infected with endophytes show higher growth promotion tolerance to unsuitable soil conditions and improved resistance towards drought stress (Malinowski et al. 2004).

Lu et al. (2000) observed that *Colletotrichum* sp., an endophytic fungus, produced growth-regulating substances such as indole acetic acid (IAA) that helped in controlling various plant processes (Lu et al. 2000). Similar results were observed in the case of *Fusarium* sp., where E5 extract functioned as an auxin (Dai et al. 2008). As defence responses are energy intensive in nature, the endophytes are also believed to provide reducing equivalents and carbon skeletons with the help of primary metabolic pathways (Bolton 2009). It is reasonable to believe that enhancement of plant growth would lead to increased protection of the plant against pathogens.

Volatile compounds such as 2, 3 butanediol and acetoin produced by bacteria have been reported to be a newly discovered mechanism that helps in promoting plant growth (Ryu et al. 2003). It was observed that endophytic bacteria produce adenine ribosides that help in stimulating growth as well as mitigating browning of pine tissues (Pirttilä et al. 2004). Hoffman et al. (2013) conducted in vitro experiment on endophyte *Pestalotiopsis* and reported that endophytic bacteria identified as *Luteibacter* enhanced indole-3-acetic acid (IAA) production, whereas the bacteria and fungi were unable to produce IAA on the media when cultured alone.

19.5.3 Ecological Effects

The plant-pathogen-endophyte interaction is largely influenced by the endophytic niche. Competition for nutrients and limited space leads to hyperparasitism and predation between diverse microorganisms that live in endophytic niche, especially

between endophytes and pathogens. Endophytic recognition and colonisation rapidly occupy ecological niche and leave no space for pathogens, which would be the common and main reason that fungal endophytes inhibit pathogen infection in plant. Fungal endophytes have the ability to colonise inter- or intracellularly and often are localised in a single cell. The colonisation of plant tissues by endophytes involves several steps including host recognition, spore germination, penetration of the epidermis and tissue colonisation (Petrini 1991). After endophytes are successfully colonised in the host tissue, the endophytic niche becomes established. In the endophytic niche, endophytes will obtain a reliable source of nutrition provided from plant fragment, exudates and leachates and protect host against other microorganisms. Fungal endophytes are generally thought to protect plant by rapid colonisation and thereby exhausting the limited available substrates so that none would be available for pathogens to grow (Pal and Gardener 2006). Furthermore, the plants produce lignin and other cell-wall deposits to limit the growth of endophytes and cause it to be a virulent (Harman et al. 2004). As a result, the cell wall becomes re-reinforced after endophytic colonisation; thus, it becomes difficult for pathogens to infest.

Hyperparasitism is another ecological strategy that endophytes provide to protect host plant. In hyperparasitism, the pathogen is directly attacked by a specific endophyte that kills it or its propagules (Tripathi et al. 2008). Fungal endophytes parasitise around hyphae of pathogens by various means such as twisting, penetrating the hyphae of pathogens and secreting lyase to decompose cell wall of pathogens. For example, *Trichoderma* are able to parasitise hyphae of plant pathogen *Rhizoctonia solani*, and many of these observations are linked with biocontrol. In contrast to hyperparasitism, microbial predation is a more general way to suppress plant pathogens. Some endophytes show predatory behaviour under nutrient-limited conditions. For example, *Trichoderma* produce a range of enzymes that are directly used against cell walls of fungi to utilise the fragment of pathogens (Benhamou and Chet 1997). It was also suggested that endophytes may protect their hosts by enhancing stress tolerance to oxidative stress. Several studies demonstrated increased production of antioxidant compounds (flavonoids and other phenolic antioxidants) in endophyte-infected plants (Herrera-Carillo et al. 2009), which was assumed to be triggered by production of reactive oxygen species (ROS) by endophytes. ROS is produced to oxidise and denature host cell membranes, thereby facilitating nutrient leakage from plant cells, which are subsequently absorbed by fungal hyphae (White and Torres 2010). Consequently, host plant resistance to ROS involving stresses, e.g. drought, diseases and metal toxicities, may likewise be improved (White and Torres 2010). Furthermore, endophytes are known to be producers of a vast variety of antioxidant compounds as well, such as phenolic acids and their derivatives, isobenzofuranones, isobenzofurans as well as mannitol and other carbohydrates. This antioxidant capacity may also contribute to the enhancement of stress tolerance in their hosts (White and Torres 2010). Stress tolerance conferred by some endophytes was shown to represent habitat-adapted phenomena. *Curvularia protuberata* was found to dominantly colonise

Dichanthelium lanuginosum, thereby conferring heat tolerance to the plant growing on geothermal soils of Yellowstone National Park, USA.

19.6 Crop Adaptation to Abiotic Stress Environment

Endophytes have been extensively studied in the recent decades for their overall impact on the plant growth and physiology. It has been observed that endophytes not only confer resistance to plants against invading plant pathogens but also aid in adapting the plant to various stress conditions such as drought, extreme heat or cold, salinity, water stress, etc. Endophytes are responsible for overall improvement in plant vigour, which confers drought tolerance and reduced transplanting shock in plants (Lazarovits and Nowak 1997). It was observed that grapevine plants exhibited increased cold stress tolerance due to bacterial endophyte *Burkholderia phytofirmans* PsJN, which was achieved by altering photosynthetic activity and increased carbohydrate metabolism (Ait et al. 2006). In the presence of the bacterium, the plant resulted in lower cell damage and accumulation of starch, proline, phenolic compounds and other cold-stress-related metabolites (Naveed et al. 2014). Endophytic bacterium *Pseudomonas pseudoalcaligenes* was reported for induce tolerance to salinity stress in rice due to the accumulation of higher concentrations of glycine betaine-like (Jha et al. 2011).

Water stress tolerance was demonstrated by Cohen et al. (2009) in maize plants due to the production of the abscisic acid (ABA) by endophytic bacterium, *Azospirillum* spp. ABA is known to be crucial for plant growth during stress conditions, as it regulates plant water balance and osmotic stress (Tuteja 2007).

When ethylene is accumulated in plants as a result of stress, it has deleterious effect on plant growth and health (Czarny et al. 2006). Endophytes may produce the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase that has no particular function in endophytic bacteria but significantly contributes to the promotion of plant growth and improvement in stress tolerance due to cleaving of ACC, which is an ethylene precursor (Campbell and Thompson 1996; Glick 2014). An endophytic bacterium, *Achromobacter xylosoxidans* AUM54, was shown to impart tolerance to its host plant *Catharanthus roseus* by producing ACC deaminase that resulted in reduced ethylene levels (Karthikeyan et al. 2012). In addition, *Achromobacter xylosoxidans* strain Ax 10 and *Pantoea agglomerans* Jp3-3 producing ACC deaminase were reported to alleviate stress of *Brassica* sp. plants that were grown in copper-contaminated soils, along with improving copper uptake by the plants (Ma et al. 2009).

19.7 Production of Bioactive Secondary Metabolites

Studies have demonstrated that endophytic fungi are able to produce a large number of bioactive compounds, hence indicating that endophytes can be used as an alternate source of these metabolites (Priti et al. 2009). The bioactive compounds

belong to a number of classes including terpenoids, alkaloids, cytochalasins, flavonoids, polyketides and steroids. The best known example till date, for the occurrence of 'bioactive photochemical' in endophytes is paclitaxel (Taxol®), an anticancer drug, that has been extracted from endophytic fungus *Taxomyces andreanae* (Stierle et al. 1993; Stierle and Stobel 1995). Paclitaxel has also been extracted from *Seimatoantlerium tepuiense*, *Seimatoantlerium nepalense* (Bashyal et al. 1999), *Tubercularia* sp. strain TF5 (Wang et al. 2000), *Metarhizium anisopliae* (Liu et al. 2009), *Periconia* sp., *Bartalinia robillardoides* and *Colletotrichum gloeosporioides* (Gangadevi and Muthumary 2008a, b).

Vincristine, another anticancer drug that was originally obtained from *Catharanthus roseus* (Apocynaceae), was recently detected in cultures of endophytic *Fusarium oxysporum* (Zhang et al. 2000). This anticancer compound arrests mitosis by binding to tubulin dimers and inhibiting their assembly to microtubule structures.

Podophyllotoxin, produced by podophyllum species, is known to be the precursor to be the anticancer drugs teniposide and etoposide. Both drugs cause apoptosis and cell death by inhibiting topoisomerase II, which in turn blocks the ligation step of the cell cycle, harming the integrity of the genome. Recently, endophytic *Fusarium oxysporum*, isolated from medicinal plant *Juniperus recurva*, was reported to produce approximately 28 μg/g dry mass of podophyllotoxin (Kour et al. 2008). Many other such bioactive compounds have been extracted from endophytes which are outlined in Table 19.4.

Former studies repeatedly indicated that endophytes are capable of biosynthesizing plant secondary metabolites in vitro, but it is not to be believed that they alone perform the production in the plant. A plausible explanation might be a horizontal gene transfer at some stage during co-evolution, thus importing the respective pathways from fungi into the host plant (Kusari et al. 2008) or vice versa. Nevertheless, it would be of great interest to determine the degree and contribution of endophytic biosynthetic pathways to the secondary metabolite profiles of plants as this would offer an additional explanation for the patchy distribution of certain natural products, such as certain alkaloids, cardiac glycosides and anthraquinones, in the plant kingdom (Wink 2008). Endophytes have a great potential in bioprospecting, indicating that many other important and interesting examples of endophyte secondary metabolism are yet to be discovered.

19.8 Biodegradation Effects of Endophytes

Even though it has not been widely recognised, endophytes possess a number of extracellular enzymes such as ligninases, pectinases, cellulases, phenoloxidase, proteinase and lignin catabolic enzymes (Oses et al. 2006; Bischoff et al. 2009). All these enzymes are instrumental in penetration and colonisation of the host plant. Later, when the plant dies, the endophytes utilise the plant litter as a source of carbon such as glucose, hemicellulose, cellulose, keratin, lignin, lipids, pectin and protein (Kudanga and Mwenje 2005; Urairuj et al. 2003).

Table 19.4 Natural products derived or produced from various endophytes

Organism	Plant association	Active agent	Activity	References
Taxomyces andreanae	*Taxus brevifolia*	Taxol	Anticancer	Strobel et al. (1993)
Pseudomonas viridiflava	Grass	Ecomycins B and C	Antimicrobial	Miller et al. (1998)
Streptomyces griseus	*Kandelia candel*	p-Aminoacetophenonic acids	Antimicrobial	Guan et al. (2005)
Streptomyces NRRL 30562	*Kennedia nigricans*	Munumbicins Munumbicin D	Antibiotic antimalarial	Castillo et al. (2002)
Streptomyces NRRL 30566	*Grevillea pteridifolia*	Kakadumycins	Antibiotic	Castillo et al. (2003)
Serratia marcescens	*Rhyncholacis penicillata*	Oocydin A	Antifungal	Strobel et al. (2004)
Paenibacillus polymyxa	Wheat	Fusaricidin A–D	Antifungal	Beck et al. (2003)
Cytonaema sp.	*Quercus* sp. *103*	Cytonic acids A and D	Antiviral	Guo et al. (2000)
Streptomyces sp.	*Monstera* sp.	Coronamycin	Antimalarial antifungal	Ezra et al. (2004)
Fusarium solani	*Camptotheca acuminata*	Camptothecin	Anticancer	Kusari et al. (2011)
Eupenicillium parvum	*Azadirachta indica*	Azadirachtin A and B	Insecticidal	Kusari et al. (2012)
Streptosporangium oxazolinicum	Unspecified orchid	Spoxazomicins A and B	Antiprotozoal	Inahashi et al. (2011)
Penicillium sp.	*Garcinia nobilis*	Penialidin C and citromycetin	Antibacterial	Jouda et al. (2016)

Three endophytic fungi, *Alternaria*, *Phoma* and *Phomopsis*, were isolated from surface-sterilised pods of *Colophospermum mopane* and showed lignocellulolytic enzyme activity. Scanning electron microscope (SEM) studies revealed that *Alternaria* and *Phomopsis* had the ability of degrading heavily lignified fibres, while *Phoma* was able to degrade those mesophyll cells that were moderately lignified. The lignocellulolytic abilities displayed by the endophytes considerably accelerate the decay of pods, resulting in effective germination of seeds in an arid environment under favourable conditions (Jordaan et al. 2006).

19.9 Formulations

Large-scale use of endophytes necessitates the availability of environmental friendly alternative approach, and hence various formulations of these beneficial microorganisms as bioinoculants are being extensively studied to find the most efficient formulation. Various experiments have been conducted on different types of inorganic and organic carriers along with their evaluation for shelf life and storage conditions, finding suitable formulation of bioinoculants (Vidhyasekaran et al. 1997; Sallam et al. 2013) that would help in increasing microbial population as well as their survival in soil (Bashan 1998). For the purpose of commercialisation of endophytes, it is important that the bioinoculants must remain viable in the prescribed formulation for a certain period of storage (Bazilah et al. 2011). The bioinoculant formulations that have been developed so far have utilised inert carrier materials (Vyas et al. 2010), which act only as carrier media while being neutral to microbial population in every aspect.

Studies have shown that viability counts of plant growth-promoting rhizobacteria (PGPR) bioinoculants decreased in a regular pattern until 210 days, when sawdust was used as a carrier (Arora et al. 2008), while talcum-based formulations exhibited better results in terms of storage as well as management of various plant pathogens (Sah et al. 2011; Shanmugam et al. 2011; Prathuangwong et al. 2013). Kumar et al. (2012) conducted an experiment to assess the application of talcum-based formulations of *Piriformospora indica* and other PGPRs and reported the overall growth promotion in *Vigna mungo*. Then that talcum powder–based formulation exhibited the best results with regard to long shelf life as well as plant growth response. Due to the application of talc-based powder formulations of *Trichoderma* at the time of planting, an increase in the survival percentage of cantaloupe plants was observed under greenhouse condition (Sallam Nashwa et al. 2014). They found that peat- and talc-based formulations were the most helpful carriers in sustaining the microbial population at the time of storage, while *Pseudomonas fluorescens* survived on the chickpea seeds for at least 180 days, when applied as talc-based formulation.

Table 19.5 A list of pollutants that have been associated with phytoremediation strategies using bacterial endophyte

Compound	Organism	Plant association	References
Mono- and dichlorinated benzoic acids	*Pseudomonas aeruginosa* strain R75 and *Pseudomonas savastanoi* strain CB35	Wild rye (*Elymus dauricus)*	Siciliano et al. (1998)
TCP and PCB	*Herbaspirillum* sp. K1	Wheat	Mannisto et al. (2001)
Volatile organic compounds and toluene	*Burkholderia cepacia* G4	Yellow lupine (*Lupinus luteus* L.)	Barac et al. (2004)
MTBE, BTEX, TCE	*Pseudomonas* sp.	*Populus* cv. *Hazendans* and cv. *Hoogvorst*	Germaine et al. (2004), Porteous-Moore et al. (2006)
Methane	*Methylobacterium populi* BJ001	Poplar tissues (*Populus deltoides nigra* DN34)	Van Aken et al. (2004)
Toluene	*Bacillus cepacia* Bu61(pTOM-Bu61)	Poplar (*Populus)*	Taghavi et al. (2005)
2,4-D	*Pseudomonas putida* VM1450	Poplar (*Populus*) and willow (*Salix*)	Germaine et al. (2006)
Arsenic	*Staphylococcus arlettae*	*Brassica juncea*	Srivastava et al. (2013)
Zinc, cadmium, arsenic and lead	*Pseudomonas koreensis AGB-1*	*Miscanthus sinensis*	Babu et al. (2015)

19.10 Endophytes in Phytoremediation

Over the years, extensive studies on various endophytes have suggested that they have the ability to break down complex and toxic pollutants in the soil, which would render them harmless to the plant. Table 19.5 outlines various studies that demonstrate the role of endophytes in phytoremediation.

It was observed by Van Aken et al., in 2004, that *Methylobacterium*, isolated from hybrid poplar trees (*Populus deltoides x nigra*), had the ability of biodegrading numerous nitroaromatic compounds such as 2,4,6-trinitrotoluene. An application of bacterial endophytes with considerable biotechnological potential was described by Barac et al. (2004) who reported an increase of plant tolerance to toluene and decrease the transpiration of toluene to the atmosphere, when engineered *Burkholderia cepacia* G4 endophytes were applied to the plants. Pseudomonas endophytes, present in pea, have the ability of degrading organochlorine herbicide,

2, 4-dichlorophenoxyacetic acid (2, 4-D), as reported by Germaine et al. (2006). This was further proved when pea plants not inoculated with *Pseudomonas* endophytes showed considerable 2,4-D toxicity with advanced visual symptoms such as reduced leaf biomass, callus development and leaf abscission.

Phytoremediation enhancement might be the outcome of increased nutrient uptake and increased pathogen protection or due to a higher degradation of heavy metals. Although few phytoremediation studies have been conducted on fungal endophytes, their utility in bioremediation clearly indicates a huge potential that is yet to be utilised.

19.11 Conclusion

Recently many scientists are focusing their research on endophytes as biocontrol agents for plant disease management. In this chapter, we addressed that the potential mechanisms of endophytes against inhibition of plant pathogen are by direct and indirect effects; also added attentions are given on its ecological effects. Due to high production cost by the use of chemical fertilizers and pesticides and its negative effect on environment, the use of endophytes may have an advantageous role in sustainable agriculture if the added inoculants are potential.

While endophyte research still has a lot of lack and is limited, only a few teams are working on it. We strongly believe that several endophytes with unique modes of action exist in our ecosystem, and only strong research can find out about them. We also need to assess whether it could be possible to affordably promote endophytes in farmers' field. Further researches are required on host–endophyte interactions, which could be a strong evidence for its implementation in the farmers field.

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20 Bioprospecting of Diseases of Horticultural Crops in India

V. Devappa, C. G. Sangeetha, and N. Jhansirani

Abstract

Biological management of plant diseases is the need of the day as it is the most feasible alternative to the present scenario of environmental pollution leading to pesticide residue in horticultural produce affecting the health of the consumers. Bioprospecting is searching for new sources of compounds, genes, microorganisms, macroorganisms, plants and other natural sources. Botanical fungicides may be effective, selective, biodegradable and less toxic to the environment. The biocontrol efficacy of antagonistic microorganisms like *Bacillus*, *Trichoderma*, *Pseudomonas* and some endophytic bacteria has been exploited for the management of the diseases of horticultural crops. Bioprospecting has also been done for the suppression of nematodes by various biological agents. Characterization of the metabolites which play a role in the suppression is also very much needed. The biocontrol efficacy of antagonistic microorganisms depends on a combination of factors such as the characteristics of the antagonistic microorganism, the epidemiology of the target pathogen and the environmental conditions in which the relationship between the pathogen and the antagonist (s) is taking place. Hence, efforts are needed to develop systems by integrating several strategies taking into consideration pathogen biology, cultivar resistance and epidemiology.

Keywords

Bioagents · *Bacillus* · *Trichoderma* · *Pseudomonas* · Ecofriendly · Safe

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20.1 Introduction

Biological management of diseases of crops deals mainly with the identification of various ecofriendly approaches to mitigate the ill effects of plant disease by using antagonistic microorganisms, naturally derived materials of plant and animal origin and inorganic and organic compounds, to reduce or replace the use of synthetic chemicals and to integrate the compatible and synergistic strategies for enhancing the effectiveness of disease suppression. These approaches will protect the environment, increase the yield of crops and provide pesticide-free products to the consumers. It is essential that disease management strategies are compatible with the present cultural practices adopted in a given geographical location to possess the acceptance of the farmers. Various concepts of biological management of diseases have evolved from time to time, supported by the knowledge and techniques to assess the interactions between pathogens, other microorganisms and the plants. The term "biological control" or "biocontrol" was earlier applied to indicate the control of "one organism by another organism." This term now has been used in a wider sense as the "use of natural or modified organisms, genes, or gene product to reduce the effects of pathogenic organisms and to favor the development of the desirable organisms such as crops, trees, animals, beneficial insects and microorganisms." Biological management is hence defined as the utilization of biotic and abiotic agents which act through one or more mechanisms to reduce the potential of the pathogen directly or indirectly by activating host defense systems and to reduce the disease incidence and also intensity.

Bioprospecting is prospecting for the new sources of chemical compounds, genes, microorganisms, macroorganisms and other valuable products from nature which could be economically valuable genetic and biochemical resources. It also involves discovering useful products derived from bioresources including plants, microorganisms, animals, etc. that can be developed further for commercialization and overall benefit of the society. Horticultural crops which are grown both under natural conditions and greenhouse are exposed to different environment and nutrient regimes. The disease problems are likely to vary widely in the crops. Hence, the extent of losses due to diseases caused by microbial pathogens depends on the susceptibility or resistance levels of cultivars and available inoculum potential. Vegetable and fruit crops are high-value crops and hence, greater attention has to be bestowed on them to protect them against diseases.

20.2 Botanical Fungicides

Botanical fungicides are the most important aspect in the recent situation which will help us to decrease the negative impacts of synthetic agents, such as residues, resistance and environmental pollution. In this aspect, botanical fungicides may be effective, selective, biodegradable, and less toxic to the environment. Some of the botanical fungicides which have been tried by various workers are as below:

Cinnamaldehyde Cinnamaldehyde isolated from cinnamon oil, it is effective against dry bubble caused by *Verticillium fungicola*, dollar spot caused by *Sclerotinia homoeocarpa* and canker caused by *Fusarium moniliforme* var. *subglutinans* (Copping 2004). According to Wang et al. (2005), the leaves of indigenous cinnamon (*Cinnamomum osmophloeum*) exhibited strong antifungal activity against wood decay fungi. Cinnamaldehyde showed 100% efficacy against both *Coriolus versicolor* and *Laetiporus sulphureus*. Cheng et al. (2008) reported the antifungal activity of cinnamaldehyde and eugenol against wood-rot fungi.

L-Glutamic Acid + Gamma-Aminobutyric Acid These two ingredients enhance the expansion of plants like almond, broccoli and onions, prevent development of mildew on grapes and suppress certain other plant diseases caused by *Monilia* sp. (Copping 2004).

Jojoba Oil Jojoba oil obtained from the jojoba bean, it has been found to be very effective and capable of controlling white flies and mildew on ornamental plants and grapes.

Laminarin Laminarin is a storage glucan (a polysaccharide of glucose) found in the blue green algae *Laminaria digitata*. Laminarin efficiently elicits defense responses in grapevine against gray mold and downy mildew caused by *Botrytis cinerea* and *Plasmopara viticola*, respectively. Laminarin application reduced *B. cinerea* and *P. viticola* infection by 55% and 75%, respectively (Copping 2004). Hu et al. (2012) reported inhibitory effects of laminarin on growth and toxin production of *Aspergillus flavus*.

Milsana The ethanolic extract from the plant *Reynoutria sachalinensis* decreased the mildew incidence on tomato by a mixture of induced resistance and direct antifungal activity. A single spray reduced mildew infection on young seedlings of tomato by 97% (Reglinski 2009). The active ingredient is reported to act as an elicitor of phytoalexins, which induces resistance (Copping 2004).

20.3 Fruit Crops

20.3.1 Banana

Fusarium wilt is the most devastating disease of banana occurring worldwide and causing lot of economic losses. Plant extracts have shown antifungal activity and reduced mycelium growth of *Fusarium* under both greenhouse and field conditions (Akila et al. 2011). Gnanasekaran et al. (2015) investigated the in vitro biological control of *Fusarium oxysporum* f. sp. *cubense* by some Indian medicinal plants. Among the five plants tested, *P. betle* L. plant extracts exhibited maximum antifungal activity against the tested *Foc* followed by *V. negundo*, *C. gigantea*, *C. asiatica*, and *O. sanctum* plant extracts.

Pre-inoculation with beneficial endophytes through banana plantlets might be an effective strategy for both biocontrol and growth promotion of banana (Ho et al. 2015). Priming the tissue-cultured banana plantlets led to a substantial reduction in the infection and severity of wilt disease. It also led to an increase in plant growth parameters (Jie et al. 2009). The combined application of endophytic and rhizospheric bacterial strains was effective in suppressing *Fusarium* wilt, in both pot and field trials. Two non-pathogenic *F. oxysporum* isolates CAV 255 and CAV 241 reduced the *Fusarium* wilt disease incidence by 87.4 and 75.0%, respectively. The known Fo47 isolate did not suppress the disease significantly. *P. fluorescens* strain WCS417 reduced the disease incidence by 87.4% under greenhouse conditions (Nel et al. 2006). The bacterial bioagents *Serratia marcescens* effectively suppressed the development of *F. oxysporum* f. sp. *cubense* race 4 (FocR4). The bacteria *S. marcescens* was formulated using montmorillonite clay (carrier), nonfat skimmed milk (NFSM), and sucrose (enrichment materials), which improved the bacterial cell viability significantly. Bioformulation of *S. marcescens* with the above materials was found to be useful for both storage and field application (Ting et al. 2009).

20.3.1.1 Leaf Spot Disease

The *Bacillus subtilis* strain B106 isolated from the rhizosphere soil of banana field in China, exhibited effective suppression of *Pseudocercospora musae* (teleomorph: *Mycosphaerella musicola*), causing banana leaf spot disease and *Colletotrichum musae* causing postharvest anthracnose disease of banana fruits during storage. Development of banana leaf spot was suppressed by 72.3% in the greenhouse experiment at 10 days after pathogen inoculation. The efficacies of strain B106 (1×10^6 CFU/ml) for controlling both the banana leaf spot diseases in the field and the anthracnose disease at postharvest stage were 48.3 and 48.6%, respectively, under optimized cultural conditions for the BCA to express its antagonistic potential.

20.3.2 Citrus Diseases

20.3.2.1 *Phytophthora* Root Rot Disease

Many workers have reported biological agents for *Phytophthora* management in citrus. Gade (2012) reported significant suppression of *P. parasitica* by *Trichoderma harzianum* and *T. virens*. There was a continuous reduction in pathogen population from 41 to 8 propagules/g soil with reduction in root rot/collar rot in *Citrus jambhiri*. Biochemically efficient strain of *P. fluorescens* PfIV was found effective to arrest the percent mycelial growth (55.20%) of *P. parasitica*. Gade and Koche (2012) reported that *P. fluorescens* in combination with fungicides and organic amendments was effective in the management of root rot and gummosis in Nagpur mandarin. Significant decrease in intensity of both root rot and gummosis was observed. Root rot intensity reduced from 36.18% to 16.70%. Similarly, significant reduction in gummosis up to 54.76% was recorded. *T. viride* inhibited the highest mycelial growth of *P. parasitica* (75.33%) in vitro, whereas, under glasshouse experiment, combined

application of *T. viride* at 4 g/Kg + garlic clove extract at 5% significantly reduced percent root rot incidence (11.32%) as compared to untreated control (44.99%) (Pente et al. 2015). Lende et al. (2015) found significant increase in shoot length (23.55 cm) and canopy volume of tree (10.89%) in Nagpur mandarin when bioagent *T. harzianum* was incorporated in combination with chemicals and organic amendments and also found significant reduction in root rot intensity. Greenhouse studies conducted by Abraham (2005) showed that the *Bacillus* and some *Trichoderma* isolates suppressed *Phytophthora* root rot disease.

20.3.2.2 Citrus Canker

Some strains of bacteria, viz., *P. syringae*, *E. herbicola*, *B. subtilis*, and *P. fluorescens*, isolated from citrus phylloplane are reported to be antagonistic in vitro to the canker pathogen (Ota 1983; Goto et al. 1979; Unnimalai and Gnanamanickam 1984).

Kalita et al. (1996) reported that three bacteria, viz., *B. subtilis*, *B. polymyxa*, and *P. fluorescens*, and three fungi, viz., *Aspergillus terreus*, *T. viride*, and *T. harzianum*, isolated from the phylloplane of citrus variety Assam lemon (*Citrus limon*) inhibited the growth of *Xanthomonas citri* subsp. *citri* in vitro. When the antagonists were tested for their efficacy under field condition by applying them over crop foliage, they also reduced citrus canker incidence. *B. subtilis* was found to be the most effective antagonist exhibiting maximum (14.70 mm) inhibition of the pathogen and reducing the disease incidence to an extent of 61.90%.

Five collections of *P. citrinum* (P1, P2, P3, P4, and P5) and three collections of *Trichoderma* spp., i.e., *T. virens* (TD1), *T. harzianum* (TD2), and *T. virens* (TD3), were tested for biocontrol activity against *Fusarium solani* (F1), *F. oxysporum* (F10), and *E. chlamydosporum* (F6) (Misra 2008). All the *Penicillium* isolates were able to suppress all the three *Fusarium* spp. A very clear zone of inhibition was formed with *T. virens* (TD1 and TD3) with all the three *Fusarium* spp. However, *T. harzianum* (TD2) made very clear zone with *F. solani* and *F. oxysporum*.

20.3.3 Guava

Guava wilt is one of the major diseases affecting guava. The filterate of the cultures and released volatile compounds of eight bioagents, comprising three isolates of *Aspergillus niger*, three isolates of *Trichoderma* spp., and two isolates of *Penicillium citrinum*, were evaluated against ten isolates of *Fusarium* spp. (five isolates each of *F. oxysporum* f. sp. *psidii* and *F. solani*) causing wilt disease of guava. It was found that all the fungal bioagents significantly reduced the growth of *F. oxysporum* f. sp. *psidii* and *F. solani* (Misra 2008). Shruthi et al. (2019) studied volatile compound production by indigenous *Trichoderma* isolates against *C. fimbriata*. Eleven isolates of *Trichoderma* (PT-I to PT-11) were positive for volatile compound production indicating their biocontrol activity. Higher concentrations of volatile metabolites

were produced in isolates PT-6 (78.84%) followed by PT-11 (78.67%) and PT-10 (71.84%), and lowest concentration of volatile metabolites was produced by PT-8 (50.00%).

20.3.4 Grapes

20.3.4.1 Powdery Mildew

Powdery mildew disease caused by *Uncinula necator* is one of the most economical diseases infecting both leaves and the berries. The basidiomycetous yeast *Pseudozyma flocculosa* (syn. *Sporothrix flocculosa*) was reported to be an efficient natural antagonist of *U. necator* (Belagner and Labbe 2002). Sawant et al. (2015) identified *T. afroharzianum* NAIMCC-F-01938, which provided about 50% reduction in disease severity. The workers also reported that replacing two late-season sulfur applications with that of *T. afroharzianum*, in a fungicide spray schedule, enhanced powdery mildew control by 31% as compared to the only fungicide schedule. Melidossia et al. (2005) reported management of powdery mildew using mycophagous studied mite, *Orthotydeus lambi*. The mites applied at 30 mites per leaf could suppress disease when applied pre-bloom or 1 week after bloom. In grapes, *B. subtilis* KSI isolated from grape berry skin could reduce downy mildew on berries and leaves (Furuya et al. 2011).

20.3.4.2 Downy Mildew Disease

Downy mildew disease of grapes is of historical and economic importance. The effect of Pen, an aqueous extract of the dry mycelium of *Penicillium chrysogenum*, was investigated on the suppression of grapevine downy and powdery mildew disease under greenhouse and field conditions. Pen extract had no direct inhibitory effect on the grapevine pathogens. Hence, it is considered that Pen might act indirectly by inducing resistance in treated plants.

20.3.4.3 Gray Mold Disease

Biocontrol methods for the suppression of the gray mold pathogen *Botrytis cinerea* can be successful only with the understanding of the ecology and epidemiology of *B. cinerea* in the vineyards. An isolate of *Ulocladium oudemansii* effectively suppressed the development of *B. cinerea* on necrotic grape tissues (Elmer et al. 2003; Shorten et al. 2003). A commercial formulation of *U. oudemansii* (BOTRY-Zen®) was found to be as effective as the fungicide program (Elmer et al. 2003). The efficacy of the chemical elicitor 5-chlorosalicylic acid and the fungal antagonist *U. oudemansii* in suppressing the development of *Botrytis cinerea* infecting grapevines was compared, when applied alone and in combination under greenhouse conditions. Mochizuki et al. (2012) isolated *B. amyloliquefaciens* strain S13-3, which showed good inhibitory activity against *C. gloeosporioides* in vitro and could decrease ripe rot caused by *C. gloeosporioides* in vineyard.

20.3.4.4 Crown Gall Disease

Crown gall disease caused by *Agrobacterium vitis* is one of the most destructive diseases of grapevines occurring in several countries. *Agrobacterium rhizogenes* strain K84 was effective against *A. tumefaciens* infecting peach, but not against *A. vitis* (Kerr 1980). *Rahnella aquatilis* isolated from vineyard soils in Beijing was evaluated for its biocontrol potential against *A. vitis. R. aquatilis* strain HX2 exhibited significant suppressive effect on the development of crown galls in grapevines. Under field conditions, immersion of the basal ends of grape cuttings with HX2 cell suspensions inhibited or completely prevented crown gall formation caused by *A. vitis* K308 in the roots of the plants growing from the cuttings.

20.3.4.5 Anthracnose

Liang et al. (2016) obtained a highly antagonistic strain of *Streptomyces atratus* PY-1, which could reduce disease severity by 92.13% in the detached leaf assay and by 83% in a field assay. Sawant et al. (2015) evaluated 34 *Trichoderma* isolates against anthracnose caused by *C. gloeosporioide*s hyphae in dual culture studies. The isolates produced volatile and non-volatile metabolites which inhibited the radial growth of *C. gloeosporioides*, but their efficacies varied. Sawant et al. (2016) isolated and screened 293 bacteria from the grape ecosystem of 43 spatially distant vineyards in the Peninsular India and identified 7 *Bacillus* isolates with significant biocontrol abilities. Narkar et al. (2017) evaluated 87 endophytic bacteria isolated from the mature shoots of Thompson Seedless for their antagonistic activity against carbendazim-resistant *C. gloeosporioides* isolates. They could identify three *Bacillus amyloliquefaciens* strains which gave good control of anthracnose under natural field conditions. Mochizuki et al. (2012) isolated *B. amyloliquefaciens* strain S13-3 which showed good inhibitory activity against *C. gloeosporioides* in vitro and could control ripe rot caused by *C. gloeosporioides* in vineyard. They attributed the antagonistic activity of S13-3 toward *C. gloeosporioides* on iturin A production by the strain.

20.4 Vegetables

20.4.1 Tomato

20.4.1.1 *Fusarium* Wilt Disease

Application of soil amendments to encourage the antagonistic microorganisms has been followed for the reduction of soilborne diseases. Chitosan has been applied as a soil amendment to suppress the development of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. The efficacy of strains of non-pathogenic *Fusarium oxysporum* in suppressing the *Fusarium* wilt of tomato was reported by Larkin and Fravel (1998). The BCA strain CS-20 reduced the *Fusarium* wilt disease at all temperature tested and four different field soils varying in texture and organic matter content. This strain was also effective against all three races and reduced the disease incidence by 48–66%. All formulations (FOR1 to FOR8) were applied to seedlings

in seedbeds at 7 days before transplanting. The percent disease reduction varied from 22 to 64% with all formulations. *Pseudomonas fluorescens* suppressed the development of tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. The Pf1 formulation at 10 ml/kg seeds and 150 ml/ha were found to be optimum for seed treatment and seedling root dip treatments to achieve effective control of tomato wilt disease (Manikandan et al. 2010). The bio-efficacy assays showed that addition of glycerol in the production medium reduced the tomato *Fusarium* wilt disease by 44–50% (Sriram et al. 2011).

Srivastava et al. (2010) observed that the combination of the bacterial *Pseudomonas* spp. and *Trichoderma harzianum* and mycorrhizal bioagents protected the tomato plants in a highly effective manner, resulting in a reduction of disease incidence by 74 and 67%, respectively, under greenhouse and field conditions. Yield of tomato fruits due to treatment was also increased by 20%. Addition of cow dung compost still further reduced disease incidence and improved the yield in all treated plots. Application of carbendazim at a low concentration (1 mg/ml) in combination with *B. cepacia* or *B. megaterium* reduced the disease symptoms by 46 and 84%, respectively, compared with carbendazim alone (77%) and untreated control. The antagonistic potential and compatibility with fungicides of *Bacillus megaterium* (strain 96) and *Burkholderia cepacia* (strain 91) were investigated for the control of tomato crown and root rot caused by F. *oxysporum* f. sp. *radicis-lycopersici* (Omar et al. 2006).

20.4.1.2 Damping-Off

Bacterial bioagents like *Pseudomonas fluorescens*, *P. putida*, *P. marginalis*, *P. corrugata*, and *P. viridiflava* reduced the incidence of damping-off caused by both *P. aphanidermatum* and P. *ultimum* (Gravel et al. 2005). Treatment of tomato seeds with *Bacillus subtilis AUBS*-1 formulations in lignite, lignite+ fly ash, and bentonite paste resulted in the effective suppression of the damping-off disease caused by *P. aphanidermatum* and enhancement of plant biomass under glasshouse and field conditions (Jayaraj et al. 2005). Soil application of *Bacillus subtilis* RB14-C protected the tomato seedlings against the damping-off disease caused by *Rhizoctonia solani* (Szezech and Shoda 2006). *Pseudomonas fluorescens* isolate CW2 in combination with fungicides azoxystrobin, metalaxyl-M, or pyraclosporin was assessed for their efficacy. The fungicides were fungitoxic to *P. ultimum*, but did not inhibit the development of *P. fluorescens* in in vitro assays (Salman and Abuamsha 2012).

20.4.1.3 Late Blight

Tran et al. (2007) showed that the compound mass A produced by *P. fluorescens* was effective in preventing tomato late blight infection and the further spread of the late blight lesions also reduced significantly. The study determined that induced systemic resistance was responsible for their results (Tran et al. 2007). Zakharchenko et al. (2011) demonstrated increased protection against phytopathogens including *P. infestans* when plants were colonized with a strain of *P. aureofaciens*. Various naturally occurring microorganisms, like *T. viride*, *Penicillium viridicatum*,

P. aurantiogriseum, *Chaetomium brasiliense* (Gupta et al. 2004), *Acremonium strictum*, *Myrothecium verrucaria*, and *P. aurantiogriseum* (Roy et al. 1991), showed antagonistic effect against *P. infestans*. The antagonistic activities of *P. fluorescens*, *Pseudomonas* sp., *Aspergillus flavus*, *A. niger*, *Penicillium* sp., *T. virens*, and *T. harzianum* showed positive inhibition of mycelial growth of *P. infestans*. *Bacillus pumilus* SE34 and *Pseudomonas fluorescens* 89B61 were employed to elicit systemic resistance in tomato against late blight disease.

20.4.1.4 Early Blight

T. viride was found to be effective against early blight of potato for reducing disease intensity under field conditions (Yadav and Pathak 2011). The bioagents *T. harzianum* and *P. fluorescens* (seed treatment + foliar spray) were effective in reducing the disease intensity of early blight of tomato and also increasing the yield (Mane et al. 2014).

20.4.1.5 Bacterial Wilt

Biocontrol of bacterial wilt by the use of antagonists such as *P. fluorescens*, *Bacillus* spp., avirulent *P. solanacearum*, and actinomycetes has been found to be effective in some countries (Mclaughlin and Sequerira 1988; Aspiras et al. 1986). Vesicular arbuscular mycorrhizae (VAM) increased growth and yield of tomatoes and reduced infection by *R. solanacearum*. This may be due to competition or the mechanical barrier in the form of VAM vesicles and hyphae that inhibit the bacterial pathogen from deeper penetration into host tissues (Halos and Zorilla 1979). Treatment of tubers with avirulent strain of *R. solanacearum* and strain of *P. fluorescens* caused a significant reduction in disease severity of bacterial wilt of potato (Kempe and Sequeira 1983).

Biological control of *R. solanacearum* has been reported in tomato (Guo et al. 2004) and in brinjal (Ramesh et al. 2009). *Pseudomonas fluorescens* induced ISR in tomato plants and prevented bacterial wilt (Kempe and Sequeira 1983). Xue et al. (2009) reported soil drenching with antagonistic strains of *Acinetobacter* sp. and *Enterobacter* sp. which reduced bacterial wilt incidence in tomato plants. *Bacillus* species are very effective in reducing bacterial wilt incidence in tomato (Li et al. 2004).

Fujiwara et al. (2011) reported effectiveness of *R. solanacearum* specific phage ORSL1, a jumbo phage belonging to *Myoviridae*, in preventing bacterial wilt in tomato. Infective phage particles were detected in plant rhizosphere and bulk soil highlighting its usefulness as BCA. Other phages with less effectiveness in bacterial wilt control include phage ORSAI (*Myoviridae*) with a very wide host range (infects race 1, 3, or 4 and biovar 1, N2, 3, 4 of *R. solanacearum*) and ORSB1, with T7-like morphology belonging to *Podoviridae* (infects race 1, 3, or 4 of *R. solanacearum* strains) (Fujiwara et al. 2011; Kawasaki et al. 2009). Phage PE226, a long lytic flexible filamentous (*Inoviridae*) with circular (+) sense single-strand DNA genome, has been isolated from tomato, potato, and pepper rhizosphere soil and infects nine different *R. solanacearum* including GMIl000 (Murugaiyan et al. 2010). A new phage ORS138 (*Siphoviridae*) has been isolated from tomato field soil lysed strains

of *R. solanacearum* (Thi et al. 2016). Co-inoculation of phage PE204 (*Podoviridae*) and *R. solanacearum* in tomato rhizosphere completely inhibited bacterial wilt infection. However, pretreatment with phage PE204 was less effective than post-treatment in bacterial wilt prevention (Bae et al. 2012). Addy et al. (2012a) reported loss of major virulence factors EPS, endoglucanase, and extracellular hydrolytic enzymes in *R. solanacearum* cells infected with phage ORSM3 (*Inoviridae*). Contrastingly, phage ORSSl (*Inoviridae*) enhanced virulence of *R. solanacearum* by inducing early expression of phcA (Addy et al. 2012b).

Frey et al. (1994) reported bacteriocin involved in bacterial wilt prevention by Hrp mutants of *R. solanacearum*. Avirulent *R. solanacearum* strains in combination with *Pseudomonas fluorescens* induced ISR in tomato plants and prevented bacterial wilt (Kempe and Sequeira 1983). Etchebar et al. (1998) studied xylem colonization by HrcV mutant of *R. solanacearum* and reported its efficacy in the prevention of bacterial wilt in tomato.

20.4.1.6 Bacterial Spot

In the field trials conducted in Alabama and Florida, *Pseudomonas syringae* strain Cit7 was found to be the most effective in suppressing bacterial spot disease in two of three trials. *Bacillus pumilus* SE34 suppressed the development of bacterial leaf spot in two field trials. Combined application of these two strains was effective against bacterial spot and bacterial speck of *Pseudomonas syringae* pv. *tomato* in all the trials. Both bacterial strains appear to enhance the level of resistance in treated tomato plants (Ji et al. 2006). *Pseudomonas fluorescens* and benzothiadiazole (BTH) were applied as seed treatment or foliar spray for the control of bacterial spot disease under field conditions. All treatments effectively reduced the severity of bacterial spot disease, compared with untreated control plants. Foliar application of *P. fluorescens* was the most effective treatment in reducing disease severity. The combined application of *P. fluorescens* and BTH reduced the pathogen population effectively and also promoted plant growth (Abo-Elyousr and El-Hendawy 2008). Phosphorus acid salts (PASs) were evaluated for their ability to suppress the development of tomato bacterial spot disease under field conditions for a period of 3 years.

20.4.1.7 Bacterial Speck

Non-pathogenic strains of *P. syringae* Cit7 most effectively reduced the disease intensity in the greenhouse conditions, when *P. syringae* strain TLP2, *Pseudomonas fluorescens* strain A506, and *P. syringae* pv. *syringae* DC3000 hrp mutants were also tested for their efficacy. The strain Cit7 provided a mean level of disease reduction of 78%, and hence, this strain was tested under field conditions at different locations in Alabama and Florida, USA, and Ontario, Canada. *P. syringae* Cit7 was the most effective in reducing disease severity. The mean level of disease reduction was 28% over ten different field experiments. *P. fluorescens* A506 available commercially as BlightBan provided a mean level of disease reduction of 18% over nine different field experiments. Commercially available plant activators like benzothiadiazole (BTH) (inducer of SAR) and plant growth-promoting rhizobacteria

have been shown to be effective, when applied individually. Application of BTH on greenhouse-grown tomatoes effectively reduced bacterial speck disease incidence and severity, both alone and in combination with the ISR-inducing bacterial product.

20.4.1.8 Bacterial Canker

The effectiveness of *Bacillus subtilis*, *Trichoderma harzianum*, and *Rhodosporidium diobovatum* in suppressing the development of tomato bacterial canker disease was indicated by Utkhede and Koch (2004). Pretreatment of tomato plants with ASM reduced the severity of the canker disease. Development of resistance to canker disease required an interval of 1–7 days between inducer application and challenge inoculation. Highest level of protection could be obtained, when plants were inoculated at 3 days after ASM application (Soylu et al. 2003; Baysal et al. 2003). Foliar sprays at 500 mg/ml of DL-ß-aminobutyric acid (BABA) suppressed canker disease development up to 54% 14 days after inoculation. Bacterial populations were reduced by 84% in planta treated with BABA (Baysal et al. 2003). In another investigation, the effect of BABA application alone or in combination with *Pseudomonas fluorescens* isolate CW2 in suppressing the development of tomato canker disease was assessed. Soil treatment with BABA or isolate CW2 significantly reduced the incidence of bacterial canker. Combined sequential treatments with BABA and isolate CW2 were found to be more effective in reducing the disease severity, compared to treatment with either BABA or isolate CW2. The combined application was effective not only for protecting the tomato plants against canker disease but also for promoting the growth of plants (Hassan and Buchenauer 2008).

20.4.1.9 Tomato Spotted Wilt Disease

Lecanicillium lecanii, *Metarhizium anisopliae*, and *Beauveria bassiana* were reported to be pathogenic on *F. occidentalis* (Vestergaard et al. 1995; Sengonca et al. 2006). A strain of *L. lecanii* originally isolated from glasshouse whitefly was also effective against thrip species *Frankliniella occidentalis* (Van der Schaaf et al. 1991). *Paecilomyces lilacinus*, a soil-inhabiting nematophagous fungus, produces chitinases and proteases capable of breaking down egg shell, facilitating penetration into insect body. This mechanism was demonstrated to operate effectively against *F. occidentalis* (Fiedler and Sosnowska 2007). In another investigation, five strains of *Beauveria bassiana* were evaluated for their efficacy against *F. occidentalis*. The strain RSB of *B. bassiana* was the most virulent causing 69–96% mortality at concentrations of 1×10^4 to 1×10^7 conidia/ml, at 10 days after inoculation of first instar larvae. In greenhouse evaluation, RSB strain applied to broccoli foliage significantly reduced adult and larval populations of *F. occidentalis* (Gao et al. 2012).

20.4.2 Potato

20.4.2.1 Late Blight

The antagonist *Bacillus subtilis* B5 was found effective in inhibiting the growth of *P. infestans* (Ajay and Sunaina 2005). Certain phyllosphere microorganisms like *Sporobolomyces* spp., *Acetobacter* spp., *Pseudomonas* spp., and *Bacillus* spp. were antagonistic to *P. infestans* (Ramos et al. 1993; Sanchez et al. 1998). *Bacillus* sp. inhibited mycelial growth of *P. infestans* both in vitro and in vivo (Sadlers 1996).

Streptomyces violaceusniger strain YCED-9 was strongly antagonistic to isolates of *P. infestans* in vitro. Rhamnolipid-based formulation (0.25%) from *Pseudomonas* spp. was tested under field trials at three different locations. The terminal disease severity in rhamnolipid formulation was 45% (compared to 100% in control). Ramos et al. (1993) and Sanchez et al. (1998) reported that certain microorganisms in the phyllosphere are antagonistic to *P. infestans*. These include the yeast *Sporobolomyces* spp. and isolates of *Pseudomonas* spp.

20.4.2.2 *Verticillium* Wilt

The efficacy of sweet corn varieties (Jubilee Sweet Corn and Jubilee Super Sweet Corn) as green manure was assessed for suppressing the *Verticillium* wilt disease of potato caused by *Verticillium dahliae*. The sweet corn varieties suppressed the disease incidence by 60–70%. These treatments did not influence the pathogen populations directly, but the colonization of *V. dahliae* on potato feeder roots and in potato tissues of stem pieces was reduced. Feeder root colonization was positively correlated with *Verticillium* wilt disease incidence ($P < 0.05$) and negatively corrected with yield. In addition, corn green manures increased the populations of several soil fungi such as *Ulocladium* and *Fusarium equiseti.* When potato was grown consecutively for 2 years, the beneficial effects of sweet corn green manures were almost entirely lost. But following two consecutive years of potato, a single sweet corn crop was enough to restore the original benefit of disease suppression and enhanced yields, although the pathogen populations had increased by fourfold. The results indicated the effectiveness of growing green manure crops that could reduce disease incidence and increase the yield as well (Davis et al. 2010a). Austrian peas, Sudan grass, rape, oats, and rye also exerted similar beneficial effects by reducing disease incidence and enhancing potato yields (Davis et al. 2010b). Bacterial isolate DF37 could reduce the *Verticillium* wilt disease by 29–43% and increased the yield of the cultivar Russet Burbank by 24% (Uppal et al. 2008).

20.4.2.3 Stem Rot

Sixteen isolates belonging to 11 species of *Trichoderma* were evaluated for their potential to suppress the development of stem rot disease. In addition, one isolate of *Talaromyces flavus* was also included in the evaluation. Spore suspensions of these fungi were sprayed on the foliage in the greenhouse assays. *T. koningii*, *T. virens*, *T. ceramicum*, and *T. viridescens* were more effective in reducing disease severity, while *T. flavus* was the least effective against the stem rot pathogen. *T. viridescens*

followed by T. *ceramicum* provided the best protection to the potato plants against S. *sclerotiorum* (Ojaghian 2011).

20.4.3 Brinjal

20.4.3.1 Damping-Off

Seed coating and soil application of *Trichoderma* formulation reduced the damping-off disease caused by *Pythium* spp. in many vegetables. Seed priming with slurry of *Trichoderma* resulted in 70% more plant stand in nursery bed as compared to non-treated plot in tomato and brinjal (Harmann and Taylor 1989). Seed treatment with talc-based formulation of *T. viride* @ 4 g/kg showed 7.0 and 12.5% pre- and post-emergence damping-off of chili, respectively, against 27.50 and 75% in control with reduction of pathogen population (Manoranjitham et al. 2000).

20.4.3.2 *Verticillium* Wilt

B. subtilis strain could effectively reduce the incidence and severity of wilt under greenhouse and field condition (Luo et al. 2010). Application of bioorganic fertilizer containing *B. subtilis* at the beginning of nursery and during transplanting control the *Verticillium* wilt by significantly changing fungal community structure and reducing pathogen population in rhizosphere (Lang et al. 2012). Karagiannidis et al. (2002) inoculated mycorrhizal fungi *Glomus mosseae* to control *Verticillium* wilt of egg-plant and found very low disease incidence due to enhanced root colonization, growth, and nutrient uptake in plant. A root- and rhizosphere-colonizing fungal strain QLP12 with broad-spectrum antifungal activity was identified in China as *Purpureocillium lilacinum* which showed excellent growth-promoting effect on eggplant seed germination (76.70%), bud growth (79.40%), chlorophyll content (47.83%), and root activity (182.02%) and reduced the incidence of *Verticillium* wilt by 83.82% in greenhouse (Lan et al. 2017). Mixing the transplant soil plug with *Paenibacillus alvei* Kl65 or non-pathogenic *F. oxysporum* F2, at a rate of 10 and 20% (v/V), respectively, reduced *Verticillium* wilt symptom development with PRI and PR4 expression in the roots of brinjal (Angelopoulou et al. 2014).

20.4.3.3 Collar Rot

The soil and seedling dip treatment with *T. viride* @4 g/kg of seedling and 50 mg with 5 g FYM/m, respectively, was found most effective in reducing the percent disease incidence of collar rot followed by summer plowing and also increased the growth (Jadon 2009). Elad et al. (1999) proposed that spore germination and germ tube elongation of *S. sclerotiorum* were hindered by protease released from *Trichoderma* spp. *Sporidesmium sclerotivorum* was detected in soil sample from fields which caused a natural decline of *S. sclerotiorum* in field soils. The increase in glucanase activity may be stimulated by the production of haustoria by *S. sclerotivorum* in the cells of sclerotia of *S. sclerotiorum*. *Talaromyces flavus* is another mycoparasite of *S. sclerotiorum* (Su and Leu 1980). McLaren et al. (1982)

obtained 92% disease control of sunflower wilt caused by *S. sclerotiorum* when *T. flavus* and *sclerotia* were buried together in the field**.**

20.4.3.4 *Fusarium* Wilt

Several species of *Trichoderma* and *P. fluorescens* were widely used for controlling vascular wilt caused by *Fusarium* spp. by several workers (Elad and Baker 1985; Biswas and Das 1999; Vyas and Mathur 2002; Najar et al. 2011).

20.4.3.5 Stem and Root Rot

Several microorganisms including *Trichoderma* spp. and *P. fluorescens* are widely used for controlling *Rhizoctonia* disease in soil by suppressing the competitive saprophytic ability. Bunker and Mathur (2001) reported that 51% more disease control was achieved by seed treatment and soil application of *Trichoderma* formulation against capsicum root rot caused by *R. solani*. Plant root colonized by VAM fungi tolerates the infection of *R. solani* to a great extent (Zambolim and Schenck 1983). Combined seed bacterization with *B. subtilis* CA32 and soil application of compatible *T. harzianum* RUO1 significantly enhanced protection of brinjal from *R. solani* (Abeysinghe 2009).

20.4.3.6 *Phomopsis* Blight

T. virens, *T. harzianum*, and *T. viride* are effective in antagonizing the mycelial growth of *P. vexans* on brinjal in vitro (Muneeshwar et al. 2011). Antimicrobial actions of *B. subtilis*, *Streptomyces griseus*, and fungal species such as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Periconia* have been tested against *Phomopsis vexans* (Varma and Bhale 2010). Antagonistic *P. fluorescens* and *T. harzianum* seed treatment and foliar applications are effective against *P. vexans* (Srinivasa et al. 2005; Das et al. 2014). Sowing of apparently healthy seeds treated with garlic bulb extract and soil treatment with *T. harzianum* completely controlled damping-off, tip over, and seedling blight in the nursery bed with 48.83% increased seed germination over control in Bangladesh (Islam and Meah 201). Rohini et al. (2016) reported that combined application of rhizosphere-colonizing bacteria *P. putida* Has-1/c and phylloplane-colonizing bacteria *B. subtilis* Br/ph-11 significantly reduced the disease incidence (18.00%) and severity (0.54) in comparison with distilled water-treated control (91.00% and 6.00%).

20.4.3.7 Bacterial Wilt

Bacteria of the genera *Pseudomonas*, *Bacillus*, *Paenibacillus*, and *Sphingomonas* isolated from bacterial wilt-resistant plants were reported to be highly antagonistic to *R. solanacearum* (Feng et al. 2013). Very high biocontrol effect against bacterial wilt and growth promotion in tomato was exhibited by *P. fluorescen*s (Seleim et al. 2011). Endophytic strains of *Burkholderia* sp., *Bacillus* sp., and DAPG-producing *Pseudomonas* sp. from brinjal were found inhibitory to *R. solanacearum* (Ramesh et al. 2009).

20.4.4 Cucurbitaceous Crops

20.4.4.1 *Pythium* Root Diseases

The bacterial strains *Bacillus subtilis*, *Pseudomonas fluorescens*, and *P. corrugata* and two fungal strains *Trichoderma viride* and *T.* (*Gliocladium*) *virens* were evaluated for their ability to suppress the damping-off disease of cucumber caused by *P. ultimum*. Among the antagonists, *Pseudomonas* spp. were superior to *Bacillus subtilis* in reducing the incidence of damping-off disease in cucumber. Combination of antagonists did not show any additive effect. The effectiveness of disease suppression was greater, when the bacterial antagonists were applied by drenching or by coating the cucumber seeds with bacteria in a peat carrier (Georgakopoulos et al. 2002).

20.4.4.2 *Fusarium* Wilt Disease

The strains of *Pseudomonas aeruginosa* isolated from the composts exhibited the greatest antagonistic activity against FORC. Further, internal stem colonization of FORC was significantly reduced by *P. aeruginosa* (Bradley and Punja 2010). Application of compost reduced the incidence of *Fusarium* wilt disease of melon. *Aspergillus* sp. isolated from the compost was the most effective one. The suppressiveness of the compost was associated with the population of *Aspergillus* spp. (Suárez-Estrella et al. 2007). The efficacy of two citrus composts composed of 40% citrus wastes, 20% sludge obtained from citrus industry wastewater treatment facility, and 40% green residues and C2 composed of 60% citrus wastes amended with *Trichoderma harzianum* T-78 was evaluated for the control of *Fusarium oxysporum* f. sp. *melonis* (FOM). Incidence of *Fusarium* wilt disease on melon and growth promotion effect of the treatments was recorded. Disease incidence was significantly reduced in C2Th (extract amended with T-78), while C1Th was not effective. Population of T-78 significantly decreased at first sampling time compared to the initial level, but later recovered over time. The results indicated that combination of citrus compost and *T. harzianum* T-78 could become a viable alternative to peat and also adoption of this strategy could minimize the chemical use for the management of *Fusarium* wilt disease in greenhouse nurseries for melon seedling production (Lopez-Mondejar et al. 2010). The bioorganic fertilizer containing an organic fertilizer and *Paenibacillus polymyxa* (3×10^7 CFU/g) and *Trichoderma harzianum* (5×10^7 CFU/g) was evaluated for its efficacy in suppressing the development of watermelon *Fusarium* wilt disease caused by *F. oxysporum* f. sp. *niveum.* The incidence of *Fusarium* wilt disease was reduced by 84.9 and 75.0% at 27 and 63 days after treatment with bioorganic fertilizer (0.5%) under greenhouse conditions (Wu et al. 2009). The incidence of *Fusarium* wilt disease was reduced by 60–100% in the greenhouse and by 59–73% under field conditions. Nursery application of B10 reduced the pathogen population in the soil significantly. The bacterial BCA *Paenibacillus polymyxa* present in the product effectively colonized the rhizosphere of watermelon and proliferated along the extending plant roots (Ling et al. 2010).

20.4.4.3 Anthracnose Disease

Plant growth-promoting rhizobacterial strains *Bacillus pumilus* strain INR7, *Bacillus subtilis* GB03, and *Curtobacterium flaccumfaciens* strain ME1 either alone or in combination of strains reduced the severity of anthracnose disease caused by *Colletotrichum orbiculare* and bacterial angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans*. *Serratia marcescens* suppressed the development of anthracnose disease caused by *C. orbiculare* in cucumber through induced systemic resistance (ISR). *Bacillus mycoides* isolate BmJ and *B. mojavensis* isolate 203-7 were tested for their ability to induce systemic resistance in treated plants against the anthracnose disease. The isolates BmJ and 203-7 delayed disease onset and reduced total (43 and 56%) and live spore production (38 and 49%) per mm^2 of lesion area by inducing systemic acquired resistance in cucumber. Field experiments (2004 and 2005) were conducted to evaluate the efficacy of applications of BmJ and fungicides for the control of anthracnose in cucumber cv. General Lee and cantaloupe. BmJ applied 7 days before inoculation with the pathogen reduced disease severity by 41% in cucumber in 2004 and by 21–14% in cantaloupe for both years, compared with water controls which were in equivalence to fungicides azoxystrobin and chlorothalonil. BmJ applied 1 week prior to inoculation with the pathogen significantly reduced the AUDPC values ($P = 0.05$) in cucumber, compared with control plots (Neher et al. 2009).

20.4.4.4 Powdery Mildew Disease

Verticillium (= *Lecanicillium*) *lecanii* is reported to effectively suppress the cucumber powdery mildew development. In addition, *V. lecanii* was also pathogenic to the aphid *Macrosiphum euphorbiae* which is an efficient vector of Cucumber mosaic virus infecting cucumber and other cucurbitaceous crops. The biocontrol efficacy of two mycoparasite-based products AQ10® containing *Ampelomyces quisqualis* and Mycotal® containing *Lecanicillium lecanii* as well as three strains of *Bacillus subtilis* was assessed for the control of the melon powdery mildew disease caused by *Podosphaera fusca*. The mycoparasites were more effective, when the relative humidity values in the greenhouse were 90–95%. The effectiveness of the mycoparasites *A. quisqualis* and *L. lecanii* was absolutely dependent on mineral oil. The mycoparasites were most effective only in combination with the mineral oil ADDIT, showing a disease reduction of 80–95%. On the other hand, the strains of *Bacillus subtilis* without any complementary additive were very effective in providing disease reduction similar to mycoparasites with mineral oil or the fungicide azoxystrobin. The results revealed the effectiveness of these BCAs for suppressing the development of powdery mildew disease of greenhouse melons/ cucurbits either as single products or as a component of integrated control programs (Romero et al. 2007). Three commercially available products Actinovate (*Streptomyces lydicus* WYEC108), Companion (*Bacillus subtilis* GB03), and Sonata ASO (*Bacillus pumilus* QRD2808) were evaluated along with the fungicide quinoxyfen for their efficacy in suppressing the development of powdery mildew in pumpkin under field conditions where the disease pressure was high. Penetration of foliar sprays of the bacterial BCA strains to the lower surfaces appeared to be restricted,

resulting in higher incidence and severity of the disease in the lower surface of leaves. Disease incidence was lower in *B. subtilis*-treated foliage than on leaves treated with *S. lydicus* or *B. pumilus*. Disease incidence on the upper surface of leaves treated with three species in rotation was similar to levels in *B. subtilis*-treated plots. The fungicide quinoxyfen was the most effective in reducing the disease severity and outperformed all three bacterial BCAs tested (Janousek et al. 2009).

20.5 Medicinal Plants

20.5.1 Cumin

Bio-efficacy of different bioagents, viz., *T. harzianum*, *T. viride*, *Aspergillus niger*, *A. flavus*, *P. fluorescens*, *Bacillus subtilis*, *Paecilomyces lilacinus*, and *P. chlamydosporia*, has been tested under in vitro and in vivo conditions (Deepak et al. 2009; Shelar 2013). With the aim of sustainable management of cumin blight, in vitro mycelial growth inhibition of *Alternaria burnsii* was achieved by using *T. harzianum* strain II (85.45%), *T. viride* (69.8%), *P. fluorescens* (45.3%), and *B. subtilis* (26.7%) separately (Vihol et al. 2009). Similarly it is evident with bioagents when applied either through seed treatment or as soil/foliar application under field conditions (Vyas and Mathur 2002; Sharma and Pandey 2011).

20.5.2 Funnel

The different antagonists, viz., *T. harzianum*, *T. viride*, and *B. subtilis*, were used for coating seed @ 5 g/kg of seeds to protect seedling from damping-off and root rot diseases (Gebily 2015). Commercial formulation of *T. harzianum*, *T. viride*, *P. fluorescens*, and *B. subtilis* is used with Tween 80 @ 0.3% for coating seed 12 h prior to sowing (Ahmed et al. 2016), while for the management of root-knot nematode (*Meloidogyne javanica*) organic amendment with neem cake @100 kg/ha or castor @1000 kg/ha is found to be effective as nematicidal treatment at Kapadvanj, Gujarat (Patel et al. 2005). Exploitation of nematode antagonists, viz., *Paecilomyces lilacinus*, *Verticillium chlamydosporium*, and *Bacillus* sp., will also provide very good options for managing root-knot nematodes in spices (Ramana and Eapen 1995).

20.5.3 Coriander

Wilt caused by *Fusarium oxysporum* f. sp. *coriandrii* is the most common problem, which severely affects the crop. *T. harzianum* was found most effective to inhibit 83.69% of mycelial growth of fungus in vitro, and seed treatment with *T. harzianum*

@ 4 g kg/seed was recorded to be more effective in reducing the wilt incidence to 20.81% and 61.75% disease control under field condition and maximum 801.33 kg/ ha seed yield (Jat et al. 2017). Similarly, *P. fluorescens*@ 10 g kg and *T. viride* (4 g/ kg) as seed treatment + soil application reduce the wilt incidence. This crop also suffers from stem gall (*Protomyces macrosporus*), which is a serious problem in Rajasthan and responsible for heavy losses in seed yield. It can be managed by seed treatment and subsequent three foliar sprays of *T. viride* and *P. fluorescens* (0.4%) at regular interval (Kumar et al. 2014). Problem of nematodes in coriander is also increasing day by day. *P. fluorescens* is proved to be best to reduce the gall index (Sultan et al. 2011).

20.5.4 Fenugreek

Bioagents like *T. viride*, *P. lilacinus*, and *P. fluorescens* are very effective to manage these soilborne pathogens. Of them, root rot (*Rhizoctonia solani*) can be effectively managed by seed treatment with *T. viride* and/or *P. fluorescens* (Reddy 2014). The efficacy of seed pelleting and soil application with *Trichoderma* sp. along with neem cake @150 kg/ha also provides better protection to root rot protection (Reddy 2014). Similarly, seed treatment with *T. viride* @4 g/kg followed by soil application @ 5 kg/ha along with 150 kg/ha of neem cake suppresses the population of root-knot nematodes.

20.5.5 Black Pepper

The antagonistic potential of bacterial isolates was also evaluated by dual culture technique against *P. capsici*, the foot rot pathogen of black pepper, and the efficient bacterial strains that showed up to 72% inhibition of *P. capsici* were shortlisted. Similarly, out of about 172 *rhizobacteria* isolated from seed spices, 25 were shortlisted based on useful agronomic traits like P solubilization, enzyme production, etc. for greenhouse trials. Based on growth promotion, two isolates were selected for large-scale testing in seed spices-growing areas (Bini et al. 2011).

Biocontrol agents *Pseudomonas fluorescens* and *Trichoderma harzianum* isolated from rhizosphere of black pepper, ginger, and cardamom have been evaluated for their efficiency, and efficient isolates for each crop were identified and used against the pathogens of respective crops. In this study the efficient isolates of *Trichoderma*, namely, IISR-1369 and IISR-1370 from black pepper, ISR-1371 from ginger, and IISR-1292 from cardamom, were made into different combinations and tested along with *P. fluorescens* strains from black pepper (IISR-11) and ginger (IISR-6) for their ability in disease suppression and growth promotion in these crops. The experiments on black pepper and ginger were performed in the greenhouse, and that of cardamom was carried out in already-existing field. Out of the 22 different treatments, 3 treatments were found to be effective in suppressing root rot (*P. capsici*) disease in black pepper, soft rot (*P. aphanidermatum*) in ginger, and

clump rot (*P. vexans*) in cardamom. The best biocontrol agents included *T. harzianum* isolate, IISR-1369, and *P. fluorescens* strain, IISR-11 or IISR- 6 in common. The maximum disease suppression obtained by the treatment combination, *P. harzianum* isolate, IISR-1369, and *P. fluorescens* strain, IISR-11, in black pepper was 63, and for cardamom, it was 36% over control. The same treatment could impart 66.2% survival of ginger tillers after challenge inoculation with the pathogen. The combination of *T. harzianum* isolate, ISR-1369, and *P. fluorescens* strain, ISR-11, could improve the vigor of the plant in both black pepper and ginger. The same treatment combination imparted maximum yield in ginger and cardamom. Our earlier studies had proved the mutual compatibility between *T. harzianum* and *P. fluorescens*. When these biocontrol agents were applied in combination, there was synergistic effect for both growth promotion and disease suppression.

The egg parasitic fungus *P. chlamydosporium* and the obligate bacterial parasite *P. penetrans* were able to check the root-knot nematode multiplication in cardamom nurseries. Significant reduction in nematode population was observed in plots where *P. chlamydosporium* was applied. There was a significant increase in the total biomass of individual cardamom seedlings treated with *P. lilacinus*, but both *Trichoderma* and *P. lilacinus*, either alone or together, significantly improved the number of quality seedlings. The rhizome rot incidence was drastically reduced wherever any of these bioagents was applied. In general, biocontrol agents performed better in solarized soil than in non-solarized beds (Eapen and Venugopal 1995; Eapen et al. 2005).

Leaf rot (*Rhizoctonia solani*) and foot rot quick wilt (*Phytophthora capsici*) are serious concerns in nursery and main field when warm humid condition prevails. These soilborne pathogens can be managed by using biocontrol agents such as VAM @ 110 cc/kg of soil mixture, *Trichoderma* sp. @ 5 g/kg of soil (cfu 10/g), and *P. fluorescens* @1 g/kg of soil (cfu 18/g). Sometimes, *T. harzianum* enriched pre-wetted neem cake or FYM @1 kg/100 kg perform very best in controlling of wilt disease, when applied during pre-monsoon season @5 kg/vine below 10 years and 10 kg/vine above 10 years (Reddy 2014).

20.5.6 Ginger

Soft rot of ginger (*Erwinia* sp.) is another serious problem that massively affects the ginger production and causes significant loss in storage, and its infestation starts from field. Hence, in situ protection strategy should be followed. To ensure health of planting materials, rhizomes should be disinfected through solarization, followed by treatment with bio-inoculant of *T. harzianum* and rhizobacterial strain in consortia for seed treatment as well as soil application. *T. harzianum* in combination with *P. fluorescens* showed a synergistic effect in reducing the soft rot infection and ensured higher yields and soft rot suppression in storage. In addition to this, application of *Glomus* spp. helps in better growth promotion.

20.5.7 Turmeric

Rhizome treatment with *T. viride* + *P. fluorescens* (@ 4 g/kg seed) and soil application of *T. viride* (2.5 kg/ha) and *P. fluorescens* (25 kg/ha) along with FYM @10 MT/ha also helps in minimizing rhizome rot (Muthulakshmi and Saveetha 2009).

20.5.8 Cardamom

Sometimes, infestation of root-knot nematode predisposes the cardamom seedlings to *R. solani* infection (Ali and Venugopal 1993). Hence, integrated strategy should be followed up for managing the problem. Solarization of nursery beds and subsequent soil application of *T. harzianum* reduce root rot infection and also nematode infection. *P. lilacinus* in combination with *Trichoderma* sp. can also be applied to suppress nematode-rhizome rot (*R. solani*) complex when incorporated in solarized cardamom nursery beds (Eapen and Venugopal 1995).

20.5.9 Coleus

20.5.9.1 Root Rot/Wilt

Management of soilborne plant pathogens in particular, by organic and biological methods, is being considered because chemical methods can result in accumulation of harmful residues which may lead to serious ecological and health problems (Singh et al. 2009). Some workers reported that the root rot/wilt of *C. forskohlii* could be reduced by the application of *Trichoderma viride*, *Pseudomonas fluorescens*, and AM fungi like *Glomus fasciculatum* and *G. mosseae* (Boby and Bagyaraj 2003; Singh et al. 2009). *T. viride* and *P. fluorescens* reduced the disease incidence by 20–21% (Paramasivan et al. 2007). Combination of *T. viride* + Neemato (neem-based product applied at 500 g/ 5 m^2) resulted in lowest wilt incidence by 12.76% (Kulkarni et al. 2007).

Botanical extracts like *Eucalyptus citriodora*, *Ricinus communis*, and *Azadirachta indica* @ 5% significantly inhibited the growth of bacterial (*Ralstonia solanacearum*) and fungal (*Fusarium chlamydosporum*) growth under in vitro condition (Divya et al. 2010). Application of neem cake also reduced disease up to 40%.

20.5.9.2 Root-Knot Nematode

Biological control agents are gaining importance in the field of nematode management. Another importance of these agents is their role as plant growth-promoting microorganism (Sharon et al. 2001a, b). *Trichoderma* spp. found in close association with roots contribute as plant growth stimulators (Ousley et al. 1994). Soil application with bioagents like *T. viride* and *P. fluorescens* significantly reduced the nematode population in soil and root and increased the growth and yield of *C. forskohlii* crop (Senthamarai et al. 2008). Integration of strategies such as stem

cutting dipping in *P. fluorescens* + soil application of neem cake @ 400 kg/ha + growing marigold as intercrop followed by their biomass incorporation during earthing up increased the yield (22.7–30.0%) and reduced the root-knot nematode population (71.2–73.8%) superiorly, followed by the integration of *P. fluorescens* + marigold intercrop, which were almost equally effective (Seenivasan and Deevrajan 2008).

20.6 Economically Important Nematode Pests of Horticultural Crops

Economically important genera of *phytonematodes* associated with various horticultural crops around the globe comprise *Meloidogyne* (root-knot nematode), *Heterodera* (cyst nematode), *Ditylenchus* (stem and bulb nematode), *Globodera* (potato golden cyst nematode), *Tylenchulus* (citrus nematode), *Xiphinema* (dagger nematode), *Radopholus* (burrowing nematode), *Rotylenchulus* (reniform nematode), and *Helicotylenchus* (spiral nematode).

Kannan and Veeravel (2012) studied the effect of different dose and application methods of *Paecilomyces lilacinus* against *Meloidogyne incognita* on okra at two different locations. In field trials maximum shoot length (60 and 90 DAS), shoot weight (90 DAS), and root length (90 DAS) were documented, and they were positively correlated with fruit yield of okra (Rao et al. 2012). Hallmann and Sikora (1993) evaluated 200 isolates of endophytic fungi, representing different genera, isolated from tomato roots. They found a reduction in gall formation by *M. incognita* between 52 and 75% after application of four endophytic strains of the fungus *Fusarium oxysporum*. They also found that *M. incognita* attraction and penetration of tomato seedlings were significantly reduced following treatment with the culture filtrate *of Fusarium oxysporum.*

Biocontrol of the root-knot nematodes (*Meloidogyne* spp.) by different species of *Trichoderma* has been reported by several scientists (Sharon et al. 2001a, b, 2007, 2011; Affokpon et al. 2011; Mascarin et al. 2012; Naserinasab et al. 2011; Al-Shammari et al. 2013).

Hallmann et al. (1995a, b) found some evidence that endophytic bacteria may contribute to control of plant parasitic nematodes. They evaluated seven isolates of endophytic bacteria isolated from cucumber and cotton roots against root-knot nematode, *M. incognita*, and they found a significant reduction of 50% in the number of galls on cucumber. Munif et al. (2000) screened the endophytic bacteria isolated from tomato roots toward *M. incognita* on tomato under greenhouse conditions. They showed antagonistic properties in the screening of 21 out of 181 endophytic bacteria toward *M. incognita*. Application of these rhizobacteria to sugar beet seed and potato seed pieces caused significant decreases in early root infection of the sugar beet cyst nematode *Heterodera schachtii* and potato cyst nematode *Globodera pallida* (Racke and Sikora 1985; Oostendorp and Sikora 1986). Zavaleta-Meija and Van Gundy (1985) found that rhizobacteria have biocontrol activity toward root-knot nematode, and they showed that more than 12% of the rhizobacteria tested

reduced the number of galls of *M. incognita* on cucumber and tomato. Sikora (1992) reported that 7–10% of the rhizosphere bacteria isolated from potato, sugar beet, or tomato root systems have antagonistic activity against cyst and root-knot nematodes. Sikora and Hoffimann-Hergarten (1993) revealed that plant health-promoting rhizobacteria influence the intimate relationship between the plant parasitic nematode and its host by regulation of nematode behavior during the early root penetration phase of parasitism which is extremely important for crop yield. Strains of *Pseudomonas chitinolytica* were also shown to reduce *M. javanica* on tomato as reported by Spiegel et al. (1991). Smith and Grenfell (1994) reported that *Bacillus* sp. strain 23a reduced *M. javanica* densities on tomato and *Pseudomonas fluorescens* strain reduced the number of galls and egg masses of *M. incognita* on tomato roots (Santhi and Sivakumar 1995).

Khan and Haque (2011) showed that application of *P. fluorescens* decreased gall index from 3.0 to 1.33 and improved plant growth of two susceptible tobacco cultivars by up to 32%. *Trichoderma harzianum* suppressed gall index from 3 to 2. Similar effects of *P. fluorescens* and *Trichoderma* spp. on different crops against *Meloidogyne* spp. have been reported (Khan et al. 2007). The bacterium is a phosphate solubilizer (Khan et al. 2009) but may also suppress pathogens through antibiosis (Nielsen et al. 1998), induced systemic resistance (Kloepper et al. 1992), and production of phytohormones (Garcia de Salamone et al. 2001). Bari et al. (2004) who reported that *T. harzianum* 1 g/plant reduced root-knot nematode and enhanced vegetative growth of lady's finger in the field. Prasad et al. (2014) reported lowest root-knot nematode *M. incognita* population and increased plant growth of carrot with *T. harzianum* at 25 g per m^2 followed by isolated *T. harzianum* and commercial *T. harzianum* at 20 g per m. Chormule et al. (2017) found that the efficacy of bioagents, viz., *P. fluorescens*, *P. lilacinus*, *Phule Trichoderma plus*, *T. viride*, and *P. chlamydosporia*, @ 20 kg/ha and organic amendment with neem cake @ 2 t/ha were effective in reducing the root-knot nematode population, number of root galls, and egg masses and increasing fruit yield at terminations.

Bacterial isolates obtained from spices were screened against nematodes initially by employing the buffer method to assess their nematode suppressing ability. Except a few isolates, most of the bacterial isolates caused very less mortality of nematodes in this test. Based on their efficacy, 30 bacterial isolates were selected for further in vitro evaluation using different methods like culture filtrate assay, direct assay of bacterial suspension, and assay of volatile and non-volatile metabolites. Culture filtrates of 77 bacterial isolates were studied for their nematode toxic activity. Out of these, 22 isolates caused >90% mortality to root-knot nematodes, while another 40 isolates possessed high (>50% mortality) nematicidal property. Metabolites of 67 bacterial isolates were also tested for their nematicidal activities. Volatile metabolites play a crucial role in killing the nematodes. Besides, the production of HCN and H_2S by these bacteria was also monitored. Out of the 98 isolates screened, only 6 isolates produced HCN. H_2S production was observed in another 6 isolates among the 50 tested. The egg parasitic fungus *P. chlamydosporium* and the obligate bacterial parasite *P. penetrans* were able to check the root-knot nematode

multiplication in cardamom nurseries. Significant reduction in nematode population was observed in plots where *P. chlamydosporium* was applied.

20.7 Conclusion

The search for alternative ways to manage plant diseases is the most important concern to protect ourselves from the widespread use of chemicals that contaminate the soil and water and leave toxic residues that affect the environment. The biocontrol efficacy of antagonistic microorganisms depends on a combination of factors such as the characteristics of the antagonistic microorganism, the epidemiology of the target pathogen, and the environmental conditions in which the relationship between the pathogen and the antagonist(s) is taking place. Hence, efforts are needed to commercialize these novel microbes to bring in a second revolution within the country. However, it's clear that the stage is set for biological control agents to play a greater part in agriculture and horticulture. This approach undoubtedly would encourage environmentally desirable products that are desired by the general public to succeed in the marketplace rapidly. Biological disease management systems have to be developed by integrating several strategies taking into consideration pathogen biology, cultivar resistance, and epidemiology.

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21 Transgenerational Plant Immunity in Plant Disease Management

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Abstract

Plants have the potentiality to transfer the message of threat to their offspring. Plants adopt such mechanisms probably due to the fact that the infants and the younger ones are particularly viewed to be vulnerable to the detrimental effects of the environment. Plants can pass on such messages to the next generation through their seeds. Parents use mostly three mechanisms that are present at disposal to the higher organisms to start and sustain the epigenetic gene regulation such as DNA methylation, histone modification, and RNA interference. Plants may be induced to bring out epigenetic modifications for their signature stress memories through a process known as "priming." Priming can induce epigenetic modifications in plants to face both biotic and abiotic stresses and the same can be passed on to their modifications. Therefore, transgenerational epigenetics is seen as a future strategy to combat both biotic and abiotic stresses in plants.

Keywords

Plant immunity · Transgeneration · Epigenetics · DNA methylation · Histone modifications

21.1 Introduction

All living organisms on this planet, that is, humans, animals, plants, microbes, along with the soil and environment are suffering from the casualties posed by the pesticidal regime that is being followed currently, and we are in search of much-needed advancement in the present agricultural practices. Infants and the younger

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ones are particularly viewed to be vulnerable to the detrimental effects of the pesticides (Eskenazi et al. 1999). Apart from that, the change in climatic condition is being expected to increase the predominance of extreme environmental conditions which poses an undeniable threat to the loss in crop yield in near future (Lobell et al. 2011). On the account of such conditions, improved tolerance to various stress and substitutes to synthetic pesticides is the need of the hour (Sarma et al. 2007; Singh et al. 2013a). Although the responses of plants to distinct stresses are relatively well understood, however, the natural occurrence of stress is incessant or recurrent and reactions to such stresses are limitedly understood. The recent studies have suggested that plants carry a stress memory which helps in adapting to the recurrent stress (Bruce et al. 2007; Crisp et al. 2016). A highly promising measure to improve tolerance to stress and to curtail the use of pesticides in agricultural field is by reinforcing the host innate immunity (Singh et al. 2013b) through improvements in the stress memory either by activating priming responses or via alteration(s) in specific regions of the epigenome.

The gene accessibility for the transcriptional process is regulated by the chromatin structure, and so it is an indispensable component of regulated expression of the gene in responses to stress and development (Struhl and Segal 2013; Zentner and Henikoff 2013). The access to separate regulatory elements and the overall packing is affected essentially by methylation of the DNA along with the positioning and spacing of nucleosomes. The chromatin consists of nucleosomes as their basic unit, which is made up of two molecules, namely, histone octamer having histones H2A, H2B, H3, and H4; wrapped around in almost two turns with 147 bp of DNA. The overall packaging is contributed by the variation in length of three unpackaged linker-DNA sections present in between two nucleosomes and the bond of linker histone H1. Posttranslational modifications of histone tail such as acetylation, methylation, phosphorylation, and ubiquitination; the decisive positioning and tenancy of nucleosomes; and inclusion of the variants of histones replacing the canonical histones lead to an alteration in the chromatin structure. Additionally, DNA modification is also achieved by methylation of cytosine which affects the DNA sequence accessibility without any change in base pairing or the genetic code. Cytosine methylation in plants take place at CpG, CHG, or CHH sequences and, in this context DNA methylation is differentiated into asymmetrical and symmetrical (Matzke and Mosher 2014; Du et al. 2015). The mechanism of inheritance of CpG or symmetrical DNA methylation is straightforward by DNA replication, resulting two daughter strands which are hemi-methylated, and the missing methylation mark can be filled by recruiting a DNA methyltransferase on newly replicated daughter strands. This symmetrical DNA methylation is a faithful model of inheritance mitotically and is often referred to as a mark of epigenetics (Lämke and Bäurle 2017).

Epigenetics is defined as the study of all the stable, reversible, heritable changes in the gene expression of an organism without any change in the DNA sequence, that is change in the phenotype of the organism without any change in their genotype. Basically, these changes or modification leads to change in pattern in how the cells read the genes. These epigenetic changes occur regularly and naturally as well as

several factors influence it including age, environment, and disease. The term "epigenetics" was coined originally by Conrad Hal Waddington in 1942. Epigenetic modification, in general, is a term used for the changes in the structure of nucleosome through modifications in histone, histone variants, or DNA modification, although, all the epigenetic modifications are not necessarily of the epigenetic phenomenon. These epigenetic modifications that are caused due to abiotic or biotic stresses lead to a stress memory which is described as the phenomenon by which message about a past stress clue is retained and as a result, a modified response is seen when the stress occurs recurrently. This stress memory can be intergenerational or transgenerational. The intergenerational stress memory can be defined as the stress memory which is passed on to just the first stress-free offspring generation of the organisms from one stressed generation, while the transgenerational stress memory can be defined as the stress memory which is noticeable after at least two stress-free generations of organisms.

21.2 Epigenetic Mechanisms in Plants

Plants use all the three mechanisms that are present at disposal to the higher organisms to start and sustain the epigenetic gene regulation – DNA methylation, histone modification, and RNA interference. In DNA methylation process, the methylation of cytosine is the sole method for epigenetic regulation, but histone modifications include numerous methods which involve acetylation, methylation, phosphorylation, ribosylation, sumoylation, and ubiquitination. RNA interference, on the other hand, is a feature of both DNA methylation and histone modifications. The three mechanisms of epigenetics have various effects and implications on the plants. The mechanism is discussed as below:

(i) *DNA Methylation*: This is a method for the epigenetic inheritance, that is, transfer of expression state of the gene from the mother to daughter cells. It is also a very effective regulatory system of gene expression (Tchurikov 2005). The process involves the addition of a methyl group in the cyclic carbon-5 of cytosine ring in DNA. The levels of methylation greatly vary among the organisms as the methylated cytosines (5mC) percentage ranges from 0 to 35 in insects to more than 30% in some plants (Adams 1996). The DNA methylation in plants is controlled by plant hormones and also influenced by various phytopathogens, and it is also specific to species, tissue, organelle, and age (Vanyushin 2005). The sites of DNA methylation in plants are CpG (cytosine–phosphate–guanine) islands, CpNpG (N = A, C, or T), and CpNpN. The methylation is restricted to symmetrical sites (CpG) in mammals, but it occurs in asymmetrical sites (CpNpN) in plants. The catalyst in the DNA methylation process is a family of enzyme which is conserved and known as DNA methyltransferases (DNA MTases). There are three types of DNA MTases, namely, maintenance methylases, de novo methylases, and domain-rearranged methylases (DRMs).

The proposed prominent roles of DNA methylation process are as follows:

- To direct the developmental process of an organism by providing a heritable epigenetic mark (Holliday and Pugh 1975; Regev et al. 1998; Wolffe and Matzke 1999)
- To implement the genomic defense responses against the parasitic mobile organism (Yoder et al. 1997)
- To inhibit the transcriptional chaos in organisms having a large number of genes (Bird 1995)
- To remember the activity pattern of the gene by stabilizing gene silencing that occurs through other mechanisms (Bird 2002)

  Despite the above-mentioned roles, we can expect the methylation process to also serve diverse functions and to perform distinct task both within and among the organisms, as it is an evolutionary tool (Colot and Rossignol 1999).

The DNA methylation is associated closely with silencing of the gene, although it is not the cause but the consequence. The silencing occurs by methylation in the specified genes or in the promoter region which results in suppression of the transcription process. Evidences to support the role of methylation in gene suppression have been shown by studies on transposable elements regulation (Fedoroff 1996; Martienssen 1996) and silencing by trans-gene in genetically engineered plants (Morel et al. 2000; Paszkowski and Whitam 2001; Fojtova et al. 2003; Matzke et al. 2004).

(ii) *Histone Modification*: The modifications in the histone protein have come out as a critical epigenetic modifier which leads to regulation of DNA-encoded information. As described earlier, the nucleosome is the basic unit of chromatin made up of histone octamer and 147 bp of DNA. Within the nucleosome, each core histone protein has a globular domain-mediating histone–histone interactions and a highly dynamic terminal tail of around 20–35 amino acid residues of which mainly are basic in nature. H2A histone additionally has approximately 37 amino acid carboxy-terminal domain which protrudes from the nucleosome. The modifications of all the histones occur inside the cell nucleus, although by far only some of the modifications have been studied. Till date, 200 and more distinct posttranslational modifications (PTMs) have been identified and the numbers are still growing. Posttranslational modifications of histones take place by an array of processes which include lysine (K) and arginase (R) acetylation and methylation; serine (S) and threonine (T) phosphorylation; lysine ubiquitination, sumoylation, and biotinylation; and ribosylation of ADP (Munshi et al. 2015).

Of the above-mentioned processes of posttranslational modifications, histone acetylation causes transcriptional activation (Jacobson and Pillus 2004), lysine methylation in histones (H1, H3–K9, H3–K27, H4–K20) causes silencing, in histones (H3–K4, H3–K79) causes transcriptional activation (Bastow et al. 2004; Kirmizis et al. 2004; Schotta et al. 2004; Schneider et al. 2004), and histone phosphorylation causes transcriptional activation (Cheng and Shearn 2004). The enormous diversity makes a “histone code” (Strahl and Allis 2000),

which arises due to the multiple combinations of different modifications and is read and interpreted by various factors of the cell. These modifications in histones lead to remodeling in chromatin which in turn regulates responses to abiotic stress (Luo et al. 2012).

(iii) *RNA Interference (RNAi)*: Epigenetic gene regulation is also influenced by the presence of non-protein-coding RNAs. After the discovery of double-stranded RNAs (dsRNAs) as a robust means of gene silencers in *Caenorhabditis elegans* and plants, RNA interference (RNAi) has evolved as a novel approach to understand gene expression regulation. RNAi also brings about gene downregulation by small RNAs (about 21–24 nucleotides) which directs proteins of Argonaute protein family to a nucleotide target sequence through complementary base pairings. Based on the effector complexes (or RNA-induced silencing complexes, RISC) protein composition and the target sequence's nature, the downregulation or silencing of genes can be through mRNA degradation, posttranscriptional gene silencing (PTGS) by repression of translation and alternative splicing of mRNA, or transcriptional gene silencing (TGS) by genome modification (Munshi et al. 2015). The role of RNA-mediated gene silencing pathways is essential in the development of plants, the structure of the chromosome, and resistance to virus (Tsaftaris et al. 2005).

21.3 Mechanism and Regulation of DNA Methylation

Methylation of cytosine occurs by the transfer of methyl group from *S*-adenosyl methionine to the 5′ position of cytosine in the presence of a covalent enzyme catalyst that transcends into the formation of 5-methylcytosine (5mC). There are comparatively high levels of 5mC in plants, that is, 6–25% of total cytosines as per the species (Steward et al. 2000). Due to the symmetrical nature of CpG and CpNpG methylation, they are copied simply after replication of DNA, although after each subsequent DNA replication cycle the non-symmetrical CpNpN methylation has to be established de novo (Karlsson et al. 2011). During the vegetative phase of plants, this epigenetic memory gets accumulated under the influence of environment and is passed to the subsequent generation by germline cells, that later gets established during development. As described earlier also, methylation of DNA takes place at promoter as well as in body regions of the gene which leads to the existence of genes in a repressed state. So, there is likely to be an increased gene expression when the level of methylation declines (Finnegan et al. 1998).

The enzymes which take part in cytosine methylation are categorized into three groups – methyltransferase1 (MET1), chromomethylase3 (CMT3), and domains rearranged methylase (DRM). MET1 is responsible for the CpG methylation and the defective plants which do not possess the enzyme lack CpG methylation (Lindroth et al. 2001). The enzyme CMT3 is responsible for CpNpG methylation at transposons and at centromeric repeats (Lindroth et al. 2001 and Tompa et al. 2002). Two of these methylation processes imprint symmetric methylation on the

parental DNA (Chan et al. 2005). There are two methyltransferases in DRM, that is, DRM1 and DRM2 which act as a catalyst in de novo methylation at CpNpNp sites and is of asymmetrical type (Ramsahoye et al. 2000; Gowher and Jeltsch 2002; Cao and Jacobsen 2002). Apart from the above-specified catalysis of enzymes, there are also reports of various functional redundancies of CMT3 and MET1 in methylation at CpNpG sites (Cao et al. 2003). There is also the implication that there exists a functional redundancy of CMT3 for methylation of CpG and CpNpG sites, like single and double mutants of met1 and cmt3 lead to the activation of CACTA transposon which is normally silenced (Kato et al. 2003).

In plants, the status of DNA methylation is regulated by various developmental processes, physiological processes, and various abiotic and biotic stresses. The process of histone and DNA methylation are interdependent as there is a loss of H_3K_9 methylation due the result of losing CpG methylation in *met1* (Soppe et al. 2002; Tariq et al. 2003), although, there was no effect on CpG methylation due to the loss of H_3K_9 methylation in *kyp* (Kryptonite) histone methyltransferase (Jasencakova et al. 2003). As from the reports, we can conclude that the methylation of H_3K_9 acts later than CpG methylation which fortifies the foundation of heterochromatin. In contrast, the CpNpG methylation is reported to be reliant partially on the *kyp* activity (Jackson et al. 2002). DNA methyltransferase and DNA demethylation enzymes both are responsible for the status of overall DNA methylation. The demethylation occurs in either an active or passive way. The active demethylation may take place through the activity of glycolyase by the removal of methylcytosines from DNA (Zhu et al. 2000, 2007; Agius et al. 2006; Morales-Ruiz et al. 2006), rather than this the passive demethylation may take place through hinderance in de novo methylation or incompetency to preserve the paternal imprints after the replication of DNA (Kankel et al. 2003). These processes may play vital functions in the prevention of the formation of epialleles with stable hypermethylation in the genome of a plant (Penterman et al. 2007).

Additionally, small RNAs also assume an imperative job in the regulation of epigenetics through the RNA-directed DNA methylation (RdDM) in reverberation to the various stresses, growth, and development by means of transcriptional gene silencing. The mechanism starts with producing the transcripts for biogenesis of siRNA after mediation through RNA Pol II and RNA Pol IV by the pathway of RNA interference. In the initial phase, the RNA-dependent RNA polymerase 2 (RDR2) converts the single-stranded RNAs (ssRNAs) into double-stranded RNAs (dsRNAs) which are previously produced from transcription of methylated DNA through RNA Pol IV; while the inverted repeat regions are targeted by RNA Pol II for a dsRNAs generation. The advanced processing of these dsRNAs is done by Dicer-like 3 (DCL3), HUA enhancer1 (HEN1), and finally loading of this processed product on Argonaut 4 (AGO4). The complex thus formed interfaces with the Pol V's largest subunit via WG/GW repeats of Nuclear RNA polymerase (NRPE1) at C-terminal domain (El-Shami et al. 2007). The DNA methylation of the homologous DNA sequence is inducted by this machinery of siRNA and affiliated proteins by DRM2. Apart from that, the binding of AGO4 to specific gene promoters also takes place with the assistance of Pol V-derived long non-coding RNAs (lncRNAs). The

avocation of this complex escorts asymmetrical CpNpN-type methylation of DNA in promoter regions that sequentially modulates the expression of the target gene (Zheng et al. 2013).

21.4 Priming: A Method to Induce DNA Methylation-Mediated Stress Memory

Endangering the plants to meagerly virulent necrotrophic pathogens/beneficial organisms or to any compound that induces resistance leads to a peculiar state of defense in plants which enables them to acquire intensified defense responses whenever they are challenged further by biotic agents or abiotic factors. The state of embellished capability to stimulate stress-induced defense responses has been named as the "primed" state of the plant (Conrath et al. 2002) (Fig. 21.1). Priming is defined as the phenomenon by which a transitory biotic or abiotic stress action on the plants leads to an altered defense response which may be typically accelerated and vigorous upon the exposure to recurring stress (Conrath et al. 2015). The word "priming" was formerly conceived in the background of plant's immunity to the biotic agents (pathogens), although later it was started as being used for the responses to abiotic stresses also (Lämke and Bäurle 2017).

The unfavorable growth conditions which repress the natural plant growth and development is defined as the stress that in extreme cases can be lethal also. These conditions can arise due to flooding, drought, excessive salt in soil or irrigation water, attack by a phytopathogen, and/or herbivore. The plants which are in primed state respond to the triggering stress cue in a modified manner in comparison to those plants which are in the unprimed (naïve) state. The mechanism of priming functions at the phenotypic level and does not alter the sequence of DNA, which makes it eventually reversible (Conrath et al. 2015; Hilker et al. 2016). In general, priming leads to a faster and stronger pattern of response, which is evident by the modified activation dynamics of expression of defense genes. The speed of plant defense is dependent on the time taken by the plant to recognize the attacker which means sooner the identification, more effective is the defense response.

The stress memory period takes place after the event of priming (Stief et al. 2014) in which the information is stored about the priming stress clue after its remission. This memory spans over a period of days to weeks in case of somatic stress memory and to the offspring (intergenerational or transgenerational). The best possible mechanisms for memory manifestation can be as follows:

- Modifications in the transcriptional response, that is, transcriptional memory, in which either sustained alterations in expression of the gene (may be either activation or repression) is induced by priming stimulant or same by a secondary stimulant (as in hyperinduction) (Light and Brickner 2013; D'Urso and Brickner 2017).

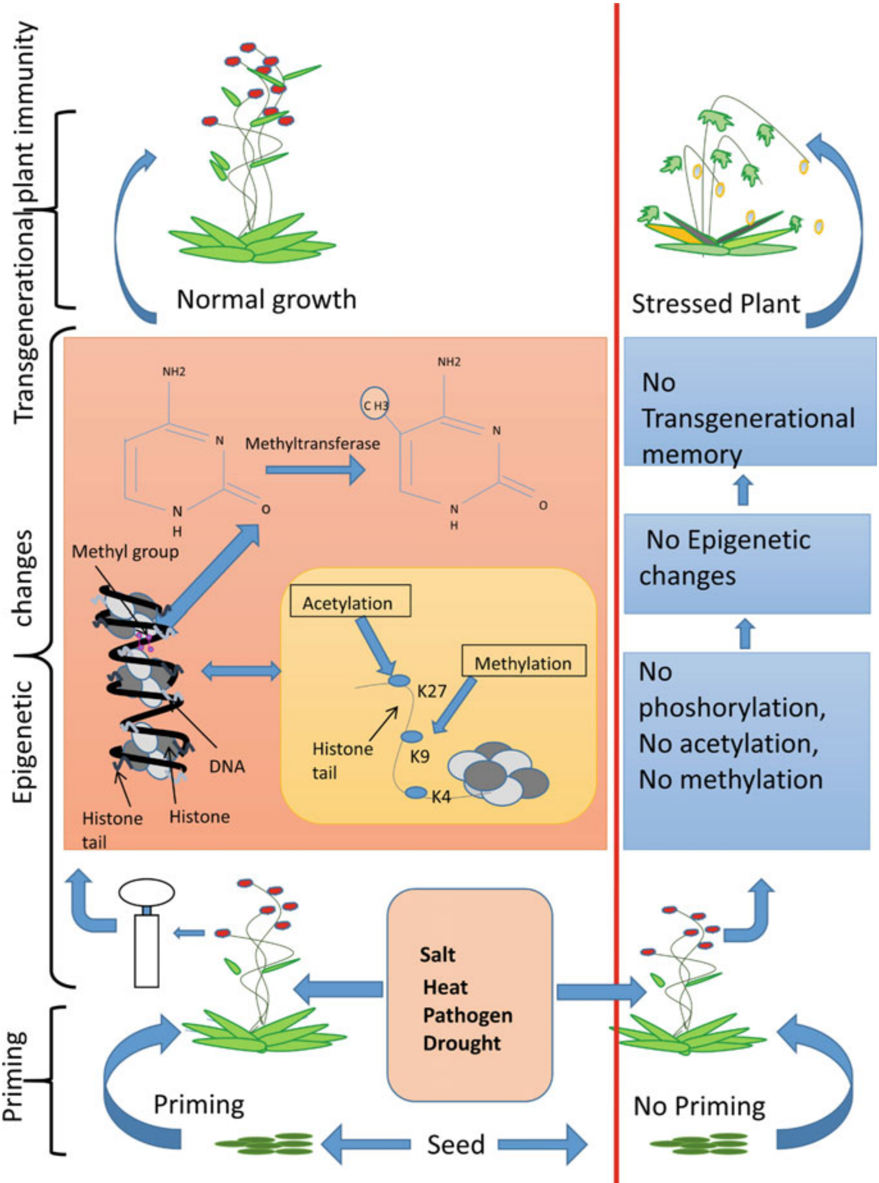

Fig. 21.1 A detail scheme of seed priming leading to transgenerational immunity via histone modifications in plants

- Involvement of transcriptional feedback loops (as self-activation of a transcription factor) or by posttranslational mechanisms (modifications of proteins) (Ptashne 2008).

- Mechanisms which are transcription independent, that is, prions transmission or prion-like proteins transmission (Shorter and Lindquist 2005; Chakrabortee et al. 2016a, b) as notably described in yeast (Tyedmers et al. 2008).

All the cases of stress memory have been confirmed with a possibility of epigenetic basis and as by the definition it requires the phenomenon to be heritable and stable, yet change-independent DNA sequence. A true transgenerational stress memory is mostly expected to be epigenetic, although it would not hold for somatic stress memory due to its shorter duration. The scientific meaning of the term “epigenetic mechanisms” contains all the specifications that have an impact on chromatin structure (may or may not be stably inheritable) including methylation of DNA. There have been many reports about transgenerational inheritance of DNA methylation of plants which grows under the stress conditions (Hauser et al. 2011; Feng et al. 2012), and this epigenetic flexibility has a crucial part in the immediate and long-term adaptation of organisms under stress (Mirouze and Paszkowski 2011). The phenomenon was reported in distant genotypes of rice (*Oryza sativa*) when treated with salt and alkaline stresses and revealed that the level of DNA methylation persisted in the progenies produced after selfing (Feng et al. 2012). As per Byoko et al. (2010), while measuring the cytosine methylated DNA level in between the progenies of treated and untreated plants in *Arabidopsis* for two generations, the levels were maintained higher in the treated plant progenies in response to both stress and control conditions as compared to the untreated plant progenies of the same generation. The study suggested that there is a decrease in DNA methylation in the absence of stress. The transgenerational inheritance of stress tolerance is stimulated even in the untreated progenies of tobacco plants through viral infection and of *Arabidopsis* plants through UV-C exposure and flagellin by the means of global genome methylation.

The progenies of *Arabidopsis* plants primed either with β-aminobutyric acid (BABA) or with *Pseudomonas syringae* pv. *tomato* (avirulent isolate *Pstavr*Rpt2) responded by showing a quicker and greater transcript accumulation of defense genes related to salicylic acid signaling pathway (Slaughter et al. 2012). These progenies also exhibited an embellished resistance to the disease on challenging with *Pseudomonas syringae* (virulent isolate) and the oomycetic pathogen *Hyaloperonospora arabidopsidis*. In addition to all these, the priming of progenies of previously primed plants leads to an even greater magnitude of defense responses. The plants of tomato (*Solanum lycopersicon*) and *Arabidopsis* too showed transgenerational induced resistance which was jasmonic acid–dependent when the plants at their vegetative growth stage were challenged by herbivory or methyl jasmonate (Rasmann et al. 2012). These effects were persistent to the second generation in *Arabidopsis* and their presence in plants belonging to Solanaceae and Brassicaceae families proves the resistance to be transgenerational and distantly dispersed among the plant kingdom (Sarma and Singh 2014). This concept is further consolidated by the study conducted by Luna et al. (2012) in which the priming of *Arabidopsis* plants was done by inoculating them with virulent *Pseudomonas syringae*, and it showed the remnants of primed state onto the succeeding generation

and even sustenance of the same over one stress-free generation. A central role is played by NPR1 in the transgenerational immunity as it is blocked in the SA signaling *nonexpressor of pathogenesis-related genes1* (*npr1*) mutant and additionally the phenomenon of immunity was shown to be associated with modifications of chromatin at promoters regions of SA-responsive genes *PR-1*, *WRKY6*, and *WRKY53*. Apart from this, the transgenerational immunity which was generated from bacterial infections is transmitted by the means of hypomethylation of genes that are responsible for directing the priming of SA-dependent genes in the succeeding generations (Luna et al. 2014).

21.5 Transgenerational Plant Immunity and Abiotic Stresses

Many complex gene regulatory mechanisms have evolved in plants which help in coping with diverse environmental stresses and among these mechanisms chromatin remodeling, DNA methylation, and small RNA-based mechanism are the major ones involved in regulation of the expression of genes responding to climatic stresses (Subbah et al. 1995; Gravitol et al. 2012). This theory was further consolidated by the report of the presence of natural epigenetic variations among the mangrove plants growing at banks of rivers having tall height and thicker stem as compared those growing at salt-marsh habitat (Lira-Medeiros et al. 2010). The analysis of methylation-sensitive amplification polymorphism (MSAP) proclaimed DNA hypermethylation in riverside plants in comparison to the salt-marsh plants, thus indicating toward the vital function played by the natural epigenetic variations among the population of plants in adapting to the environment. In another study consisting of genome-wide MSAP analysis performed for distinct rice genotypes which have a differential response to salt-stress revealed differing methylation and expression of salt-related genes and genes related to chromatin modification (Karan et al. 2012). An investigation consisting of genome-wide analysis of two divergent rice lines disclosed cytosine variations which are site-specific, recoverable, and reversible; is regulated epigenetically; and related to drought adaptation (Wang et al. 2010). Under the water-deficit condition also cytosine hypermethylation has been seen in rice cultivars that are drought-tolerant and cultivated on lowlands (Suji and Joel 2010).

There have also been reports of locus-specific changes in methylation in leaf tissues of plant sustaining N-deficiency (Kou et al. 2011). In forest trees, heat stress tolerance was discovered as the cork oak (*Quercus suber*) leaves exhibited interaction of specific methylation of DNA and acetylation of H3 histone as an adaptation to high temperature (Correia et al. 2013). The DNA methylation gets altered also by the global warming phenomenon and stresses associated with nitrogen deposition in the soil, thus offering a molecular basis for adaptation to these stresses in the naturally occurring plant population as inspected in *Leymus chinensis* Tzvel. (Yu et al. 2013a). The hypermethylation of transposable elements was recognized during these environmental stresses when compared to other regions of the plant genome. The physiological processes of plants like photosynthesis and development of

reproductive organs are modulated by the epigenetic mechanisms under the conditions of stresses leading to its acclimatization (Yaish et al. 2011). Epigenetic processes have been acknowledged as the functional and elemental factor of abscisic acid (ABA)-regulated processes and the same phytohormone has been revealed to operate in the expression of genes which are dependent on DNA methylation/ demethylation through small RNAs (Khraiwesh et al. 2010). The same study conducted on moss *Physcomitrella patens* showed that miR1026 accumulation and PpbHLH gene (miR1026 target gene) hypermethylation at CpG sites are induced by ABA and eventually lead to decrease in expression of *PpbHLH*. Apart from these, numerous researchers have identified ABA as controller of histone modifications and thus as a regulator of DNA methylation (Chinnusamy et al. 2008; Yaish et al. 2011).

21.6 Transgenerational Plant Immunity and Biotic Stresses

As described earlier, the exposure to biotic stresses activates the plants' immune system and basal defense machinery by the process of defense priming (Muthamilarasan and Prasad 2013). DNA methylation has emerged as a major approach of defense strategy in plants against the biotic stresses. The progenies which were obtained from diseased *Arabidopsis* showed enhancement in resistance against the downy mildew pathogen and other biotrophs also (Luna and Ton 2012). Apart from that, there are also reports of DNA methylation playing a role in defense mechanisms of *Arabidopsis* against the bacterial pathogens (Dowen et al. 2012; Yu et al. 2013b). The offspring of *Arabidopsis* inoculated with *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000) exhibited an increased plant immunity when they were further challenged by the pathogens. The *Pst*DC3000 inoculation activated salicylic acid (SA)-inducible defense genes and repressed jasmonic acid (JA)-inducible genes of the plants, and the enhanced resistance of the offspring was not only limited to *Pst*DC3000 but also to a (semi)biotrophic pathogen *Hyaloperonospora arabidopsidis*. As mentioned earlier, it has been shown that signal transduction is required via the *NPR1* gene for transgenerational immunity/ defense response in the plants (Luna et al. 2012).

During the infection of the pathogen, DNA hypomethylation has also been reported to influence the expression of the defense-related gene as the chemically demethylated *Xa21G* (R gene) gene of rice showed heritable resistance against *Xanthomonas oryzae* pv. *oryzae* (Akimoto et al. 2007). A very interesting report disclosed that regulation of formulation of crown gall tumor is controlled by DNA methylation through ABA-dependent stress defense in *Arabidopsis* plants (Gohlke et al. 2013). Advanced epigenetic researches revealed that the plants use siRNA-mediated methylation strategy systematically as a mechanism of defense against viral pathogens through methylation of viral genomic components (Bian et al. 2006; Tougou et al. 2007; Yadav and Chattopadhyay 2011; Emran et al. 2012; Sharma et al. 2013) and there is a positive correlation between the high methylation of viral DNA and recovery of symptoms after infection from virus (Rodríguez-Negrete et al.

2009). A separate study in soybean showing resistance to *Mungbean yellow mosaic India virus* (MYMIV) identified a higher level of DNA methylation specific to Intergenic Region (IR) (Yadav and Chattopadhyay, 2011). In addition to this, research conducted to study the dynamics of tomato cultivar tolerant to *Tomato leaf curl New Delhi virus* (ToLCNDV) also showed substantial methylation at IR and part of replication-associated protein (rep) gene in the cultivar (Sahu et al. 2010, 2012).

There has been a report of a correlation between genomic DNA hypomethylation in plants and the abundance of transcripts of genes related to defense (Wada et al. 2004). In tobacco plants infected with *Tobacco mosaic virus* (TMV), it was found that 24 hours after the infection of viral pathogen, the transcript accumulation of *NtAlix1* (pathogen-responsive gene) was higher and there were also changes in methylation level (Sahu et al. 2013). The study suggested that expression of defense-related factors is regulated by plants through DNA methylation when the pathogen infestation takes place. A very effective tool for the introduction of DNA methylation in any gene (endogenous) is virus-induced gene silencing (VIGS) that relies upon double-stranded transcripts generation. An artificial alteration of DNA methylation through the *Cucumber mosaic virus* (CMV)-based gene silencing system is illustrated as a substitute for epigenetic changeover in plants' endogenous gene (Kanazawa et al. 2011). Hence, it can be said that the epigenetics lead to priming of defense mechanisms in plants, allowing them to secure their succeeding generations against biotic stresses which occurs repeatedly without any stable heritable fixation of the trait.

21.7 Conclusions and Future Perspectives

Many reports have consolidated the response of the plant to abiotic and biotic stresses through DNA methylation, but there are many gaps which pose many unanswered questions regarding the methylation pathways, activation of adaptive mechanisms, and sensing of stresses. The study of DNA methylation is much easier in context of the model plant *Arabidopsis thaliana* having small genome size and simple genetics but the true challenge for the plant biologists lies in trying to understand about the epigenetic mechanisms in response to various stresses and its feasible utilization in the plants' genetic manipulation. The transgenerational epigenetics which are inducible can be further utilized to boost up the production and protection of agricultural crops. Moreover, it can be seen as a tool for the development of an easy and convenient technique to bring desirable changes in plants. Epigenetics can also be seen as promising machinery which can be utilized in understanding the functional genomics of plants as it has the capability to elucidate and confer tolerance and/or resistance to stresses among the various species of plants including the agricultural crops. The transgenerational inducible epigenetic changes in some of the genes' expression pattern enable us with the freedom to exploit and to bring out the desired inheritable changes for better production and protection in the target crops. A better understanding of the epigenetic phenomenon will aid in

making more developed strategies for regulating plant genes according to the necessities of agriculture. Apart from the epigenetics, identification and characterization of promising priming agents (including both abiotic and biotic agents) and their receptor sites on the host will make the implementation of this compelling biological mechanism successful in future. Seeds being the primary vehicle for the production and propagation of plants make the ones harvested from the primed plants to show a stronger defense potential against the different abiotic and biotic stresses. Thus, we can conclude that invigorating the plants' innate immunity by the transgenerational epigenetics will be significantly beneficial in reducing the application of synthetic pesticides against the biotic stresses. The present knowledge and the future prospects of transgenerational epigenetics in plants have inspired the researchers to fantasize about a comparatively pesticide-free environment and which is also the demand to save the ecological balance of our mother earth.

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22 Concept of Effectors and Receptors in Improving Plant Immunity

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Abstract

Plants employ two distinct layers of immunity to encounter pathogen invasion. The first layer PAMP-triggered immunity (PTI) involves the perception of evolutionarily conserved pathogen structures, termed pathogen-associated molecular patterns (PAMPs), at the plasma membrane through PRRs, to evade this PTI pathogens evolved to secrete effector molecules. In response to pathogen effectors, plants have acquired additional receptors that specifically recognize the effectors, establishing a second layer of immunity known as the effector-triggered immunity (ETI). Various strategies have been developed to effectively integrate ETI into crop improvement programs in different ways. 'Effectoromics' is a large-scale screening approach that uses effector candidates to identify host resistance (*R*) genes. Stacking multiple NLRs confers resistance for durable resistance. Engineering new NLR-mediated resistance specificities can be carried out by altering either NLRs' domains or host proteins which are guarded by NLRs. Synthetic TALE nucleases and CRISPR/Cas9 (clustered regularly interspaced short palindrome repeats) mediated genome editing of host susceptible genes which are TALE targets were also used to engineer resistance against plant SWEET sugar transporters. Resistant genotypes are developed, in which TAL effectors are recognized by plant cells through trap promoters, which are coupled to an executor-type resistance gene (*E*-gene). This recognition triggers hypersensitive (HR) reaction, and this limits the further growth of the pathogen.

Keywords

PAMP-triggered immunity · Effectors · Effector-triggered immunity · CRISPR/Cas9 · Hypersensitive reaction (HR)

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22.1 Introduction

Plant pathogens, be it bacteria, fungi or viruses, have different lifestyles and infection strategies, but one similarity is that they try to colonize and live at the expense of their host. Essentially, all of these pathogens either evade or suppress the immune system or modify host physiological processes in the process of infection. To counteract them, plants evolved to employ two distinct layers of immunity, viz. PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl 2006). The first, evolutionarily ancient, layer involves the recognition of evolutionarily conserved pathogen structures, termed pathogen-associated molecular patterns (PAMPs), at the plasma membrane through conserved and ubiquitous receptors generally defined as pattern recognition receptors (PRRs). Binding to these receptors to PAMPs initiates an active defence response, the so-called PAMP-triggered immunity (PTI), in both host and nonhost plants. In the next round of host-pathogen conflict, several adapted pathogens escape from detection or suppress PTI, by passing effector proteins inside the host cells to incite disease, known as effector-triggered susceptibility (ETS). In response to these pathogen effectors, plants have acquired additional receptors that precisely recognize the effectors, establishing a second layer of immunity known as the effector-triggered immunity (ETI).

22.2 From PTI to ETI: An Overview of Plant Defence Response

In fact, pathogens approaching to enter the cellular cytosol must initially overcome the first layer of plant immune system called PAMP-triggered immunity (PTI), also known as surface immunity, in which PAMPs are recognized by PRRs at the cell membrane to activate PTI to prevent further entry and colonization of the host cells (Ionis and de Witt 2009). But these basal defences are only partially effective at restricting pathogens. Once pathogen evolves itself to detect and suppress PTI, then it can transfer its effector proteins inside the host cells and cause disease. In the process of evolution, plants also develop mechanisms to overcome the effect of effector molecules of pathogens; this forms the second layer of plant immunity called as effector-triggered immunity (ETI). ETI is often associated with a hypersensitive response (HR), at the infection sites; in many cases, it is followed by systemic acquired resistance (Fig. 22.1).

22.3 The Following Are Some of the Terms Which We Need to Understand Before Going Through This Chapter

PAMPs (Pattern-Associated Molecular Patterns) are the highly indispensable molecules for the survivability of the pathogens; impair the viability of pathogens, if they are lost, since these are evolutionarily stable; form a core component of the microorganism; and are conserved across larger groups of pathogens, which are not

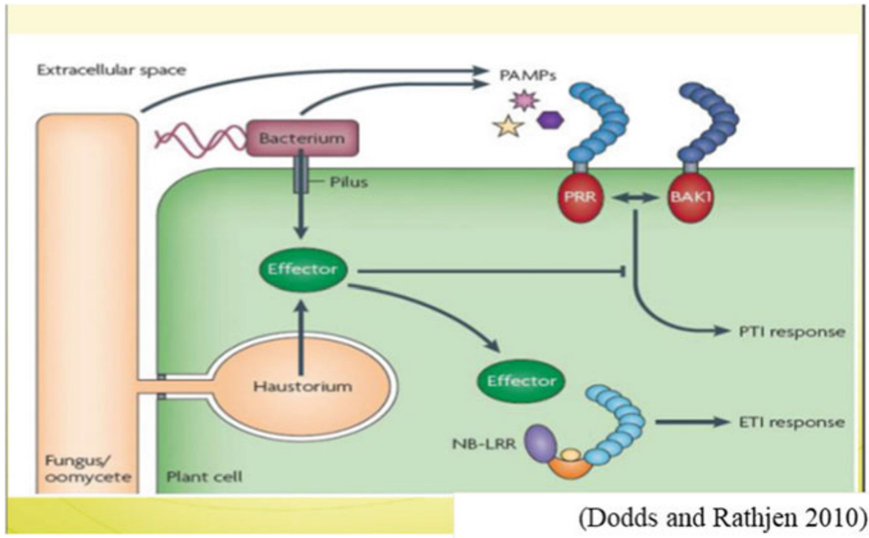

Fig. 22.1 Overview of PTI and ETI

found in the host. Some of the examples include bacterial flagellins, lipopolysaccharides or elongation factor Tu, fungal chitin or oomycete Pep-13 (Boller and Felix 2009).

Pattern Recognition Receptors (PRRs) like FLS2, ERF, CEBiP etc. are the plasma membrane-localized receptors, which recognize the presence of PAMPs in the extracellular environment located in the plasma membrane.

PTI It is the situation in which a cascade of responses are commenced leading to development of immunity in host plants upon detection of PAMPs (conserved pathogens molecules important for their reproduction and survival) that elicit a physiological changes in the host cell activated by the Pattern Recognition Receptors (PRRs).

Effectors Effectors are any regulatory pathogen-secreted molecule that can modify the host cell structure and function, thereby facilitating infection or triggering defence responses (Kamoun 2006). Mainly, effectors perform three functions: First is the structural role, for example, Avrblb2 of fungi, which is secreted as extrahaustorial molecules. The second function is to cause nutrient leakage, for example, *P. syringae* HoPM effector protein. The third role is to act in pathogenicity process, for example, HopA1 dephosphorylates MAP kinase which results in the inhibition of PTI. These include PWL2, pep1, Avr4, AVR2, P6 protein of *Cauliflower mosaic virus* (CaMV), and cyst nematode (*G. rostochiensis*) expansins (Leisner and Schoelz 2018).

ETS (Effector-Triggered Susceptibility) is the situation in which susceptibility/disease is induced in the plant due to the action of effectors on the host system. The outcome of the deployment of effector molecules favours pathogen virulence.

Effector-Triggered Immunity (ETI) is the situation in which plant defence response or the immunity is elicited by effector recognition in the host cell. The effector molecules are recognized by R protein which are governed by R genes: NB-LRR (nucleotide-binding leucine-rich repeats) and Ser/Thr kinases. ETI occurs when there is highly specific, direct or indirect interaction of pathogen effectors and the products of plant R genes. Localized ETI can also induce systemic acquired resistance (SAR).

When bacterial pathogen comes in contact with plant surface, these cells detect bacterial molecules (PAMPs) by receptors called PRRs; then, a signalling pathway named PTI/basal defence is activated. Later, molecular events occur and stop bacterial growth. If this pathogen has the ability to overcome this PTI effect, then it can inject effectors directly into the plant cells through type III secretion system, which often bacterial pathogens possess. Once these effectors enter inside the host cells, they manipulate the host physiological conditions, which may favour for disease development. If host plants are resistant, then plant cells have got proteins that can recognize effectors; they are called resistance proteins and are specific to one particular effector, and then signalling pathway named ETI is activated. If ETI is activated, then hypersensitive response (HR) occurs and stops bacterial growth; ultimately, plant becomes resistant to the bacterium (Fig. 22.2).

22.4 Phases of Plant-Pathogen Interaction

There exists always a coevolutionary arms race between pathogens and plants, during which pathogens respond by mutating effectors or by developing new effectors that can avoid or subdue ETI, whereas plants also develop novel R proteins, facilitating recognition of novel effectors; therefore, new effectors and receptors keep on evolving in plant-pathogen interaction. This is explained through the following zigzag model of different phases of the plant-pathogen interaction (Fig. 22.3).

22.5 Apoplastic Effectors Target Host Defences

The apoplastic space lies between the plant cell wall and the plasma membrane, constituting an important space where plant and pathogen interact. To overcome such harsh conditions in the plant apoplast, pathogens have evolved various immune responses. During infection, plant pathogens secrete a large number of effector proteins into the apoplastic space, some of which are recognized by the plant surveillance system, and, thus, plant innate immunity will be activated. Some of the effectors, which evade plant perception, act in modifying plant apoplast

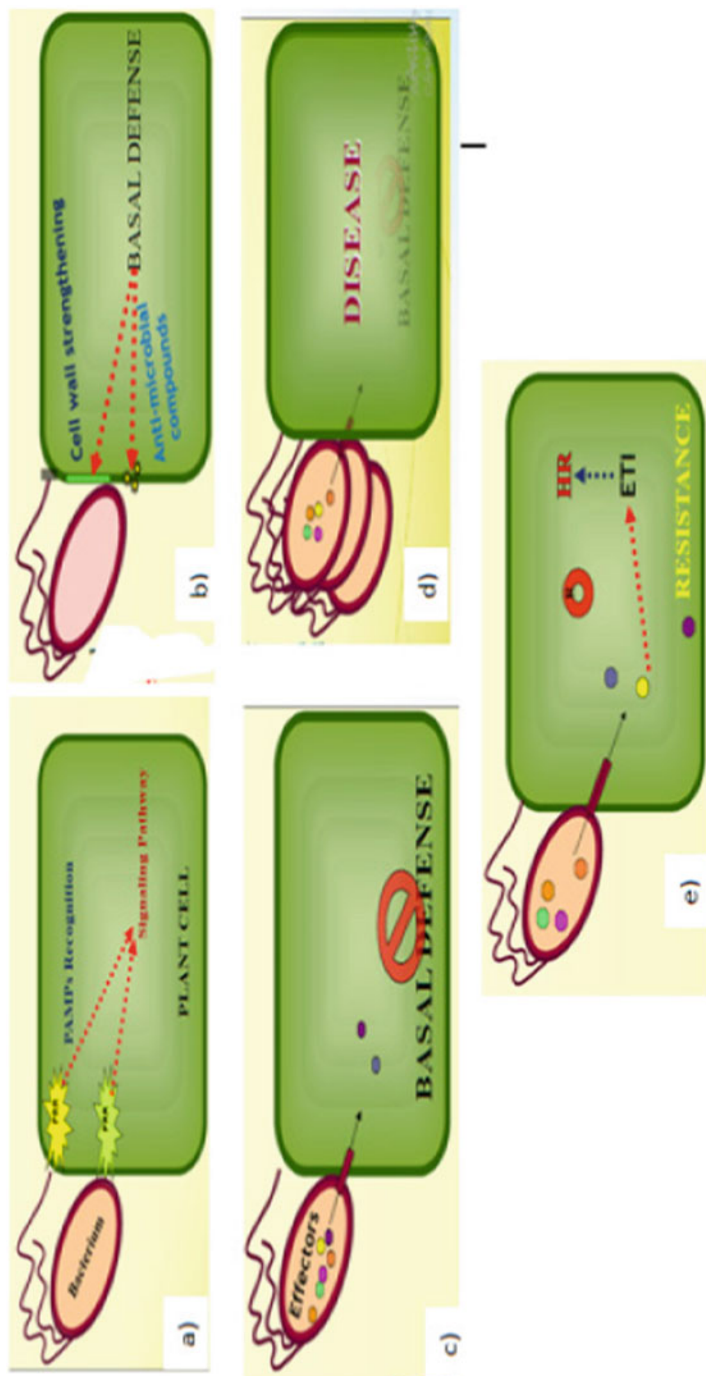

Fig. 22.2 Main steps involved in plant immunity

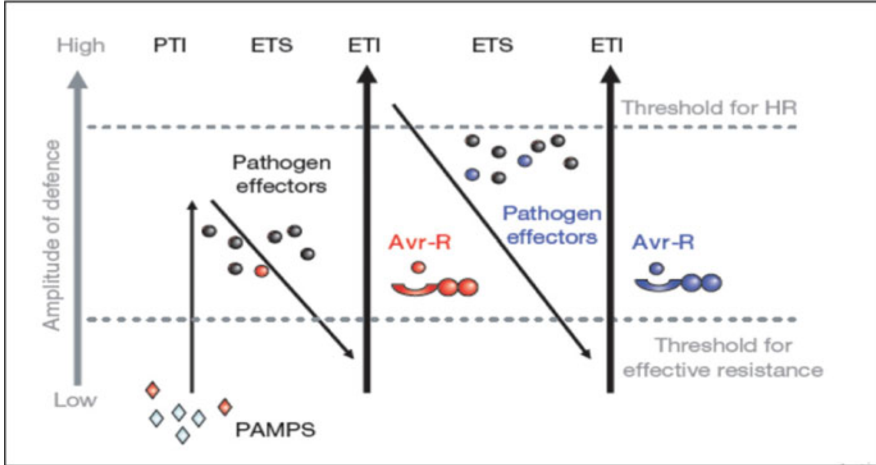

Fig. 22.3 Zigzag model depicting different phases of plant-pathogen interaction

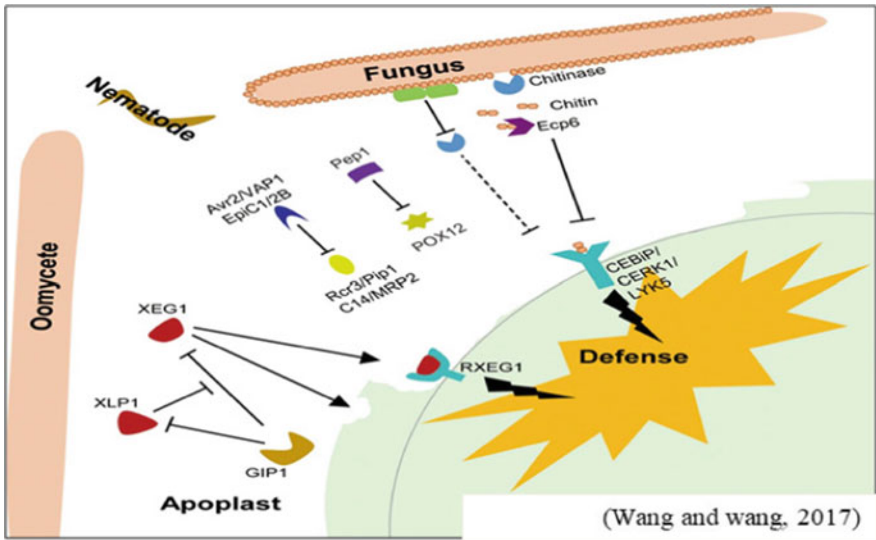

Fig. 22.4 Microbial effectors modify plant immunity in the plant apoplast. Modes of action of several apoplastic effectors with their corresponding plant proteins during interaction are depicted

immunity and favour effective pathogen infection. The concerted actions of apoplastic effectors often define the consequences of plant-pathogen interactions (Fig. 22.4). Some of the apoplastic effectors include protease inhibitors, peroxidase inhibitor of *U. maydis* and chitin-binding effectors like LysM and Ecp6 effectors of *C. fulvum*.

Protease Inhibitors Papain-like cysteine proteases (PLCPs) are essential constituents of the plant immune response in the apoplast (Doehlemann and Hemetsberger 2013), and various pathogens secrete PLCP inhibitors. Tomato plant-derived cysteine protease RCR3 is inhibited by the apoplastic AVR effector Avr2 of *C. fulvum.* Indeed, the activation of hypersensitive response occurs when Avr2-RCR3 complex is recognized by the CF-2 resistance protein in tomato plants (Rooney et al. 2005).

Chitin-Binding Effectors Fungi secrete apoplastic effectors to block chitin-induced immunity. Various pathogens secrete apoplastic effectors that either avoid the release of chitin oligosaccharides from fungal cell walls or sequester these released oligosaccharides to avoid recognition by the plant surveillance system. For example, apoplastic effectors LysM and Ecp6 secreted by *C. fulvum*, which sequester chitin oligosaccharides which are released from the fungal cell wall (de Jonge et al. 2010), and one more apoplastic effector of *C. fulvum*, i.e. Avr4, can bind to plant chitinases with its different chitin-binding domain and functions to protect the fungal cell wall from degradation by plant chitinases (van den Burg et al. 2004)

Peroxidase Inhibitor The apoplastic effector Pep1 of *U. maydis* protects fungal hyphae from reactive oxygen species (ROS), which constitute the major component of the plant immune response by surrounding the hyphae in the apoplast and concentrating as rings around hyphae at cell-to-cell passage sites (Hemetsberger et al. 2012).

22.6 Damage-Associated Molecular Patterns (DAMPs)

In addition to biotic attack, plants also need to cope up with a range of abiotic assaults too such as mechanical or cellular damage, as well as environmental stresses like drought and salinity. Some endogenous molecules activate the innate plant immune system when they are released into the extracellular space (including plant apoplast) from their normal location due to damage; these molecules are referred to as DAMPs or damage-associated molecular patterns (Bianchi 2007). DAMPs are the endogenous biomolecules that are passively released by the host upon external damage or infection-induced necrosis. While MAMPs are derived from microbes and activate the innate immune system, DAMPs are host cell–derived, and both initiate and perpetuate innate immune responses. It is understood that these defences help to protect the damaged tissue by preventing microbial ingress, which is otherwise vulnerable to infection due to the disruption of physical barriers.

DAMPs in plants are mainly cytosolic proteins, nucleotides, peptides and amino acids, which are released from damaged cells or secreted by intact cells, which are undergoing pathogen invasion. In addition, DAMPs also include the oligomeric fragments of plant cell wall polysaccharides released when tissues are disrupted by physical injuries or attacks of pathogens and herbivores. As the case of PAMPs,

DAMPs also initiate PRR-mediated immune responses in local sites surrounding a wound and pathogen invasion and regulate systemic immune signalling (Fig. 22.5).

Examples: Systemin, hydroxyproline-rich systemin, oligogalacturonides (OGs), extracellular ATP (eATP) and plant elicitor peptides (Peps), i.e. *Arabidopsis* AtPep1, a peptide upon recognition by its receptor PEPR1; the plants get alerted and activate immune responses.

22.7 Plant Defence Responses Associated with PTI

PTI is associated with various plant defence responses, like calcium influx, callose deposition, oxidative burst and activation of a mitogen-activated protein kinase (MAPK) cascade, to induce defence gene expression (Nicaise et al. 2009). Cellular and physiological responses are elicited by patterns in plants. Plant cell surface-resident pattern recognition receptors (PRRs) perceive microbe-associated molecular patterns (MAMPs) or DAMPs and recruit the coreceptors, leading to a series of intertwined cellular and physiological responses. PRR complex formation is followed by a rapid transphosphorylation in the complex as well as phosphorylation of receptor-like cytoplasmic kinases (RLCKs). The activation of PRR complexes also leads to the activation of mitogen-activated protein kinase (MAPK) cascades and calcium-dependent protein kinases (CDPKs), which regulate gene transcriptional changes and other cellular responses. The hallmarks of PTI responses include ion efflux, calcium influx, actin filament remodelling, callose deposition, plasmodesmata (PD) and stomatal closure and production of reactive oxygen species (ROS), phosphatidic acid (PA), nitride oxide (NO), phytoalexins and phytohormones. Collectively, these responses contribute to plant resistance against a variety of pathogens (Fig. 22.6).

22.8 Plant Defence Responses Associated with ETI

ETI is also associated with various plant defence responses, like localized programmed cell death, autophagy and transcriptional reprogramming of defence-responsive genes. The striking characteristic of ETI is the HR, which exhibits a rapid induction of programmed localized cell death at the infection site. The primary purpose of this cell death is against biotrophic pathogens, which derive nutrients from living cells. Localized PCD is regulated by salicylic acid concentration gradient and NPR proteins. In *Arabidopsis*, PCD is regulated by SA, NPR1 and SA receptors, viz. NPR3 and NPR4; in this case, low level of SA suppresses cell death, while over-accumulation of SA leads to cell death. The finding that PCD is regulated by autophagy is one of the important discoveries related to ETI. Liu et al. (2005) showed that in *Nicotiana benthamiana* downregulation of ATG6 and ATG7 (autophagy genes) leads to an extended cell death in TMV-infected plants. Catalase 2 (CAT2) and no catalase activity 1 (NCA1), which are involved in catalase activities in plants, should, in theory, prevent PCD. However, both CAT2 and

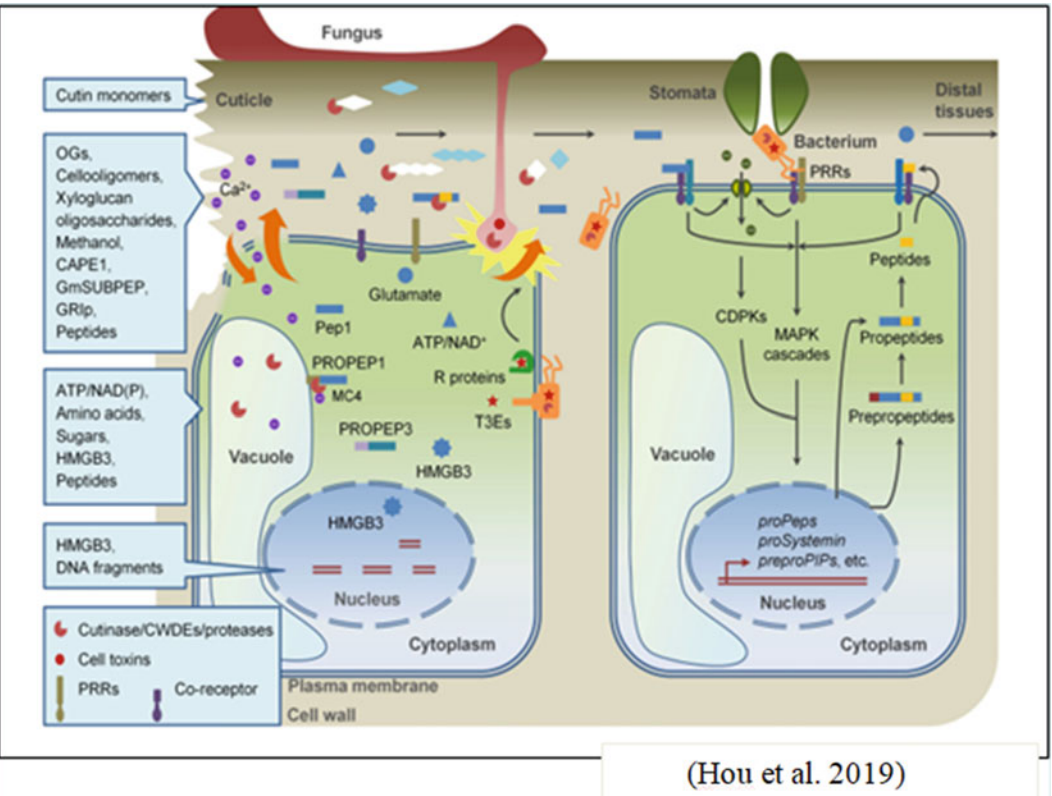

Fig. 22.5 DAMP-triggered immunity in plants is depicted. Pathogen invasion as well as environmental stresses disrupt plant cell wall and plasma membrane, leading to the release of DAMPs, including fragments of cell walls and apoplastic proteins, and cytoplasmic components. Perception of these DAMPs as well as PAMPs by PRRs in cells surrounding of the damaged cells also promotes the production and release of new DAMPs. These DAMPs join together with PAMPs to modulate immune responses locally as well as systemically

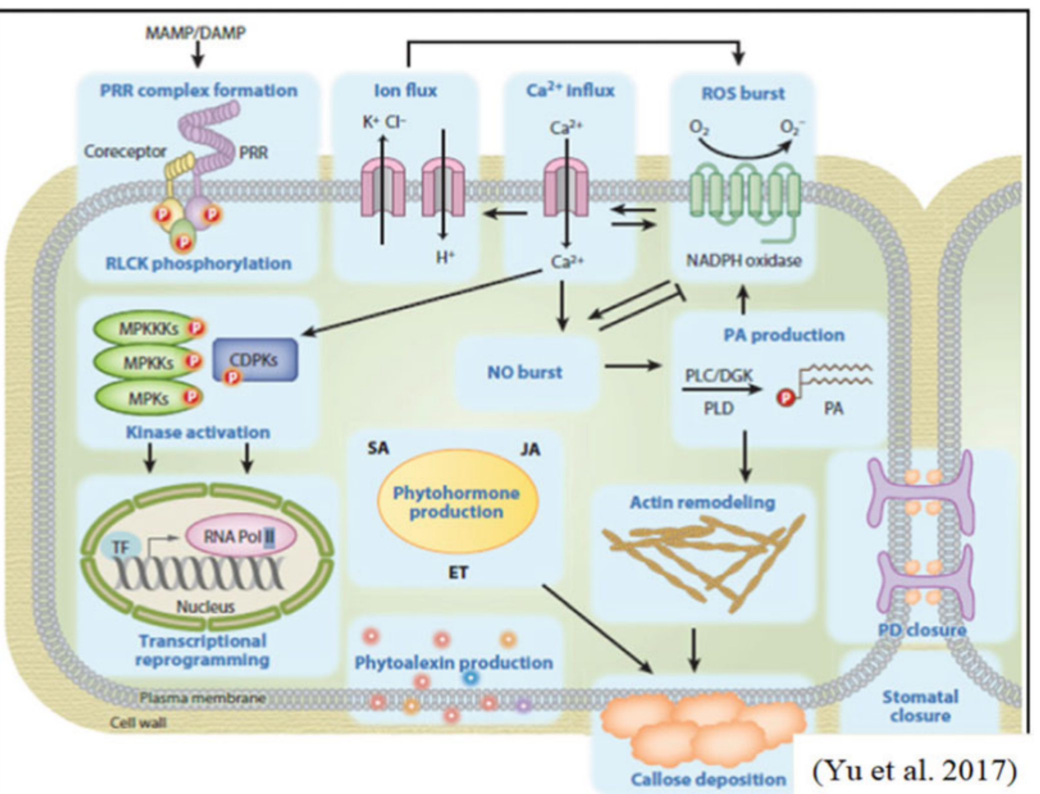

Fig. 22.6 Defence responses developed in plant due to PTI. Abbreviations: *DGKs* diacylglycerol kinase, *JA* jasmonic acid, *ET* ethylene, *PLC, PLD* phospholipase D, phospholipase C; *TF* transcription factor, *SA* salicylic acid

NCA1 contribute to autophagy-dependent PCD, which shows that PCD is regulated by autophagy (Fig. 22.7).

ETI is a strong plant immune response associated with extensive transcriptional reprogramming that involves numerous transcriptional regulators (Caplan et al. 2008; Bhattacharjee et al. 2013). WRKY transcription factors reprogramme transcription of defence-responsive genes. During ETI, active recruitment of transcriptional regulators into R protein-mediated signalling pathways is as an important signalling event just after the recognition of Avr effectors (Rivas 2012; Rivas and Deslandes 2013) Rapid transcriptional changes in ETI can be extensively influenced by chromatin modifications using different mechanisms, viz. methylation of cytosine residues located in DNA, ATP-dependent chromatin remodelling and post-translational histone modifications.

22.9 Modes of Pathogen Effector Recognition by Receptors or Modes of Pathogen Effector and Receptor Interaction

There are two ways by which pathogen effectors are recognized by plant receptors.

1. **Direct perception of pathogen effectors by R proteins.** In this mechanism of interaction, effectors directly come in contact with the receptors (Fig. 22.8a). Direct interaction is seen between *P. infestans* effector Avrblb1 and Rpi-blb1 in potato plants.
2. **Indirect perception of pathogen effectors by R proteins.** An alternative model "guard hypothesis" postulates that R proteins recognize effectors indirectly via their conformational changes in the guarded host targets (Van der Biezen and Jones 1998). It was proposed that effectors target host proteins instead of R proteins directly and that conformational changes of those host proteins are the trigger that leads to R protein activation. Thus, these types of R proteins guard the target of effectors and induce defence responses when those targets are disturbed due to the interaction with effectors. This kind of interaction is evident between *Pseudomonas syringae* effector protein (AvrPphB) and RPS5 (R protein) of *Arabidopsis thaliana* (Fig. 22.8b). In this case, protein kinase PBS1 acts as a guardee. Similarly, RIN4, which acts as a guardee protein, has the ability to interact with several effector proteins, viz. AvrB, AvrRpm1, Rpm1, AvrRpt2 and Rps2 (Fig. 22.8c).

   Effectors can influence the various crucial cellular processes and manipulate as well as direct them towards the growth and colonization of the pathogens and infection in the host cells; the following are the few examples of key cellular processes that are being affected by the effectors of pathogens. Effectors can facilitate an effective penetration and early invasion of host tissues. For example, *Ustilago maydis* secretes an effector called Pep1 from its hyphae that is required for an effective invasion of the host tissue; Pep1 inhibits plant peroxidases to suppress the early maize defence responses (Doehlemann et al. 2009).

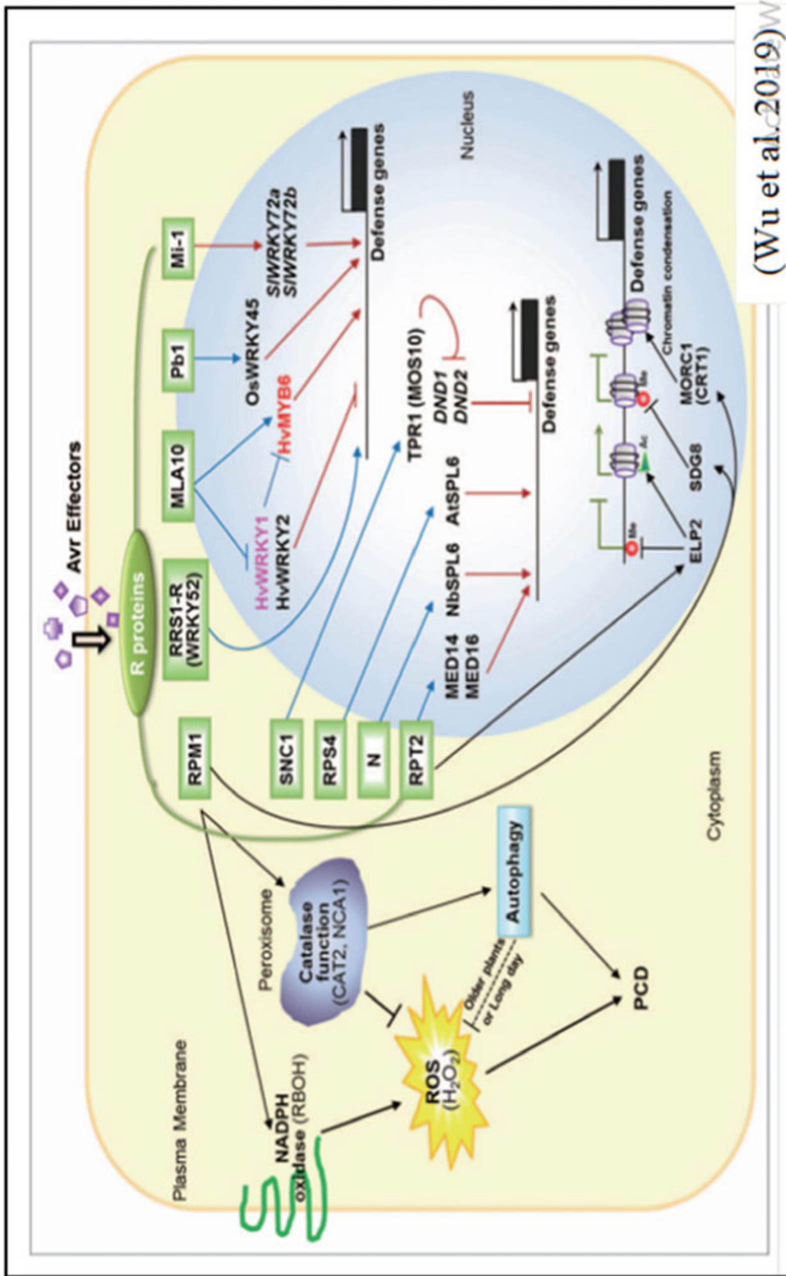

Fig. 22.7 Plant defence responses associated with ETI

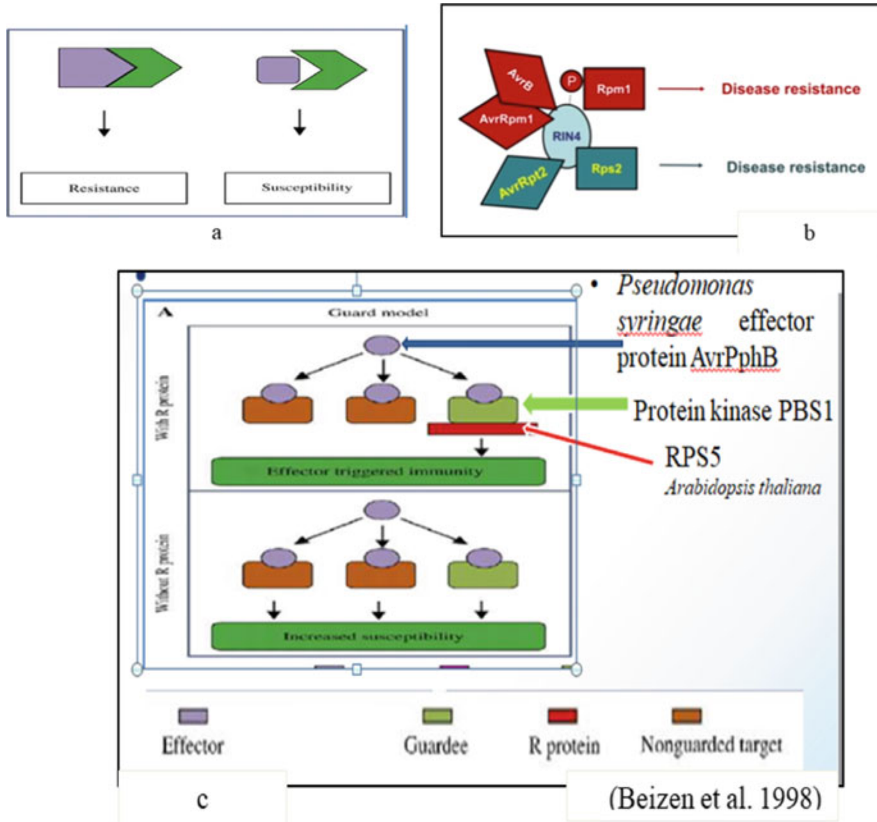

Fig. 22.8 Modes of pathogen effectors and host receptors interaction: (**a**) compatible reaction resulting in resistance development and incompatible interaction results in susceptibility; (**b**) direct interaction between effectors and receptors; (**c**) indirect interaction between effectors and receptor via guard protein

3. **Bacterial effectors for stomatal manipulation.** The tobacco wildfire pathogen *P. syringae* pv. *tabaci* secretes the effector HopX1, a cysteine protease (CP) that can degrade multiple JAZ transcriptional repressors, leading to the activation of JA-regulated genes (JA signalling) and inducing stomatal reopening on the leaf exterior (Melotto et al. 2006).
4. **Effectors for enhancing tissue colonization.** Cytosolic effectors of *M. oryzae*, such as PWL2 and BAS1, can translocate from cell to cell, possibly through plasmodesmata, to enhance subsequent colonization (Khang et al. 2010).
5. **Effectors interfere with plant hormones metabolism.** *P. sojae* and *Verticillium dahliae* secrete the virulence-promoting effectors Pslsc1 and Vdlscl, respectively; these are isochorismatases, enzymes that can hydrolyse the SA precursor isochorismate to disrupt SA metabolism (Liu et al. 2014).
6. **Manipulating host gene expression is carried out by TALEs (transcriptional activator-like effectors),** which act as plant transcription factors present in multiple *Xanthomonas* and *Ralstonia* bacterial pathogens, for example, *Xanthomonas oryzae* pv. *oryzae* TALE PthXo1 binds to the promoter region of

a sucrose transporter gene called OsSWEET11 to induce its expression and to promote bacterial pathogenicity (Yang et al. 2006).

7. **Effectors targeting RNA silencing machinery as plant viruses** have evolved suppressors of RNA interference machinery, which helps them to multiply well inside the host system, for example, P1/HC-Pro protein (helper component proteinase) from *Tobacco etch potyvirus*, 2b from *Cucumber mosaic virus* and P19 from *Tomato bushy stunt virus* (Csorba et al. 2015).

22.10 Effect on Various Cellular Processes of the Host by the Effectors

Effectors can influence various crucial cellular processes and manipulate as well as direct them towards the growth and colonization of the pathogens and infection in the host cells. Following are the few examples of key cellular processes that are being affected by the effectors of pathogens:

1. **Effectors can facilitate effective penetration and early invasion of host tissues.** For example, *Ustilago maydis* secretes effector called Pep1 from its hyphae that is required for effective invasion of host tissue; Pep1 inhibits plant peroxidases to suppress early maize defence responses (Doehlemann et al. 2009).
2. **Bacterial effectors for stomatal manipulation.** The Tobacco wildfire pathogen *P. syringae pv. tabaci* secretes the effector HopX1, a cysteine protease (CP) that can degrade multiple JAZ transcriptional repressors, leading to activation of JA-regulated genes (JA signalling) and to induce stomatal reopening on the leaf exterior (Melotto et al. 2006).
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22.11 Different Ways to Utilize Effectors as Well as Receptors for Enhancing Plant Immunity

1. **Effectors for screening R genes:** As the great amount of research work has been taking place in the field of molecular plant pathology over the last 30 years, it has enabled scientists to effectively integrate ETI into crop improvement programs. It will first be essential to identify multiple NLRs, which can recognize conserved effectors of pathogens in order to aptly harness ETI for the development of disease resistance. If effector repertoires attained through genomics studies are opted, they are helpful in breeding programmes for disease resistance. Effectors can be employed to identify R genes/NLRs in plant systems, so such extensive screening approach that uses effectors to identify specific unknown *R* genes is known as 'effectoromics' (Vleeshouwers et al. 2008). This method mainly depends on the transient expression of candidate effector gene in plant leaves; subsequently, the appearance of cell death responses due to hypersensitive reaction (HR) indicates the recognition of the effector by a suitable matching plant immune receptor in the host. This approach has the ability to quicken the identification of a large number of immune receptors when the matching specific effectors are present in the plant host system, since it has the ability to replace the slow process of stable transformants' development (Vleeshouwers et al. 2008). This approach was initiated for *P. infestans* and potato; in the last few years, a catalogue of Avr (Effector) and R (receptors) genes has become available. Specific HR responses to AVRblb1 were quickly detected in *S. stoloniferum* that is directly crossable with cultivated potato *S. tuberosum* and was found to carry Rpi-sto1, a functional homologue of *Rpi-blb1*; presently, the *Rpi-sto1* gene is efficiently utilized for the classic introgression into cultivated potato breeding material (Hein et al. 2009).
2. **Stacking multiple NLRs to confer resistance for durable resistance:** Multiple NLRs recognizing the core effectors can be stacked into one genotype, since the pathogen would rarely be able to mutate or lose multiple core effectors simultaneously. For example, three Rpi genes have been stacked transgenically into potato simultaneously, and it resulted into the development of resistance against *P. infestans* (Zhu et al. 2012).
3. **Engineering new NLR-mediated resistance specificities:** To engineer novel resistance specificities, much effort has targeted at the receptor level and defined mutations in the NLRs' nucleotide-binding site. NLR receptors have developed for enhanced effector recognition: Tomato NLR I2, which weakly responds to Avr3a effector of *P. infestans*. A point mutation in coiled-coil domain of I2 was carried out, which resulted in enhanced perception of Avr3a (Giannakopoulou et al. 2015). A great promise for synthetic NLR engineering for designing of NLR receptors to recognize diverse pathogen effectors has seen when mutation in the *Rx* CNL expanded the recognition of *Potato virus X* (PVX) strains. In *Arabidopsis*, RPS5 NLR guards the host kinase PBS1. AvrPphB effector of *P. syringae* is a protease which can cleave PBS1 at a defined region. The resulting conformational change due to cleavage will be detected by RPS5 (Fig. 22.8c).

Since effector proteases are common in both bacterial and viral pathogens, recently, Kim et al. (2016) engineered host proteins guarded by NLRs to generate new resistance specificities, by substituting the cleavage site of AvrPphB within PBS1 with those from other bacterial or viral proteases, which enabled RPS5 recognition of these proteases upon infection. This approach could also be employed to engineer resistance against a wide variety of other pathogens using well-characterized NLRs.

4. **Combining NLR-mediated resistance (ETI) with pattern recognition receptors (PRRs)-mediated resistance (PTI):** The transfer of *Arabidopsis* EFR, which recognizes elongation factor Tu (EF-Tu) to *Nicotiana benthamiana* and tomato confers responsiveness to EF-Tu, resulted in resistance against bacterial pathogens from different genera (Lacombe et al. 2010). This research suggests that PRRs could be used to engineer broad-spectrum disease resistance to diverse pathogens, potentially enabling more durable resistance in the field. Additional layers of disease resistance can also be combined with stacks of PRRs and NLRs.
5. **Genome editing of susceptibility loci:** TALEs, also called as transcription activator-like effectors, are mostly found in *Xanthomonas* spp. These are delivered into host cells during infection which later bind to loci of susceptible genes in the host cells and induce expression of susceptibility genes, by acting as transcription factors, thereby facilitating bacterial pathogen growth and virulence (Streubel et al. 2013), to provide resistance against susceptibility genes, viz. plant SWEET sugar transporters, which are TALE targets. Genome editing mediated by CRISPR/Cas9 approach and engineering of synthetic TALE nucleases have been carried out (Fig. 22.9). Jiang et al. (2013) developed resistance to *Xanthomonas citri* pv. *citri* through CRISPR/Cas9-targeted genome editing of citrus susceptibility gene *CsLOB1* and its promoter.
6. **Promoter traps and executor genes:** In resistant host genotypes, TAL effectors are recognized by plant cells through trap promoters, which are coupled to an executor-type resistance gene (*E*-gene). Trap promoters possess recognition sites for specific TAL effectors, so that, upon infection, the delivery of that TAL effector by pathogen induces the expression of the executor gene. Expression of the executor genes triggers hypersensitive (HR) reaction, and this limits the further growth of the pathogen. The first such E-type resistance gene shown to be triggered by a TAL effector was the *Xa27* gene of rice; it has a binding site for the *X. oryzae* pv. *oryzae* TAL effector AvrXa27 in its promoter region (Fig. 22.10a). This principle can be further utilized by combining different EBEs for multiple TAL effectors from individual and different pathogen strains and species into one promoter to achieve broad-spectrum resistance (Fig. 22.10b). Hummel et al. (2012) demonstrated that combining six EBEs that correspond to three TAL effectors from *Xoo* and three from *X. oryzae* pv. *oryzicola* into the *Xa27* promoter resulted in a gene inducible by all six of the TAL effectors and plant lines resistant to both the bacterial pathogens.
7. **TAL effector-based antiviral approaches:** TAL effectors are typically known to bind to double-stranded DNA. There are only very few double-stranded DNA

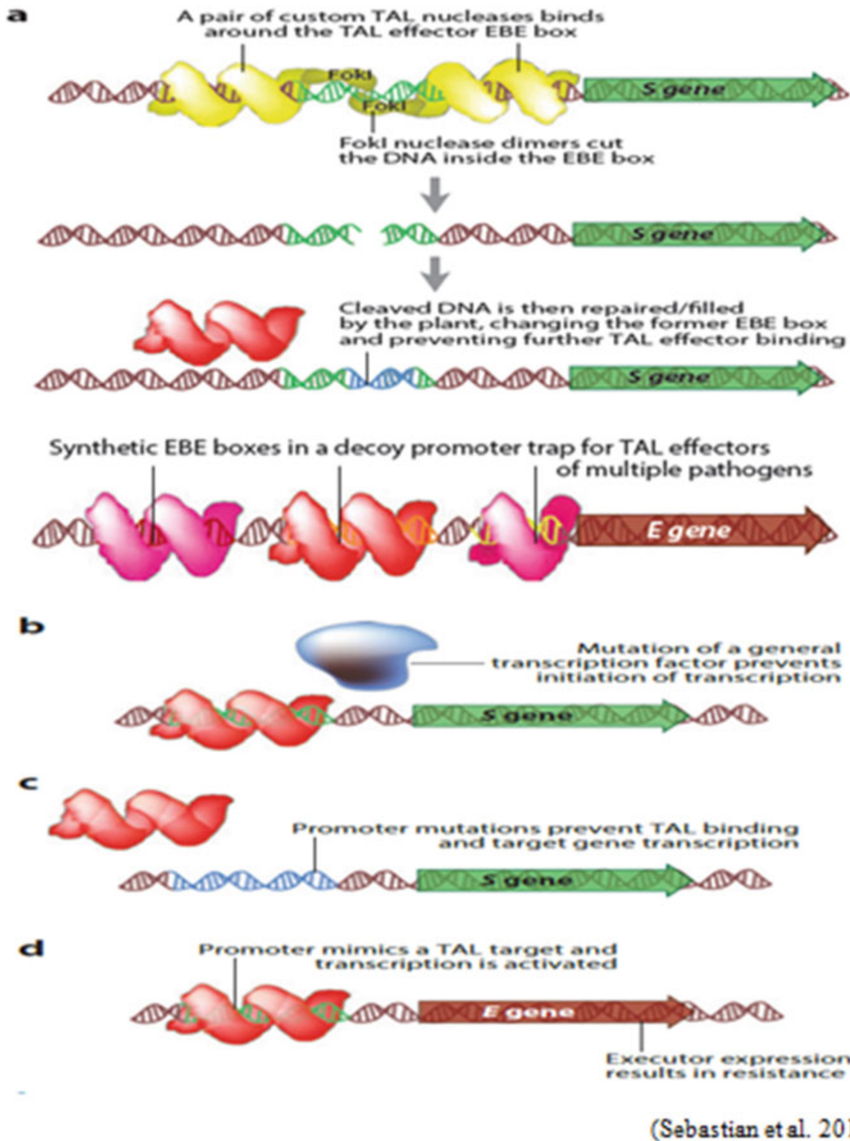

Fig. 22.9 Engineering resistance to *Xanthomonas* by utilizing TAL effector knowledge. (**a**) Customized TAL effector pairs fused to FokI nuclease domains are used to mutate a TAL effector-binding element (EBE) upstream of a key host susceptibility gene (*S* gene). (**b**, **c**, **d**) Mechanisms of plant resistance against transcription activator-like (TAL) effectors

plant viruses; *Geminiviruses* possess single-stranded DNA but go through a double-stranded DNA rolling circle replicative stage. Examples include *Maize streak virus*, *African cassava mosaic virus* and *Tomato yellow leaf curl virus*

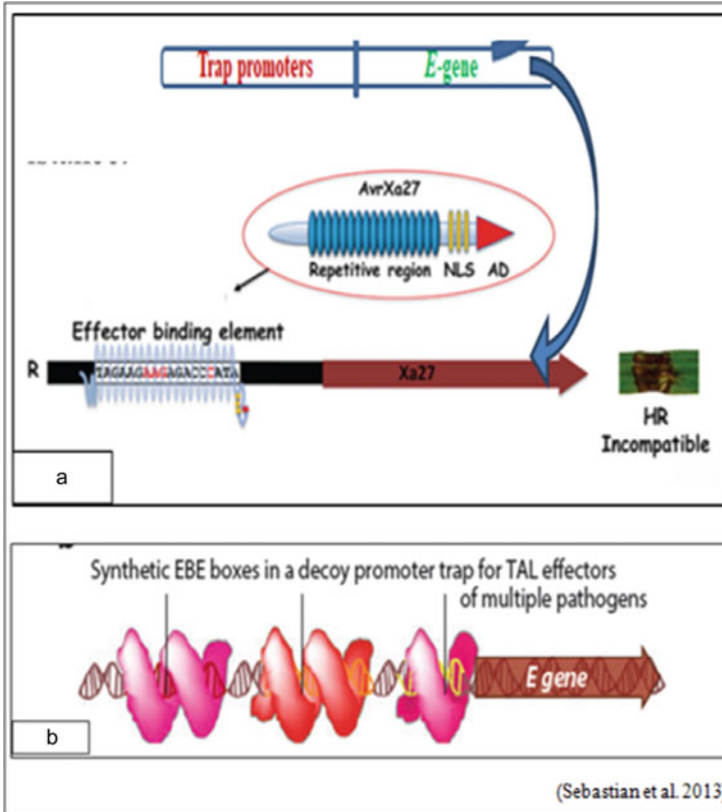

Fig. 22.10 EBEs for TAL effectors of pathogen strains are construed and inserted into promoters driving executor gene (*E* gene)

(TYLCV). Rolling circle replication could be obstructed in transgenic plants expressing one or more TAL DNA-binding domains designed against the origin of replication sequence (ori). In addition, pathogen genomes can also be destroyed through TAL nucleases, which are designed to cleave sequences within the viral genomes. Transgenic plants engineered with TAL nucleases targeting the specific DNA of other pathogens, like fungi and nematodes, may also be feasible, provided such molecules (TAL nucleases) can be delivered to the pathogen, for instance, through transfer across haustorial membranes to fungi or parasitic plants or by feeding in nematodes.

8. **Host-induced gene silencing (HIGS):** It is based on exploiting an RNA interference mechanism to target a selected pathogen gene, i.e. effector gene, via the host plant to nullify the effector production capacity. Plants can be transformed with hairpin RNA constructs that target selected pathogen effector genes to suppress their expression and production of effectors. For example, HIGS in barley expressing dsRNA targeting the effector gene Avra10 from the fungus *Blumeria graminis* led to reduced disease incidence (Nowara et al. 2010) (Table 22.1).

Table 22.1 Receptors present in different plant species and their corresponding effector (Avr) proteins present in different pathogen species

NLR	Type	Host species	Avr	Pathogen species	References
Pi2	NB-LRR	Rice	Unknown	*Magnaporthe oryzae*	Zhou et al. (2006)
Pi37	NB-LRR	Rice	AvrPi37	*Magnaporthe oryzae*	Lin et al. (2007)
RPI-BLB1	CNL	*Solanum bulbocastanum*	AvrBLB1	*Phytophthora infestans*	van der Vossen et al. (2003)
R1	CNL	*Solanum demissum*	Avr1	*Phytophthora infestans*	Vleeshouwers et al. (2011)
R8	CNL	*Solanum demissum*	Avr8	*Phytophthora infestans*	Vossen et al. (2016)
Rpi-sto1	NB-LRR	*Solanum stoloniferum*	AvrBLB1	*Phytophthora infestans*	Wang et al. (2008)
N direct	TNL	Tobacco	Helicase domain	*Tobacco mosaic virus*	Padgett and Beachy (1993)
RPG1b	CNL	Soybean	AvrB	*Pseudomonas glycinea*	Kessens et al. (2014)
Prf	CNL	Tomato	AvrPto/ AvrPtoB	*Pseudomonas syringae*	Rathjen et al. (1999)
Lr10/ RGA2	CNL/CC-NB-NBLRR	Wheat	AvrLr10	*Puccinia triticina*	Loutre et al. (2009)
sr22	CNL	Wheat	PGTAUSPE-10-1	*Puccinia graminis*	Upadhyaya et al. (2014)
RPS1k	CNL	Soybean	Avr1k/ Avr1b	*Phytophthora sojae*	Dou et al. (2008)
Pm3b	CNL	Wheat	AvrPm3b	*Blumeria graminis*	Yahiaoui et al. (2004)
Sw5b	CNL	Tomato	NSm	*Tomato spotted wilt Virus*	Yahiaoui et al. (2004)
Bs4	TNL	Tomato	AvrBs4	*Xanthomonas campestris*	Schornack et al. (2004)
I2	CNL	Tomato	Avr2-SIX5 pair	*Fusarium oxysporum*	Ma et al. (2015)
RPG1r	CNL	Soybean	AvrRPM1	*Pseudomonas glycinea*	Ashfield et al. (2003)
R3A	CNL	*Solanum demissum*	Avr3a	*Phytophthora infestans*	Engelhardt et al. (2012)
Pi9	NB-LRR	Rice	AvrPi9	*Magnaporthe oryzae*	Wu et al. (2015)

22.12 Limitations

Even though we identify different NLRs in wild germplasm, it is difficult to transfer into commercial cultivars through conventional plant breeding approaches; to achieve this, we need to rely on transgenic approaches, but transgenic-based crops are not widely accepted in most of the countries in the world. Sometimes combining multiple NLRs together may negatively regulate each other, for example, Rye *Pm8* and wheat *Pm3* resistance genes form heteromeric receptor complex.

22.13 Conclusion

Since the discovery of H. H. Flor's gene-for-gene concept, there has been a significant progress in understanding the genetic and molecular basis of ETI. Accelerating climate change is predicted to generate new strains of plant pathogens carrying novel effectors, demanding rapid responses by plant breeders. Rational design of plant immune systems will be one tool among many that enables agricultural systems to keep pace with pathogens. Thus, it will be necessary to utilize concept of effectors and receptors to improve plant immunity in combination with different types of resistance acting at different stages in pathogen infection.

22.14 Future Prospects

For better understanding of how individual NLR domains interact with one another, we need to understand how NLRs activate downstream (signalling) events, How disease resistance and cell death are triggered remains, remarkably, a black box. To gain deeper insights, the modes of action of individual effectors and their interactions with effector targets and matching immune receptors should be studied.

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Transgenic Technology for Disease Resistance in Crop Plants

23

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Keywords

Transgenic for disease resistance · Pathogen-derived resistance · RNAi · Genome editing tools

23.1 Introduction

The ever increasing demand for food production proportionate to exponential growth of global population evoked the need for applying innovative techniques for developing disease-resistant crop varieties, as the pest and pathogen attack causes considerable yield loss which in turn putting the agriculture sector and crop production in crisis. This is highly significant when the resistance conferred by conventional measures, like artificial hybridization, mutation breeding, marker-assisted selection, etc. appears to be inadequate in many cases, especially due to the evolution of new/more virulent strains/ pathovars/ isolates of pathogens and their unexpected host range expansion. Development of recombinant DNA technology, transgenic expression, and RNA silencing strategies lead to a new era of transgenically engineered resistance in several crop species, many of which succeeded in field trials and got commercialized. Elucidation of genetic and molecular mechanisms underlying host-pathogen interactions, resistance, susceptibility, and different levels of plant immune responses (PTI, ETI, HR, etc.) revealed the key genes in the host as well as pathogens that can be manipulated by transgenic approaches/ techniques for conferring effective resistance. Reprogramming of phytohormonal regulatory pathways determining defense response and remodeling of molecular receptors/

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transcription factors involved in resistance or disease development can be brought about by transgenic methods to enhance the resistance in host plants. Targeting of conserved sequences or molecular components could provide broad-spectrum resistance in certain cases. Mining of R genes, transgenic expression of foreign R gene, etc. is another useful strategy. Potential of genome editing based on engineered nucleases like ZFNs, TALENs, and CRISPR/ Cas9 to precisely mutate the genomic sequence of interest can be exploited for specific targeting of the host/ pathogenic genes associated with the biotic stress response. Application of all these approaches for the management of plant pathogens were discussed in this chapter.

During the earlier times, resistant varieties were created by artificial hybridization with wild-type resistant varieties, which was purely depended on phenotypic selection. Diminishing genetic variability and lack of resistant germplasm limited the possibilities of resistant gene introgression via conventional breeding. Moreover, it is time-consuming and associated linkage drag may result in introgression of undesirable traits too. Mutation breeding was an alternative to this, where chemical mutagens are used for inducing mutation in target genes to create desirable phenotype. Resistance to powdery mildew has been successfully engineered in this way by mutating Mlo locus. As mutagenesis is random, the chances of off-target mutations is high. QTL mapping and marker-associated selection and breeding are other ways to create disease-resistant varieties but require large mapping population and are time-consuming and labour-intensive as in the case of hybridization. Even though these conventional strategies enabled crop protection to a great extent, over the centuries, rapid genetic variation and adaptive evolution of pathogens engendered for novel, robust and sustainable methods for controlling pathogens and pest management.

The development of genetic engineering (GE) technology for designing tailor-made plants by transforming plant with the desired gene from other organism was a major breakthrough in crop improvement programs. Initially, transgenic experiments were confined to model plants merely for demonstration and proof-of-concept purpose; later, species wide dispersal of technology was made possible by the development of optimum transformation and regeneration protocols, and this widened the applicability of GE for many economically important crops. Since disease resistance is an important yield determinant, transgenic technologies have given considerable focus on developing/reinforcing resistance in economically important crop species. Crop improvement program for disease resistance gained a significant progress by deploying various GE techniques. Rapid introgression of promising candidate genes, even into phylogenetically unrelated species, precise alteration or modification of host factors involved in the perception of pathogenic effectors and deployment of defence signals, metabolic pathways and biomolecules involved in direct defence responses as well as targeted by pathogen effectors have been successfully implemented using transgenic strategies. Similarly, multitude of pathogenic genes and effectors have also been manipulated transgenically, in order to reduce virulence and pathogenicity. Furthermore, transgenic strategies permit precise spatio-temporal regulation of trait of interest. The emergence of new breeding techniques (NBT), including targeted mutagenesis and precision breeding,

further revolutionized crop improvement strategies. Genome editing tools, like ZFNs, TALENs and CRISPR/Cas9, based on site-directed nucleases enabled precise editing of targeted genomic loci to confer/modify resistance traits in many of the crop plant species. Altogether transgenic strategies facilitated the fine-tuning of the plant immune system for ensuring sustainable production. In this chapter, we will describe about the strategies adopted for creating resistance in transgenic plants against virus, bacteria and fungi and at then the upcoming strategies based on the information obtain from plant–pathogen interactions.

23.2 Transgenic Technology for Virus Resistance

Viruses are notorious plant pathogens causing devastating damage to crop yield. These obligate parasites invade, replicate and proliferate in host cell and result in infection, which reduces fitness and productivity of crop plants and decreases market and aesthetic values of the products. Attributes like great evolvability, large population sizes, error-prone replication and efficient host-dependence render the control of plant viruses extremely difficult. Development of sustained resistance against pathogenic virus, broad-spectrum as well as durable, throughout the productive stage while infection pressure persists is always a challenge in crop improvement. Farmers rely on combining traditional cultural management practices, such as field sanitation, crop rotation, planting of trap plants, spraying for vector, rouging and manual removal of infected plants upon detection of disease symptoms, use of certified virus-free seeds or planting materials (Kamala and Makeshkumar 2015; Deepthi and Makeshkumar 2016), as there is no specific direct control strategy; even chemical pesticides are not available. All these measures are laborious and time-consuming. Chemical control of vectors, in addition, causes health and environmental hazards. The most efficient practical solution available is the use of resistant varieties. But elite cultivars might not always be endowed with resistance traits, which further make the problem complicated and uneconomical. Even though resistance genes can be introgressed from wild varieties, either by conventional breeding or marker-assisted selection, linkage drag and other inherent shortcomings of these techniques render it inadequate for successful crop improvement. Resistance genes in unrelated species or sometimes related species cannot be introgressed by hybridization due to barriers, like sexual incompatibility, male sterility, etc. Similarly, attaining broad-spectrum resistance requires stacking of resistance genes from different sources, which is again a laborious and less efficient strategy. Moreover, virus resistance conferred by R genes is less durable due to the suppression of R–Avr interaction by creating variant Avr protein, which might be unrecognizable by the host R protein, by quickly evolving viral genome, rendering the host susceptible for compatible host-pathogen interaction. However, transgenic approaches have succeeded to a great extend in engineering efficient virus resistance in crop species.

Recessive resistance, conferred by mutating host factors required for infection cycle completion and thereby making the host non-permissive to virus, is more efficient and durable strategy. Resistance thus conferred is passive as there is no

active involvement of plant immune system. Resistance mechanism, to be adopted, and respective targets vary with stage of infection (Johnson 1981).

Transgenic antiviral approaches used so far were based on the expression of various viral proteins; RNAs; nonviral genes like nucleases, antiviral inhibitors and plantibodies; plant defence response elicitors; host-derived resistance genes (dominant resistance genes and recessive resistance genes); and various factors involved in host defence responses. Viral proteins usually used for engineering resistance are capsid protein, replicase proteins and movement proteins. Several RNA molecules, viz. sense RNAs, antisense RNAs, satellite RNAs, defective interfering RNAs, hairpin RNAs and artificial microRNAs, noncoding RNAs, antisense RNAs, ribozymes, double-stranded RNAs (dsRNAs) and inverted repeat RNAs (irRNAs), have been employed for conferring virus resistance by post-transcriptional or transcriptional gene silencing. Transgenic virus resistance strategies are deployed principally by three mechanisms: pathogen-derived resistance, pathogen-targeted resistance and RNA interference.

23.2.1 Pathogen-Derived Resistance

Pathogen-derived resistance (PDR) is protein-mediated resistance conferred by viral protein expressed in host cells (Sanford and Johnston 1985). PDR is accomplished by different a mode of action, which varies with strains and stages of infection cycle, like whether it is in movement/transport/replication phase. It can be inhibition of replication and viral particle accumulation in the early stages of infection while limiting the spread via apoplastic/symplastic/phloem stream during the movement stage (Galvez et al. 2014). The first type of PDR is based on the silencing of pathogenic gene by expression of a part of that gene in host.

23.2.2 Coat Protein–Mediated Resistance (CPMR)

Since coat protein (CP) has implications in almost all stages of infection, like uncoating, systemic movement, long-distance transport, replication, symptom development, etc., it can be the ideal candidate for engineering PDR, even in non-host plants (Galvez et al. 2014). CPMR is manifested by expression of coat protein gene in the host cell and the subsequent interaction between transgenic CP and viral CP (Koo et al. 2004). The very first time application of CPMR was showed in tobacco (*Nicotiana tabacum*) plants, expressing the capsid protein-encoding sequences of *Tobacco mosaic virus* (TMV), and this resulted in partial resistance to TMV (Abel et al. 1986). Later it was expanded to tomato, using the same CP construct, with resistance to *Tomato mosaic virus* (ToMV) (Nelson et al. 1988). Similarly, capsid protein gene of a potyvirus was expressed in a non-host plant, tobacco, conferring resistance to other potyviruses. CPMR has been used to confer resistance to at least 35 viruses, representing more than 15 different taxonomic groups (Table 23.1). Several virus-resistant transgenic crop plants were developed by using a suitable

Table 23.1 Transgenic plant species showing coat protein-mediated resistance

Host plants	Virus	References
Citrus	*Citrus mosaic virus* (CiMV)	Iwanami et al. (2004)
	Citrus psorosis virus (CPsV)	Reyes et al. (2011)
	Citrus tristeza virus (CTV)	Domínguez et al. (2002)
		Febres et al. (2008)
		Loeza-Kuk et al. (2011)
Cucumber	*Zucchini yellow mosaic virus* (ZYMV)	Wako et al. (2001)
Squash	*Squash mosaic virus* (SqMV)	Pang et al. (2000)
Watermelon	*Cucumber green mottle mosaic virus* (CGMMV)	Park et al. (2005)
	Cucumber mosaic virus (CMV) and *Watermelon mosaic virus* (WMV)	Lin et al. (2012)
	WMV	Wang et al. (2003)
	Zucchini yellow mosaic virus (ZYMV) ZYMV	
	ZYMV and *Papaya ringspot virus* -W (PRSV-W) PRSV-W	Yu et al. (2011)
Brinjal	CMV	Pratap et al. (2011)
Lettuce	*Lettuce big-vein associated virus* (LBVaV)	Kawazu et al. (2006)
Melon	CMV	Xu et al. (2005)
	ZYMV	Wu et al. (2009)
	ZYMV and PRSV-W	Wu et al. (2010)
Soybean	*Bean pod mottle virus* (BPMV)	Reddyet al. (2001)
	Soybean dwarf virus (SDV)	Tougou et al. (2007)
		Wang et al. (2001)
	Soybean mosaic virus (SMV)	Furutani et al. (2006)
Rice	*Rice stripe virus* (RSV)	Park et al. (2012)
	Rice tungro bacilliform virus (RTBV)	Ganesan et al. (2009)
	Rice tungro spherical virus (RTSV)	Verma et al. (2012)
	Rice yellow mottle virus (RYMV)	Kouassi et al. (2006)
Maize	*Maize dwarf mosaic virus* (MDMV)	Liu et al. (2005)
	Sugarcane mosaic virus (SCMV)	Liu et al. (2009)
Wheat	*Wheat streak mosaic virus* (WSMV)	Sivamani et al. (2002)
		Li et al. (2005)
Orchid	*Cymbidium mosaic virus* (CymMV)	Chang et al. (2005)
	CymMV	Liao et al. (2004)
Papaya	*Papaya ringspot virus* (PRSV)	Lines et al. (2002)
		Ferreira et al. (2002)
		Fermin et al. (2004)
		Souza et al. (2005)
		Kertbundit et al. (2007)
	PRSV and *Papaya leaf-distortion mosaic virus* (PLDMV)	Kung et al. (2009)
		Kung et al. (2010)

(continued)

Table 23.1 (continued)

Host plants	Virus	References
Groundnut	*Peanut stripe virus* (PStV)	Higgins, et al. (2004)
	Tobacco streak virus (TSV)	Mehta et al. (2013)
Capsicum	CMV	Lee et al. (2009)
		Pack et al. (2012)
	CMV and *Pepper mild mottle virus* (PMMoV)	Shin et al. (2002)
	CMV and *Tomato mosaic virus* (ToMV)	Shin et al. (2002)
Sugarcane	*Sugarcane mosaic virus* (SCMV)	Guo et al. (2008)
	Sugarcane yellow leaf virus (SCYLV)	Zhu et al. (2011)
Sweet potato	*Sweet potato feathery mottle virus* (SPFMV)	Okada et al. (2001)
		Okada and Saito (2008)
		Okada and Yoshinaga (2010)
Potato	*Potato virus Y* (PVY)	Rachman et al. (2001)
Plum	*Plum pox virus (PPV)*	Scorza et al. (1997); Ilardi and Tavazza (2015)
Sweet pepper	*Cucumber mosaic virus*	Zhu et al. (1996)
Nicotiana tabacum cv. Petit Havana	*Cucumber mosaic virus* (CMV) subgroup IA	Dubey et al. (2015)
Tomato	CMV	Pratap et al. (2012)
	Physalis mottle virus (PhMV)	Vidya et al. (2000)
	Tomato leaf curl virus (ToLCV)	Raj et al. (2005)
	Tomato leaf curl Taiwan virus (ToLCTWV)	Sengoda et al. (2012)
	Tomato spotted wilt virus (TSWV)	Gubba et al. (2002)

coat protein gene (Mundembe et al. 2009; Nomura et al. 2004). CPMR has been successfully established in host plants, including potato, tomato, tobacco and papaya, exhibiting resistance to *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV), *Cucumber mosaic virus* (CMV) and *Papaya ring spot virus* (PRSV) (Makeshkumar et al. 2002). The efficiency of CPMR varies for each virus with different stages of infection cycle (Bendahmane et al. 2007). The underlying molecular mechanism of resistance manifestation is either through recoating of invading viral particles or by blocking of receptors in transgenic plants (Saharan et al. 2016).

23.2.3 Replicase- or Rep-Associated Protein-Mediated Resistance

Replicase is another potential candidate to engineer resistance against viral genome. Expression of intact or truncated or mutant virus-encoded replicase in host can confer resistance to that virus. It was reported for the first time when a 54 kDa truncated protein of TMV replicase protein was expressed in *N. benthamiana*, where it conferred high level of resistance against TMV infection (Golemboski et al. 1990).

Table 23.2 List of various transgenic plants developed with replicase-mediated resistance

Plant species	Virus
Potato	*Potato leafroll virus* (PLRV)
	Potato virus Y (PVY-N) tobacco rattle virus (TRV)
Rice	*Maize dwarf mosaic virus* (MDMV)
	Rice tungro spherical virus (RTSV)
	Rice yellow mottle virus (RYMV)
Tomato	CMV
Watermelon	CMV, *Zucchini yellow mosaic virus* (ZYMV), and WMV
Wheat	*Wheat streak mosaic virus* (WSMV)
	Wheat yellow mosaic virus (WYMV)
Papaya	*Papaya ringspot virus* (PRSV)
Citrus	*Citrus tristeza virus* (CTV)
Cucumber	*Cucumber fruit mottle mosaic virus* (CFMoMV)
Barley	*Barley yellow dwarf virus* (BYDV-PAV)

This strategy has been extended to many food crops, including rice, bean, potato etc., and mostly resulted in narrow-spectrum resistance towards a particular race of the pathogen (Saharan et al. 2016) (Table 23.2). Rep-associated protein, which interacts with host DNA polymerase during replication of ssDNA virus, can also be manipulated in a similar manner. Resistance to two ssDNA viruses, *Tomato yellow leaf curl virus-Israel* (TYLCV-Is [Mild]) and *Bean golden mosaic virus* were conferred by transgenic expression of a truncated replication-associated protein gene and rep gene, respectively, in tomato and *P. vulgaris* (Brunetti et al. 1997; Faria et al. 2006). As in the case of CPMR, the active entity conferring resistance can either be protein or RNA or both.

23.2.4 Movement Protein–Mediated Resistance

Movement proteins (MP) facilitate the intracellular or cellular movement of viral particles through plasmodesmata, by modifying the gating channels of plasmodesmata. Resistance conferred by dysfunctional or mutated MP is mostly broad-spectrum in nature when compared to CP or replicase-mediated resistance (Prins et al. 2008). The first MP-mediated resistance was shown in transgenic tobacco plants expressing a 30 kDA mutant defective MP (dMP), which competed with the wild-type virus-encoded MP for the binding sites in the plasmodesmata and conferred resistance against eponymous virus infection. dMP conferred broad-spectrum resistance by preventing the systemic spread of distantly related and unrelated viruses (Lapidot et al. 1993; Cooper et al. 1995). Similar broad-spectrum resistance was observed in transgenic potato expressing wild-type *Potato leafroll virus* (PLRV) movement protein against PLRV, PVY and PVX (Tacke et al. 1996), while narrow-spectrum resistance was shown in transgenic *N. benthamiana* expressing wild-type movement proteins of *Cowpea mosaic virus* (CPMV) and transgenic tobacco expressing PVX movement protein (Sijen et al. 1995).

23.2.5 Other Viral Protein-Mediated Resistance

Transgenic expression of viral proteins other than those discussed previously, such as replication-associated protein, NIa protease, P1 protein and HC- Pro, has been tried out in order to achieve resistance against viruses (Cillo and Palukaitis 2014). Transgenic expression of partial or complete Rep gene was found to confer resistance against *Tomato golden mosaic virus* (TGMV), *Tomato yellow leaf curl virus* (TYLCV) (Yang et al. 2004; Antignus et al. 2004; Lucioli et al. 2003), *African cassava mosaic virus* (ACMV) (Chellappan et al. 2004), *Bean golden mosaic virus* (BGMV) (Faria et al. 2006), *Maize streak virus* (Shepherd et al. 2007), *Cotton leaf curl virus* (CLCuV) (Hashmi et al. 2011) and *Tomato leaf curl Taiwan virus* (ToLCTWV) (Lin et al. 2012) in transgenic plants.

Transgenic tobacco plants expressing the NIa protein of *Tobacco vein mottling virus* (TVMV) exhibited resistance against TVMV, whereas it failed to confer resistance to two other potyviruses, *Tobacco etch virus* (TEV) and PVY (Maiti et al. 1999). Transgenic tobacco lines expressing paired NIa protease coding sequences for two viruses, like TEV–PVY, TEV–TVMV and TVMV–PVY, were assessed for virus resistance and mostly resulted in the recovery-type resistance (Fellers et al. 1998). However, expression of multiple genes (NIa/NIb/capsid protein) from a single potyvirus using a single construct failed to confer any enhanced resistance in most cases (Maiti et al. 1999).

Transgenic expression of complete or partial sequence encoding P1 protein conferred resistance, of varying degree, against viruses like PVY, PPV, TVMV and PVA. A recovery-type resistance was obtained in most cases rather than complete resistance (Germundsson and Valkonen 2006). Expression P1 coding sequence of PVY-O and PPV, respectively, showed either complete resistance or a recovery-type resistance against PVY-O infection in potato and PPV infection in *N. benthamiana* (Maki-Valkama et al. 2001; Tavert-Roudet et al. 1998).

Expression of viral HC-Pro protein resulted in recovery phenotypes instead of conferring resistance to PVA and PPV in transgenic *N. benthamiana* and SMV in transgenic soybean (Savenkov and Valkonen 2002; Barajas et al. 2004; Lim et al. 2007). Expression level of transgene considerably influenced the resistance mediated by HC-Pro in most of the experiments, wherein low expression levels are mostly favoured for resistant or recovery phenotype rather than high expression levels. Deletion of central domain of HC-Pro protein has found to confer recover disease symptoms of *Cowpea aphid-borne mosaic virus* (CABMV) in transgenic *N. benthamiana* compared to intact protein (Mlotshwa et al. 2002).

Other viral genes, such as VPg-protease coding region of *Tomato ringspot virus* (ToRSV), capsid protein domain of BNYVV (Andika et al. 2005) and p23 silencing suppressor protein of *Citrus tristeza virus* (CTV) (Fagoaga et al. 2006), were also used as potential transgene candidates for providing resistance against ToRSV infection in *N. benthamiana*, *olymyxa betae* infection in transgenic *N. benthamiana* plants and CTV infection in transgenic Mexican lime plants, respectively (Table 23.3).

Table 23.3 List of transgenic crop plants with hairpin-mediated resistance against viruses

Transgenic host plant	Disease/virus	Hairpin construct used	References
Cassava	ACMV	Rep gene of ACMV	Vanderschuren et al. 2012
	Cassava brown streak virus	Capsid protein of CBSV	Ogwok et al. (2012)
	Cassava brown streak Uganda virus	Rep gene of CBSV	Chauhan et al. (2015), Wagaba et al. (2017), Vanderschuren et al. (2012), Yadav et al. (2011)
	SLCMV	AV1 and AV2	Ntui et al. (2015)
Citrus	*Citrus psorosis virus* (CPsV)	RNA3 of CPsV	Reyes et al. (2009)
		p24 gene on RNA1	Reyes et al. (2011)
	CTV	p23 gene and 3'NTR	Lopez et al. (2010)
		Capsid protein gene	Muniz et al. (2012)
		RNA silencing suppressor (CTV)	Soler et al. (2012)
Cucurbits	PRSV	Capsid protein coding sequences of PRSV	Krubphachaya et al. (2007)
	CGMMV	Capsid protein gene of CGMMV	Kamachi et al. (2007)
	Melon necrotic spot virus (NSV)	Cm-eIF4E translation initiation factors (eIF)	Rodríguez-Hernández et al. (2012)
	ZYMV	HC-pro encoding sequences of ZYMV	Leibman et al. (2011)
Common bean	BGMV	C1 gene of BGMV	Bonfim et al. (2007)
Soybean	*Soybean mosaic virus*	Coat protein, VSR Hc-pro, P3 cistron	Kim et al. (2016), Gao et al. (2015), Yang et al. (2018)
	Soybean dwarf virus (SbDV)	Coat protein	Tougou et al. (2006)
Cowpea	*Cowpea aphid-borne mosaic virus*	Coat protein	Cruz et al. (2014)
White clover	WCIMV	Replicase gene of WClMV	Ludlow et al. (2009)
Maize	SCMV	NIb coding sequences of SCMV	Zhang et al. (2010)
	Maize dwarf mosaic virus (MDMV)	P1 coding sequence or capsid protein coding sequence of MDMV	Zhang et al. (2011)
Potato	PVY-N, certain isolates of PVY-NTN and PVY-O	3'part of the PVY-N capsid protein coding sequences of PVY-N	Missiou et al. (2004)
	PVX, PVY-O, PVY-N, PVY-C	Fused PVX capsid protein and PVY Nib encoding region	Bai et al. (2009)

(continued)

Table 23.3 (continued)

Transgenic host plant	Disease/virus	Hairpin construct used	References
	PVX, PVY and PLRV	Fused sequences from PVX, PVY and PLRV	Arif et al. (2012)
	PVY-O and PVY-NTN	PVY capsid protein	McCue et al. (2012)
	PVA, PVY, PLRV	Fused hpRNA sequences of three viruses (PVA, PVY and PLRV)	Chung et al. (2013)
Sweet potato	SPFMV and SPCSV	Capsid protein of *Sweet potato feathery mottle virus* (SPFMV)	Nyaboga et al. (2008)
Tomato	TYLCV	C1 (rep) gene	Fuentes et al. (2006)
	TYLCV	TYLCV capsid protein	Zrachya et al. (2007)
	TYLCV (Sardinia variant), TYLCV	Rep gene of TYLCV (Sardinia variant)	Tamarzizt et al. (2009)
	PSTVd	hpRNAs derived from PSTVd	Schwind et al. (2009)
	CMV	Replicase	Ntui et al. (2015)
Wheat	WSMV	NIa coding sequence (WSMV)	Fahim et al. (2010)
	BYDV (PAV)	Polymerase gene of BYDV (PAV)	Yassaie et al. (2011)
Maize	*Maize dwarf mosaic virus* (MDMV)	Coat protein	Zhang et al. (2011)
Barley	*Barley yellow dwarf virus* (BYDV)	Polymerase gene	Wang et al. (2000)
Banana	*Banana bunchy top virus*	Master replication initiation protein of bunchy top virus	Elayabalan et al. (2013)
Rice	*RDV*	Pns4 or Pns12 genes of RDV	Shimizu et al. (2009)
	RSV	pC1 (replicase), pC3 (nucleocapsid) and pC4 (movement protein) of RSV	Shimizu et al. (2011)
	RSV	Nucleocapsid protein gene or disease-specific protein gene of RSV	Ma et al. (2011)
			Zhou et al. (2012)
			Park et al. (2012)
	Rice black streaked dwarf virus (RBSDV)	Non-structural gene (P9–1) of *Fijivirus* (RBSDV)	Shimizu et al. (2012)

(continued)

Table 23.3 (continued)

Transgenic host plant	Disease/virus	Hairpin construct used	References
	Rice gall dwarf virus	Non-structural gene (Pns9) of *Rice gall dwarf virus*	Shimizu et al. (2012)
	Rice ragged stunt virus	Nucleocapsid protein or movement protein gene of *Rice ragged stunt virus*	Shimizu et al. (2013)
Tobacco	CMV	Coat protein	Chen et al. (2004)
	PPV	VSR P1 and HC- pro	Di Nicola-Negri et al. (2005)
	CGMMV	Coat protein	Kamachi et al. (2007)

23.2.6 Viral RNA-Mediated Resistance

Although virus resistance has been successfully manifested in several crop species by transgenic expression of intact or dysfunctional or mutated structural proteins of virus, resistance in many plants has been found to be mediated by corresponding mRNAs rather than the encoded protein moieties (Saharan et al. 2016). The active role of RNA in conferring resistance was first revealed when an untranslatable coat protein gene in transgenic tobacco plants provided resistance against *Tobacco etch virus* (TEV) (Lindbo and Dougherty 1992a, b). Many cases of transgenic expression of untranslatable viral proteins further substantiated the direct participation of RNA in developing resistance. Later, it has been shown that the virus resistance can be RNA-mediated, and viral protein expression is not necessary for the same (Dougherty et al. 1994). Viral RNA-mediated resistance is carried out by transcriptional gene silencing (PTGS), RNA interference or RNAi or RNA silencing, where a transgenic viral RNA introduced into the host plant drives sequence-specific homology-dependent degradation of viral genomic RNA or viral mRNAs by employing the small interfering RNA (siRNA) pathway of host cell machinery (Voinnet 2008; Waterhouse et al. 2001; Lindbo and Dougherty 2005). This homology-dependent gene silencing mechanism is conserved among higher eukaryotes and operates for gene expression regulation and in host defence against transposable elements and viruses (Hannon 2002; Mawassi and Gera 2012).

23.2.7 RNA Interference: Mechanism

RNA interference is widely accepted as a potential gene silencing strategy that can be easily manipulated to obtain desirable trait in organism of interest. RNA silencing process requires a double-stranded RNA precursor to trigger the silencing process. This dsRNA precursor is derived from viral RNA intermediates or viral RNA secondary structures. After recognition of this dsRNA by the RNase III-like enzyme, namely, DICER, it is cleaved into 21–25 nucleotide duplexes, called as small

interfering RNAs (siRNAs). The siRNAs thus formed are incorporated and converted to ssRNAs by Argonaut (Ago) containing multisubunit ribonuclease named as RNA-induced silencing complex (RISC). Subsequently RISC targets the specific mRNAs that share sequence similarity with siRNA through degradation of transcript (Waterhouse et al. 1998; Hamilton and Baulcombe 1999; Voinnet 2008).

RNA-mediated resistance engineering approaches include expression of non-coding regions of viral genome, viral CP mRNA, satellite (sat) RNA, defective interfering (DI) RNA and viral sequences in sense or antisense orientation or in double-stranded forms in host plants (Cillo and Palukaitis 2014).

Non-coding ssRNA Viral non-coding RNAs like 5′ and 3′ NTRs, intergenomic regions and non-coding RNAs, either in sense or antisense orientation, have been used for engineering resistance in various crop species. Transgenic expression of 5′ and 3′ NTRs and intergenomic regions could successfully confer resistance in crop plants. Sometimes simultaneous expression of non-coding NTR regions with mRNAs also confer resistance. For instance, transgenic tobacco expressing 3′-NTR of *Andean potato mottle virus* (APMoV), which was expressed with a smaller portion of capsid protein-coding region, showed excellent resistance in several lines (Vaskin et al. 2001). Transgenic oilseed rape plant expressing 3′-NTR of *Turnip yellow mosaic virus* (Zaccomer et al. 1993) and intergenic region of PLRV expressing transgenic potato (Dong et al. 1999) showed resistance to respective pathogens.

Non-translatable Sense RNAs Non-translatable sense RNAs of coat protein created by frameshift mutation have been widely used to confer resistance to a large number of crops, including tobacco, rice, papaya, peanut, grape, sugar cane, etc. Expression of frameshifted, capsid protein coding sequences of PVY-NTN in transgenic potato was shown to be resistant to PVY^{NTN} infection (Rachman et al. 2001). There are some other viral proteins which can be suitable candidates to be manipulated in similar manner to engineer resistance. Potential of non-translatable coding sequences of movement protein, nucleoprotein, Vpg, NSm, etc. to mediate viral gene silencing has been proved through different experiments. Expression of non-translatable form of the capsid protein gene has been conferred resistance to *Papaya leaf-distortion mosaic virus* (PLDMV) (Kung et al. 2010), *Zucchini yellow mosaic virus* (ZYMV) and PRSV-W (Wu et al. 2010; Yu et al. 2011) and *Sugarcane yellow leaf virus* (Zhu et al. 2011). Transgenic tobacco expressing a non-translatable form of the NSM coding region of TSWV and a non-translatable form of the TEV 6-kDa/VPg encoding region exhibited resistance towards TSWV and TEV, respectively (Prins et al. 1996; 1997; Swaney et al. 1995). Moreover, hybrid constructs, designed by a fusion of non-translatable coding sequence of coat protein of different viruses, have found to be efficient for developing broad-spectrum resistance.

Antisense RNAs Transformation of host plant with antisense RNAs of certain viral genes is a powerful strategy to silence invading viral genes. Transgenic plants expressing sense RNAs for viral replicase, transcription activator protein (TrAP),

replication enhancer protein (Ren), AV1, etc. had been developed. Antisense RNA-mediated silencing mostly resulted in attenuated or delayed symptoms and complete resistance in some cases. Trials to engineer resistance to *Tomato leaf curl virus* (Praveen et al. 2005), *African cassava mosaic virus* (Zhang et al. 2005) and *Mung bean yellow mosaic India virus* (Singh et al. 2013) have been performed.

Satellite RNA Some viruses exhibit a supernumerary RNA component that does not show any apparent homology to the viral RNA genome but depends on its helper virus for replication, encapsidation and transmission and is defined as a satellite RNA (Simon et al. 2004). These sequences have found to have a highly variable range of effects on various components like viral replication, pathogenesis and symptom expression in certain host-pathogen interactions. Sat variants with attenuating effects are regarded as potential biocontrol agents in transgenic plants. Transgenic expression of an attenuating CMV-associated satRNA suppressed viral replication and symptom development and thereby conferred tolerance to CMV in tobacco and other solanaceous plants including tomato (Baulcombe et al. 1986; Harrison et al. 1987; Kim et al. 1997; Kim et al. 1995)). Transgenic *N. benthamiana* and *A. thaliana* plants expressing *Bamboo mosaic virus* (BaMV) satRNA showed high resistance to helper virus (Lin et al. 2013).

Defective Interfering DNAs and RNAs Defective DNAs and RNAs are produced during the replication of certain viral species, which can reduce the full-length genome accumulation and results in the denomination of DI nucleic acids, associated with the modulation of symptom expression, and thus serves as a source for transgenic resistance strategies. ssDNA geminiviruses, like ACMV, and ssRNA *Tombusvirus*, like *Cymbidium ringspot virus* (CymRSV), are known to form DIs. Interfered viral replication and milder symptoms were observed in transgenic *N. benthamiana* expressing naturally occurring subgenomic ACMV DNA B upon ACMV infection (Stanley et al. 1990). Tolerance to other viral species, including *Beet curly top virus* (BCTV) (Frischmuth and Stanley 1998; Stenger 1994), *Cucumber necrosis virus* (CNV), *Carnation Italian ringspot virus* and CymRSV (Kollàr et al. 1993; Rubio et al. 1999), has been obtained by employing DI DNA and DI RNA.

Silencing by Ds Inverted Repeat Sequence/hpRNA Combined expression of sense and antisense transcripts in the same transgenic plants is advantageous for obtaining more transgenic lines exhibiting resistance. It is more efficient than the expression of either strand alone (Waterhouse et al. 1998; Smith et al. 2000; Waterhouse and Helliwell 2003). This was made possible by designing a single transcript of both polarities, with sense and antisense sequences separated by introns or other spacer sequences, to generate dsRNAs with loops, known as hpRNAs, intron hpRNAs (ihpRNAs) or irRNAs, which stabilize the inverted repeat DNA sequences in *Escherichia coli* (Smith et al. 2000; Wesley et al. 2001). dsRNA thus produced is then acted upon by siRNA pathway, resulting in virus resistance (Castel and Martienssen 2013; Csorba et al. 2009; Ding 2010; Eames et al. 2008; Pumplin

and Voinnet 2013). Broad-spectrum resistance can be achieved by creating a chimeric, fused hpRNAs expressing sequences of several viruses from the same vector (Cillo and Palukaitis 2014). The application of hpRNAs as a transgenic resistance strategy was first demonstrated in transgenic tobacco and barley expressing a hpRNA specifically targeting NIa protease coding sequences of PVY and polymerase coding sequences of BYDV, respectively (Smith et al. 2000, Abbott et al. 2000). Development of viral resistance in crop plants was tremendously advantaged from the establishment of simple and efficient technique for stable integration of self-complementary hairpin construct, designed specifically for the cognate RNA target. dsRNA and siRNA are generated in host cell and silence the pathogen gene expression by cleaving target RNA. The major crop plants, like rice, maize, citrus, cassava, legumes, etc., have been conferred durable and efficient resistance using this method (Cillo and Palukaitis 2014). Although RNAi confers efficient and durable resistance, plant viruses have evolved smart measures, like suppressors of RNA-induced gene silencing, to escape RNAi which is a challenge before the advancements of transgenic resistance (Table 23.3).

Silencing by Artificial MicroRNAs MicroRNAs are small (20–250 nt), non-coding RNAs present in eukaryotic cells and facilitate gene regulation at post-transcriptional or translational level (Bartel 2004). The precursor miRNA transcripts are processed into mature miRNA and directed to target mRNA by the same mechanism involving DICER, Ago and RISC, as that of siRNAs (Jones-Rhoades et al. 2006). Customized miRNA precursors producing target-specific siRNAs, which are having no similarity with mature endogenous miRNAs, known as artificial microRNAs (amiRNAs), can be transgenically expressed to target invading viral sequences. amiRNA-mediated virus resistance was first reported in transgenic *Arabidopsis* showing resistance against *Turnip yellow mosaic virus* (TYMV) and *Turnip mosaic virus* (TuMV) by stable expression of amiRNAs targeting the RNA sequences encoding silencing suppressors P69 and HC-Pro of the virus (Niu et al. 2006). amiRNAs targeting RNA silencing suppressors of virus have found to be more efficient in conferring resistance either complete resistance or delayed infection/susceptibility, when compared to those targeting CP or other structural proteins (van Vu et al. 2013). Silencing suppressors Hc-Pro and TGB1/p25 of PVY and PVX, respectively, were targeted efficiently by amiRNAs (Ai et al. 2011). amiRNAs have been demonstrated to confer resistance against a vast range of viruses, including positive-sense ssRNA genome such as CMV, PVY and PVX; negative-sense ssRNA viruses like WSMoV (genus *Tospovirus*); and ssDNA viruses of the genus *Begomovirus* like *ToLCV New Delhi* variant (van Vu et al. 2013) and *Cotton leaf curl Burewala virus* (Ali et al. 2013). Efficiency of amiRNA-induced viral gene silencing is determined by several factors, like expression levels of pre-miRNA backbone and sequence complementarity of amiRNA with target and structural features of target RNA and accessibility of amiRNA to target RNA (Ali et al. 2013; Cillo and Palukaitis 2014; Simón-Mateo and García 2011; Duan et al. 2008). Viruses may quickly evolve new strains with mutations in amiRNA target site and escape silencing, which can be circumvented by simultaneous targeting of

multiple regions of highly conserved RNA motifs of viral genome with multiple amiRNAs. A polycistronic amiRNA designed from a modified rice miRNA395, targeting different conserved regions of the WSMV, conferred resistance to WSMV in wheat (Fahim et al. 2012).

Silencing by Co-suppression Resistance to virus can be achieved by activating co-suppression by introducing surplus amount of viral transcripts in the host, where overabundance of sense strand induces downregulation or suppression of transgene and invaded viral gene. Such overabundance of sense strand leads to the removal of all homologous transcripts, beyond a critical threshold, by respective cellular machineries (Stam et al. 1997). Overexpressed transcripts, when recognized by plant RNA-dependent RNA polymerase, act as primers for dsRNA synthesis, which then subjected to cleavage and degradation by DICER and RISC complex (Dougherty and Parks 1995).

23.2.8 Transgenic Technology to Engineer Pathogen-Targeted Virus Resistance

PDR and R gene often do not confer complete resistance to invading viruses, and mostly resistance is not stable over several generations. Pathogen-targeted resistance is manifested by introducing silencing constructs into host plants, where it targets viral genome. Synthetic constructs designed to target viral sequences are neither of plant origin nor pathogen-derived. Such non-viral-mediated resistance is manifested by transgenic expression of synthetic nucleases like zinc finger nucleases, transcription activator like effector nucleases, CRISPR/Cas9, antiviral inhibitor proteins like ribosome-inhibiting proteins, peptide aptamers and plantibodies (Bastet et al. 2018).

Nucleases Resistance against *Fijivirus* RBSDV and TMV was conferred by transgenic expression of an *E. coli* dsRNase gene (Cao et al. 2013), bovine pancreatic RNase (Trifonova et al. 2007) and an inducible extracellular RNase from *Zinnia elegans* (Trifonova et al. 2012). *E. coli* dsRNase (RNase III) conferred high-level resistance to a tomato isolate of TSWV also (Langenberg et al. 1997). Feasibility of transgenic expression of antiviral pathways or invading nucleic acid targeting pathways from heterologous systems in host plant for targeting infectious viruses has been demonstrated using OAS system 2,5A-oligoadenylate synthetase (OAS)/ RNase L system, which is also called as 2,5A oligoadenylate pathway, an animal antiviral pathway induced by interferons in mammalian cells. The OAS catalyses the polymerization of ATP producing 2-5-linked oligoadenylates, pppA (2_p5_A) nor 2,5A, when it recognizes a dsRNA, which can be replicative intermediates of single-stranded RNA viruses or viral dsRNA genomes. The subsequent activation of the latent ribonuclease, RNAse L, by the 2,5A oligonucleotides produced by OAS, carries out the degradation of both viral and cellular RNAs, thereby hampering the viral replication and infection (Floyd-Smith et al. 1981). The efficacy of this RNA targeting mechanism to confer broad-spectrum resistance has been demonstrated in

tobacco plants (Mitra et al. 1996). Resistance to several DNA and RNA viruses has been engineered employing ZFNs, TALENs and CRISPR/Cas9. Artificial TALE proteins and FokI nuclease expressing *Nicotiana benthamiana* plants showed resistance to different *Begomoviruses*. Transgenic expression of yeast-derived dsRNase, Pac1, confer broad-spectrum resistance to phytopathogenic viruses. Pac1 has been successfully used to confer resistance to CMV, *Tomato mosaic virus* (ToMV) and PVY in tobacco (Watanabe et al. 1995) and PSTVd in potato (Sano et al. 1997).

Ribosome-Inactivating Proteins Ribosome-inhibiting proteins or ribosome-inactivating proteins found in certain plant species possess anti-viral activities. Transgenic expression of RIP from the pokeweed (*Phytolacca americana*) conferred broad-spectrum resistance to virus infection in several plant species, including tobacco, potato and *N. benthamiana* and *Brassica napus* against PVX, PVY and TuMV (Lodge et al. 1993; Zhang et al. 1999), respectively. Transgenic expression of RIP from *Dianthus caryophyllus*, *Trichosanthes kirilowii* and *Iris hollandica* also was observed to provide varying degrees of resistance to ACMV, TMV and CMV and TEV and TMV, respectively. However, any of the RIPs evaluated so far has not found to have an efficient broad-spectrum anti-viral activity.

Peptide Aptamers There are some short-peptide molecules, which can confer low-level broad-spectrum resistance and are advantageous in that the need for producing high level of transgene to induce PTGS can be avoided. Such short-peptide-mediated resistance sometimes provides better resistance than RNAi- and protein-mediated PDR as in the case of transgenic *N. benthamiana* with an introduced target-specific peptide aptamer showing broad-spectrum resistance to *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV) and *Chrysanthemum stem necrosis virus* (CSNV) (Rudolph et al. 2003). Similarly, a conformationally constrained peptide aptamer was found to interfere the replication of *Tomato golden mosaic virus* (TGMV) and *Cabbage leaf curl virus* (CaLCuV) (Lopez-Ochoa et al. 2006). Further investigation of such peptides and understanding of their mode of action enable their application for engineering broad-spectrum resistance.

Plantibodies Antibody-mediated resistance to a plant virus was first demonstrated in transgenic *N. benthamiana* plants expressing an scFv against *Artichoke mottled crinkle virus* (Tavladoraki et al. 1993). Later, transgenic expression of scFv in different crop plants, like Chinese cabbage, citrus, gladiolus, potato and tomato, conferred them with virus resistance. Initial experiments resulted in delayed or reduced disease symptoms only, and complete resistance was not observed. Improvements in plantibody targeting and stabilization and development of non-structural viral proteins targeting plantibodies enhanced the level of resistance provided by them (Gargouri-Bouzid et al. 2006; Nickel et al. 2008, Boonrod et al. 2004; Gil et al. 2011). Transgenic *N. benthamiana* expressing scFvs targeted against the TBSV replicase showed broad-spectrum resistance to TBSV, two members of

family *Tombusviridae* (CNV and TCV) and *Dianthovirus Red clover necrotic mosaic virus* (Boonrod et al. 2004).

23.3 Engineering Resistance to Vectors

Many pathogens, particularly viruses, spread by different insect vectors, mostly hemipterans, aphids, dipterans, etc. Efficient vector control strategies significantly reduce the infection and crop damage. In certain cases, a single vector may harbour multiple pathogens, which are infecting same or different species. Transgenic strategies for vector control can be implemented by taking cues from vector-pathogen–host interactions and underlying genetic, molecular and biochemical processes. Persistent, semi-persistent and non-persistent vectors differ highly in their feeding nature, subcellular localization of virus in them and the status of virus in vector, whether replicating or non-replicative, circulating (Jones 2014; Brault et al. 2010). Viruses themselves influences host interaction with vectors and environment and modulate host immune response accordingly to regulate the feeding pattern and preference in order to manage the acquisition as well as injection of pathogens from viruliferous and non-viruliferous plants (Ingwell et al. 2012; Stafford et al. 2011). All these are accomplished by regulating host defence signalling hubs, manipulation of plant biochemistry for enhanced vector performance and virus-mediated suppression of plant immunity. Suppression of JA- and SA-mediated immune response to reduce insect repellents signals, i.e. different volatile compounds is a typical example for how pests reprogramme plant immunity for enhancing infection (Westwood et al. 2013). Interestingly, such negative regulation of defence-related signalling can be manipulated transgenically to reverse the effects so that resistance to both pests and pathogens can be achieved.

Understanding of involvement of semiochemicals and subsequent and signal generation and transduction can give an insight into potential semiochemical targets that can be modified transgenically to control vectors. Transgenic plants constitutively emitting the semiochemical (E)-b-farnesene, an aphid alarm pheromone and predator attractant, showed repulsion to aphid species and attraction to aphid-parasitizing wasps under controlled conditions (Bruce et al. 2015). However, the metabolically engineered plants showed no differences in aphid infestation or presence of natural enemies in field. Stimulated expression or mixed expression of different semiochemicals can be an alternative strategy to effectively control vectors.

Introgression of R genes conferring vector resistance, like the melon Vat (‘virus aphid transmission’) gene and the tomato Mi-1.2 gene, is useful for controlling vectors. Vat confers effective resistance to multiple viruses but only when transmitted by specific aphid species (Goggin et al. 2006), whereas Mi-1.2 provide resistance to the nematode *Meloidogyne incognita* and many phloem-feeding insects, including *B. tabaci* (Guo et al. 2016; Peng et al. 2016). As the vectors that are identified by NB-LRR protein domains of plants are limited, modifying these receptors in order to expand the range of defendable vectors using transgenic methods can be a promising strategy (Harris et al. 2013; Kim et al. 2016). The vector genes/transcripts encoding

effectors can be targeted by transgenic expression of cognate RNA molecules in host, which is taken up by feeding invertebrates and silences the respective target. Fusion protein consisting of luteoviral coat protein–spider toxin was expressed in host, where viral coat protein moiety facilitates the uptake of toxin by vector (Whitfield and Rotenberg 2015; Bonning et al. 2014). Disruption of transmission of three groups of vectors – whiteflies, aphids and mealy bugs – was obtained by phloem-specific expression of a spider-derived toxin and a lectin (Javaid et al. 2016). Engineering plant as vectors of viruses, which are pathogenic on aphids and leafhoppers, including *Densoviruses* (DNA) and *Dicistroviruses* (positive-sense RNA), is a potential strategy, where host plants act as biocontrol agents. It was tested using cricket paralysing virus, and it disrupted normal aphid responses to olfactory cues from other aphids, causing them to scatter and predisposing them to attack by predators and parasitoids or promote transition to winged morphs, enhancing virus dissemination (Kerr et al. 2015; Ban et al. 2008; Ryabov et al. 2009).

23.4 Transgenic Approach for Resistance Against Viroids

Viroids are small (~250 to 400 nt) non-protein-coding, circular, single-stranded RNAs, which autonomously replicate through an RNA–RNA rolling-circle mechanism that is catalysed by host enzymes (RNA polymerases, RNases and RNA ligases) in the nucleus (*Pospiviroidae*), or in plastids, primarily chloroplasts (*Avsunviroidae*). These small infectious nucleic acids can infect economically important higher plants potato, tomato, citrus, hop and temperate fruits, like peach, apple and pear. Systemic infection of viroids involves entry into specific subcellular organelles, replication, exit of the organelles and cell-to-cell trafficking, access into and long-distance trafficking within the vasculature and exit and invasion of non-vascular tissue to restart the cycle. Interaction of the viroid RNA with cellular proteins involved in its replication/trafficking or from defensive responses is triggered by the host results in infection and disease development (Navarro et al. 2012; Flores et al. 2005). As conventional control measures sound insufficient to offer complete protection against these pathogens, transgenic approaches, like targeting the viroid RNA for degradation or manipulating the host defensive response in order to disrupt host–viroid interactions, may efficiently alleviate the disease symptoms. A peculiar resistance mechanism, namely, cross-protection, wherein a previous infection from mild strain induces development of resistance against further infection from a severely virulent strain, operates against viroids. The underlying molecular mechanism of cross-protection can be sequence specific, which may be related to RNA silencing (Niblett et al. 1978; Khoury et al. 1988; Flores et al. 2005).

As viroid replicates via dsRNA intermediates, yeast-derived dsRNA-specific RNase pac1 has been engineered to target this dsRNA of PSTVd in potato, which resulted in reduced infection and symptoms. Such RNAi approach can confer additional resistance to some RNA viruses as well. These pac1 expressing transgenic potato lines showed resistance to *Tomato spotted wilt virus* also (Flores et al. 2017). Expression of a catalytic single-chain variable antibody (3D8 scFv) with intrinsic

RNase (and DNase) activities, in chrysanthemum plants, resulted in resistance to CSVd infection. This strategy is advantageous in that antibody accumulates to very low levels and antibody expression does not induce any phenotypic alteration, even though it acts in sequence-independent manner, and protects plants not only against CSVd but also confers resistance to DNA and RNA viruses (Lee et al. 2013; Tran et al. 2016). Different trials for antisense RNA-mediated silencing of viroid genomic RNA couldn't establish a stable resistance strategy based on RNAi. Even so, CSVd resistance was conferred by *Agrobacterium*-mediated transformation of a commercial chrysanthemum cultivar with four different constructs carrying sense or antisense CSVd-specific RNAs of 75–82 nt (Matoušek et al. 1994; Jo et al. 2015). Although initial attempts to engineer hammerhead ribozymes to target the plus and minus strands of CEVd did not confer viroid resistance in vivo, hammerhead ribozyme with shorter recognition sequences (9–11 bases) engineered to target PSTVd minus RNA suppressed viroid accumulation in transgenic potato lines (Yang et al. 1997). Tertiary stabilizing motifs (TSMs) of hammerhead ribozymes is suspected to play a critical role in their potential to cleave specific RNA in trans, and this was substantiated when hammerhead ribozymes, with their tertiary stabilizing motifs (TSMs) preserved, engineered to target PSTVd minus RNA successfully interfered with systemic PSTVd infection in in transgenic *N. benthamiana*. Target accessibility of the substrate and the subcellular co-localization of ribozyme and substrate are major hurdles to extract the complete potential of these small RNAs for crop protection (Flores et al. 2017). RNA interference strategy for targeting viral genome can be applied to viroids also. Transgenic tomato and *N. benthamiana* expressing hpRNA construct with near full-length (340-nt) and specific truncated sequences of PSTVD, respectively, showed resistance to this viroid. As RISC is active in cytoplasm, it may not interfere with viroid replication and accumulation host in nucleus but can be targeted while they move to cytoplasm for invasion into neighbouring cells (Schwind et al. 2009). Overexpression of AGO also has found to attenuate viroid accumulation (Minoia et al. 2014). Artificial small RNAs, artificial miRNAs (amiR-NAs) and synthetic trans-acting siRNAs (syn-tasiRNAs) are another efficient transgenic strategy for controlling viroids, when stably expressed in transgenic plants (Carbonell et al. 2014). Concurrent application of more than one strategy might have synergistic effects, resulting in enhanced resistance to viroids.

23.5 Transgenic Technology for Resistance Against Fungi

Fungal pathogens cause several diseases in plants resulting in catastrophic effects on crop yield (10% of global crop loss). Conventional control strategies include the application of chemical fungicides and biocontrol agents, phytosanitation, crop rotation, destruction of intermediate hosts, etc., which are inadequate to provide complete protection from infection. Transgenic strategies can be adopted to confer/enhance the resistance to fungal pathogens so that yield loss and disease management expenses can be considerably reduced. Several plants naturally produce

antifungal compounds, and genes encoding these compounds or regulating their biosynthesis can be manipulated to confer fungal resistance. Prime plant-derived antifungal compounds are chitinase and glucanases, enzymes that degrade major components of fungal cell wall, chitin and glucan, thereby imparting resistance (Silva et al. 2018, 2019). Stacking of these two genes can yield enhanced resistance against fungal pathogens. Transgenic carrot expressing chitinase and β1,3-glucanase, together with AP24 gene, exhibited broad-spectrum resistance against fungal pathogens (Ram and Mohandas 2003). Transgenic grape and cotton plants constitutively expressing chitinase from *Trichoderma* species conferred resistance to several fungal diseases (Rubio et al. 2015; Emani et al. 2003). Plant-derived inhibitors of microbial cell wall degrading enzymes are other potential candidates for engineering resistance. Transgenic expression of inhibitors of cell wall degrading enzymes has conferred resistance against many dreaded fungal pathogens in crop plants. Reduced susceptibility to necrotrophic fungus *Botrytis cinerea* has been observed in PGIP overexpressing transgenic tomato, grapevine, tobacco and *Arabidopsis thaliana* (Ferrari et al. 2012; Liu et al. 2017; Manfredini et al. 2005). PGIP degrades microbial polygalacturonases and thereby delays plant cell pectin hydrolysis, which in turn restricts fungal infection (Ferrari et al. 2013). Xylanase inhibitor protein controls the xylanases, which degrades the main component of cell wall, xylan, as in the case of effective inhibition and counteraction of the *F. graminearum* necrotic xylanase activity by constitutive expression of *Triticum aestivum* xylanase inhibitor III (TAXI-III) in GM wheat (Tundo et al. 2016). Expression of pectin methyl esterase (PME) inhibitors in *Arabidopsis* could prevent damage to the plant cell wall during *Botrytis cinerea* infection (Lionetti et al. 2017). Transgenic strategies to control insect pests, including cry genes from *Bacillus thuringiensis*, provide dual protection by reducing the chances of fungal infection and mycotoxin (fumonisin, *Fusarium* spp.; aflatoxin ergotoxine, *Claviceps* spp.; aflatoxins, *Aspergillus* spp.) contamination by reducing the rate of insect wounding. This would reduce management costs and yield loss considerably. Overexpression of PnAMP-h2 gene from *Pharbitis nil* and barley chitinase (chi-2) genes were found to provide enhanced resistance to fungal pathogens. Antimicrobial peptides derived from non-plant, non-phytopathogenic microbes can also be used for imparting disease resistance as in the case of enhanced resistance to *R. solani* and soil-borne pathogen *A. alternata* observed in transgenic cotton and tobacco plants expressing endochitinase gene from a mycoparasitic fungus *Trichoderma virens*. Phytoalexin capsidiol provided resistance to the potato blight pathogen, *Phytophthora infestans* (*P. infestans*) in pepper (*Capsicum* spp.) (Lee et al. 2017) (Table 23.4).

Defensins are cysteine-rich antimicrobial peptide of plant origin, which is a potential candidate to protect crop plants with phytopathogenic fungal infection by their transgenic expression in plants. Constitutive expression of an alfalfa seed defensin MsDef1 and NmDef02 defensin in potato provided strong resistance to *Verticillium dahliae* and *Phytophthora infestans*, respectively (Gao et al. 2000). Constitutive expression of defensins mostly causes undesirable side effects, including reduced growth and yield performance (Chen and Chen 2002). Regulation of defensin expression by using (native or heterologous) pathogen-induced promoter

Table 23.4 List of transgenic plants developed for resistance to fungal pathogens

Plant species	Antifungal genes transferred	Resistance against	References
Rice	Chitinase (chi11); glucanase (gluc)	*Rhizoctonia solani*	Sridevi et al. (2008)
	stress-inducible b-glucanase (Gns1)	*Magnaporthe grisea*	Nishizawa et al. (2003)
	Ribosome-inactivating protein (MOD1) Chitinase (RCH10)	*Rhizoctonia solani*	Kim et al. (2003)
	Chitinases (RCH10 and RAC22); glucanase (b-Glu); ribosome-inactivating protein (B-RIP)	*Magnaporthe grisea*	Zhu et al. (2007)
	ER-CecA; Ap-CecA (cecropin A)	*Magnaporthe grisea*	Coca et al. (2006)
	Dahlia merckii defensin DM-AMP1*)*	*Magnaporthe oryzae* and *Rhizoctonia solani*	Jha et al. (2009)
Potato	Chitinase (ChiC)	*Alternaria solani*	Khan et al. (2008)
	Chitinase (CHIT); glucanase (GLUC)	*Rhizoctonia solani*	Moravcikova et al. (2007)
			Moravcikova et al. (2004)
	Chitinase (BjCHI1); glucanase (HbGLU)	*Rhizoctonia solani*	Chye et al. (2005)
	Cationic peptide (msrA3)	*Phytophthora infestans* *Phytophthora erythroseptica* *Erwinia carotovora*	Osusky et al. (2004)
	RPI-BLB2	*Phytophthora infestans*	van der Vossen et al. (2005)
Tomato	Glucanase (GLU); antifungal protein (alfAFP); glucanase (GLU-AFP)	*Ralstonia solanacearum*	Chen et al. (2006)
Carrot	Chitinase383; glucanase638; cationic peroxidise (POC1)	*Botrytis cinerea* and *Sclerotinia sclerotiorum*	Wally et al. (2009)
Taro	Chitinase (ricchi11)	*Sclerotium rolfsii*	He et al. (2008)
Cucumber	Chitinase (RCC2)	*Botryti cinerea*	Kishimoto et al. (2002)
Nicotiana benthamiana	Polygalacturonase-inhibiting protein from grapevine	*Botrytis cinerea, Bipolaris sorokiniana*	Joubert et al. (2007)
Tobacco	Polygalacturonase-inhibiting protein (PGIPs) (pepper)	*Phytophthora capsici*	Wang et al. (2013)

(continued)

Table 23.4 (continued)

Plant species	Antifungal genes transferred	Resistance against	References
Alfalfa	*RCT1* from (*Medicago truncatula)*	*Colletotrichum trifolii*	Yang et al. (2008)
Potato	RPI-BLB2 (*Solanum bulbocastanum)*	*Phytophthora infestans*	Van der Vossen et al. (2005)
Wheat	*Fusarium Tri101*	*F. graminearum*	Okubara et al. (2002)
Peanut	Chitinase	*Cercospora arachidicola*, *Aspergillus flavus*	Prasad et al. (2013)

like barley GER4c promoter or tissue-specific promoter, which mainly depends on the infection biology and tissues affected, would be advantageous to avoid the negative fitness effects associated with their constitutive expression (Himmelbach et al. 2010; Rushton et al. 2002). Expression of antifungal defensins under root-specific promoter confers resistance to sudden death syndrome in soybean, because it is caused by a root-colonizing pathogen *Fusarium virguliforme*. Similarly, lifestyle-specific expression of defensins by fine-tuning their subcellular localization, which in turn depends upon whether pathogen is biotrophic or hemibiotrophic, is also effective to confer durable resistance without compromising yield. Extracellularly targeted defensins enable protection from biotrophic fungi, whereas extra- and intracellularly targeted defensins should be coexpressed in order to confer resistance to hemibiotroph. However, the effect of transgene encoded antimicrobial compound on animal/mammalian system should be assessed before employing this strategy for transforming edible crops. A detailed understanding of the underlying molecular mechanisms of their mode of action would permit the development of much sophisticated strategies for the regulated expression of antifungal defensins to confer broad-spectrum durable resistance against many notorious fungal pathogens (Kaur et al. 2011). Synergistic effects by coexpression of different plant defensins with different mode of action or plant defensin with antifungal pathogenesis-related protein (PR) or HIGS with plant defensins enhance resistance to a wide range of fungal infection (Chen et al. 2009; Ntui et al. 2011).

Silencing of pathogen genes by RNAi-based host-induced gene silencing (HIGS) is another efficient transgenic strategy to develop resistance. Resistance to *Blumeria graminis* and *F. verticillioides* was achieved by HIGs of their endogenous gene and fungal transgene, respectively (Nowara et al. 2010; Tinoco et al. 2010).

Plant resistance genes have extensively used for engineering resistance against phytopathogenic fungi. Introgression of R genes from related or unrelated species is facilitated by transgenic technologies. Overexpression of NPR1, a key regulator of SAR, conferred resistance to blight and blast disease causing fungal and bacterial pathogens, respectively (Chern et al. 2005; Yuan et al. 2007). Transgenic rice plants overexpressing AtNPR1 gene and translational suppressor uORFs from the TBF1 gene displayed resistance towards bacterial and fungal pathogens and reduced negative fitness costs associated with constitutive expression of NPR (Xu et al.

2017). In another case, introgression of the gene involved in non-host resistance, *Phytophthora soaje* susceptible 1 (AtPSS1), from *Arabidopsis* into soybean by transformation enhanced resistance to the soybean host fungal pathogen *Fusarium virguliforme*, by a suspected autophagy mechanism without yield penalty (Wang et al. 2018). Transgenic expression of R gene RPI-BLB2 from *Solanum bulbocastanum* in cultivated potato showed resistance to *Phytophthora infestans* (van der Vossen et al. 2005). Resistance conferred by single R gene is mostly not/less durable as pathogen is smart enough to circumvent the resistance. Stacking of multiple R genes, either targeting single or different pathogens, in single plant is an efficient alternative to impart durable and/or broad-spectrum resistance against destructive fungal pathogens. Robust transgenic strategies for gene pyramiding are required for executing this. Host genes manipulated by fungus for apressoria formation and haustoria development can be a potential target to be modified in order to confer pre-haustorial resistance. *Arabidopsis* PENETRATION genes involved in haustoria establishment is a suitable candidate in that regard (Fonseca and Mysore 2019). Integrated transgenic approach combining resistance genes, defensins and antifungal proteins is expected to confer effective durable resistance.

23.6 Transgenic Technology for Bacterial Resistance in Crop Plants

Bacteria are causing many diseases in economically important plants, where it severely impairs the growth, development and yield potential of plants. Plants defend bacterial pathogens with common components of immunity, viz. R genes, ETI-associated HR, antimicrobial compounds, inhabitants of bacterial enzymes, etc. Transgenic approaches for engineering resistance to bacteria in plants can be executed in the following ways: introduction of host R genes and bacterial avirulence genes, incorporation of pathogen-derived genes for resistance to bacterial phytotoxins and expression of antibacterial proteins from plants, insects or bacteriophages as bactericidal or bacteriolytic agents (Panopoulos et al. 1996). Transfer of maize R gene RESISTANCE TO XANTHOMONAS ORYZAE 1 (RXO1) to rice conferred enhanced resistance to the rice host pathogen *Xanthomonas oryzae* pv. *oryzicola* causing bacterial streak disease. Similarly, single R gene obtained from pepper was introgressed to tomato in order to control highly destructive bacterial leaf spot (Zhao et al. 2004, 2005; Tai et al. 1999). Unravelling novel candidate receptors or enzymes or structural proteins from bacteria that can be targeted by transgenic methods will have promising results in controlling bacterial diseases (Table 23.5).

Antimicrobial peptides of plant origin as well as those from heterologous non-plant systems are efficient candidates for engineering disease resistance. Plant-derived antimicrobial peptides contain cysteine-rich short amino acid sequence and target the outer membrane structures. Overexpression of pepper antimicrobial peptide-encoding gene CaAMP1 in *Arabidopsis* conferred broad-spectrum resistance to bacterial, fungal and oomycete pathogens (Lee et al. 2008).

Table 23.5 List of transgenic plants developed for resistance to bacterial pathogens

Plant species	Transgene	Target species	Reference
Nicotiana tabacum var. Petit Havana	Magainin analogue, Myp30, MSI-99	*P. syringae* pv. *tabaci*	Li et al. (2001) De Gray et al. (2001)
Tobacco Potato Apple, Poplar	Cecropin B, mutant (SB37, MB39) and synthetic (Shiva-1, D4E1)	*P. syringae* pv. *tabaci* *Erwinia carotovora* subsp. *atroseptica* *E. amylovora* *Agrobacterium tumefaciens* and *Xanthomonas populi*	Huang et al. (1997) Arce et al. (1999) Norelli et al. (1998) Mentag et al. (2003)
Tobacco	LTPs (lipid transfer protein)	*Pseudomonas syringae* pv. *tabaci.*	Sarowar et al. (2009)
Arabidopsis	Plant PRRs (RLPs) *N. benthamiana* (NbCSPR)	*Pseudomonas syringae*	Saur et al. (2016)
Tomato	R proteins (NB-LRR) pepper Bs2	*Xanthomonas perforans*	Horvath et al. (2012)
Tomato	*Pto*	X. campestris pv. vesicatoria	Tang et al. (1999)
	Bs2	X. campestris pv. vesicatoria	Tai et al. (1999)
Tomato (tomato snakin-2)	Plant antimicrobial defence proteins (plant encoded defensins (PR-12), thionins (PR- 13), lipid transfer proteins (PR-14), snakins, cyclotides, knottins and hevein-like proteins	*Clavibacter michiganensis*	Balaji and Smart (2012)
Banana	*Hrap* and *Pflp* (sweet pepper)	*X. campestris* pv. *musacearum*	Tripathi et al. (2010, 2014), Namukwaya et al. (2012)
Rice	*Rxo1*	X. oryzae pv. oryzicola	Zhao et al. (2005)
	Npr1	*X. oryzae pv. oryzae*	Chern et al. (2005)
	NH1	*X. oryzae pv. oryzae*	Yuan et al. (2007)

A significant reduction of fire blight symptoms in pear and partial resistance to bacterial blight in tomato was achieved by expression of lactoferrin, a mammalian glycoprotein (Malnoy et al. 2003). Attacin is another heterologous protein that has been used for conferring resistance against *Erwinia amylovora*, causing fire blight in

transgenic apple (Aldwinckle et al. 2003). Transgenic expression of another protein, pectate lyase 3 (PL3), in potato resulted in increased resistance to *Erwinia* soft rot (Wegener 2002).

Cecropins are antimicrobial amphipathic peptides of 31–39 aa long and interact with bacterial membranes or induce pore formation in membrane. Transgenic tomato expressing high level of cecropin displayed enhanced resistance against bacterial speck disease. An increased resistance to bacterial blight in rice was engineered by targeting cecropin towards intercellular species (Alan et al. 2004; Jan et al. 2010).

Increased expression and accumulation of ROS, hydrolytic enzymes, antimicrobial peptides and proteins in transgenic host plant can enhance resistance, including HR, to bacterial pathogens and avoid the energy and resource loss in inducing immune responses, which in turn enable plant to channelize the saved energy and nutrients/metabolites into growth and development process so that yield is not compromised. Resistance to several bacterial and fungal pathogens has been achieved by transgenic expression of genes encoding cell wall degrading hydrolytic enzymes, defence-related proteins with protease inhibitor activity, etc. (Shin et al. 2008; Senthilkumar et al. 2010).

23.7 Genome Editing Techniques for Engineering Disease Resistance

Genome editing technology, which emerged in the 1990s, enabled targeted mutagenesis of genomic loci of interest by exploiting cell's intrinsic DNA repair pathways. Site-directed nuclease fused with sequence-specific DNA-binding protein domains or RNAs creates double-stranded breaks in target genomic site. DSBs thus formed are subsequently repaired either by error-prone DNA repair pathway non-homologous end joining (NHEJ) or high-fidelity homology-directed repair pathway (HDR). NHEJ usually results in point mutations at the site of DSB, and HDR enables replacement or introduction of a DNA fragment, while a repair template is provided. Mutations thus induced are stably inherited over generations. Genome editing techniques are useful for functional genomic studies as well as for creating or modifying desired phenotype or trait in organism of interest. Meganucleases, zinc finger nucleases (ZFN), TALENs and CRISPR/Cas are the commonly used genome editing tools. ZFNs and TALENS are fusion proteins in which specific DNA-binding proteins domains are fused with endonuclease domain of FokI nuclease, so that protein guides FokI to respective DNA targets. CRISPR/Cas9, in contrast, is an RNA-guided engineered nuclease, where a single guide RNA with 5′ target-specific 20 nt spacer directs Cas nuclease to target DNA. CRISPR/Cas is a part of bacterial adaptive immune system, providing resistance to invading viruses or nucleic acids. There are different classes of CRISPR/Cas systems, of which dsDNA targeting class 2, type II system seen in *Streptococcus pyogenes*, popular as CRISPR/Cas9 is the most common CRISPR system exploited for genome editing. Several variants of CRISPR systems and Cas protein have been discovered, each of which varies in their target recognition and cleavage properties.

Genome editing techniques have been successfully employed to develop resistance against bacterial, fungal and viral pathogens. ZFNs conferred broad-spectrum resistance to various *Begomovirus*es, including *Tomato yellow leaf curl China virus* (TYLCCNV) and *Tobacco curly shoot virus* (TbCSV) by targeting a single site in viral genome (Chen et al. 2014). Modified ZFN called as artificial zinc finger protein (AZP), which lacks the cleavage domain compared to ZFN, has been used to confer resistance to *Beet severe curly top virus* (BSCTV, family *Geminiviridae*) and *Rice tungro bacilliform virus* (RTBV) in *Arabidopsis* by targeting the intergenic region (IR) of BSCTV and blocking viral promoter sequences of RTBV (Ordiz et al. 2010). TALEs have been engineered to confer resistance to TbCSV and TYLCCNV in tobacco (Cheng et al. 2015). Complexity of protein engineering and off-target effects limited the use of these modular protein toolboxes. CRISPR/Cas9, whose genome editing potential was revealed recently only, quickly superseded and emerged as a promising tool for crop improvement with high efficiency, precision, robustness and simple designing strategy.

CRISPR/Cas9 has been widely used for engineering disease resistance in many crop plants by targeting pathogenic as well as host factors. Pathogenic genes that are inevitable for survival and infection cycle are appropriate candidate targets for CRISPR/Cas9-mediated knockout. Adaptive potentials enable pathogens to overcome the dominant R gene-mediated resistance. Thus, susceptibility genes are considered as potential candidates to be targeted in order to develop durable resistance. Disruption of susceptibility genes or S proteins by CRISPR/Cas9-mediated targeted mutagenesis has been successfully demonstrated. The first report of the application of CRISPR/Cas9 for virus resistance came when double-stranded replicative from geminiviral DNA was targeted to confer resistance. Later, this strategy was used to provide resistance to several DNA viruses. Apart from the demonstration for proof of concept of the CRISPR/Cas9 in model plants, highly efficient resistance against different viral, bacterial and fungal pathogens has been obtained in various crops, including major food crops like wheat and rice (Table 23.6, 23.7 and 23.8). For instance, mutagenesis of ERF transcription factor (OsERF922) gene using CRISPR/Cas9 imparted resistance in rice against fungal pathogen *Magnaporthe oryzae* (Wang et al. 2016). Similarly, CRISPR/Cas9-induced mutation in homeoalleles of mildew resistance locus (MLO), encoding a transmembrane protein, and enhanced disease resistance 1 (EDR1), encoding a Raf-like mitogen-activated protein, conferred resistance to powdery mildew in hexaploid wheat (Wang et al. 2014). CRISPR/Cas9-mediated loss-of-function mutation in eukaryotic translation initiation factor eIF4e, which is essential for infection of *Potyviridae* family viruses, conferred resistance against potyvirus (*Zucchini yellow mosaic virus* and *Papaya ringspot mosaic virus-W*) and *Ipomovirus* in *Arabidopsis* and cucumber (*Cucumber vein yellowing virus*) (Chandrasekaran et al. 2016). CRISPR/Cas9-mediated targeted mutagenesis of promoter sequence of susceptibility gene CsLOB1 conferred resistance to bacterial pathogen *Xanthomonas citri* subsp. *citri* in citrus (Peng et al. 2017). This tool has also been shown to be effective to control dsDNA viruses, as in the case of resistance to *Cauliflower mosaic virus* (CaMV) in *Arabidopsis* (Liu et al. 2018).

Table 23.6 Crop plants harnessing CRISPR/Cas-mediated resistance to viruses

Virus/viruses	Plant	Target (viral/host)	Function	Strategy	GM/ transgene-free	References
BSCTV	*N. benthamiana* and *A. thaliana*	IR, CP and rep	RCA mechanism	*Agrobacterium*-mediated transformation of leaves with Cas9/gRNA expression plasmid vectors	GM	Ji et al. (2015)
BeYDV	*N. benthamiana*	LIR and rep/RepA	RCA mechanism	*Agrobacterium*-mediated transformation of leaves with Cas9/gRNA expression plasmid vectors	GM	Baltes et al. (2015)
TYLCV, BCTV and MeMV	*N. benthamiana*	IR, CP, and rep	RCA mechanism	*Agrobacterium*-mediated transformation of leaves with a TRV vector in Cas9 overexpressing plants	GM	Ali et al. (2015)
CLCuKoV, TYLCV 2.3, TYLCSV-Logan, BCTV-Worland, MeMV	*N. benthamiana*	IR, CP, and rep	RCA mechanism	Agrobacterium-mediated transformation of leaves with a TRV vector in Cas9 overexpressing plants	GM	Ali et al. (2016)
TuMV	*A. thaliana*	Host factor eIF(iso)4E	Host factor for RNA virus translation	*Agrobacterium*-mediated transformation with Cas9/ gRNA recombinant plasmid binary vectors (floral dipping)	Transgene-free	Pyott et al. (2016)
CVYV, ZYMV and PRSMV	*Cucumis sativus*	Host factor eIF4E	Host factor for RNA virus translation	*Agrobacterium*-mediated transformation of cut cotyledons (without embryo) with Cas9/gRNA binary vectors	Transgene-free	Chandrasekaran et al. (2016)

(continued)

Table 23.6 (continued)

Virus/viruses	Plant	Target (viral/host)	Function	Strategy	GM/ transgene-free	References
CMV, TMV	*Nicotiana benthamiana* and *Arabidopsis thaliana*	ORF1, 2, 3, CP and 3′UTR	Replication mechanism	*Agrobacterium*-mediated transformation of leaves with FnCas9/gRNA expression binary vectors floral dipping for *Arabidopsis*	GM	Zhang et al. (2018)
RTSV	*Oryza sativa* L. *japonica*	eIF4G	Host factor for RNA virus translation	*Agrobacterium*-mediated transformation of immature embryos with Cas9/gRNA expression plasmid vectors	GM	Macovei et al. (2018)
TYLCV	*Solanum lycopersicum*	CP, rep	RCA mechanism	*Agrobacterium*-mediated transformation of cotyledons with Cas9/gRNA expression vectors	GM	Tashkandi et al. (2018)
TuMV	*Nicotiana benthamiana*	GFP1, GFP2, HC-Pro, CP	Replication mechanism	Agrobacterium-mediated transformation of leaves with a TRV vector in Cas13a overexpressing plants	GM	Aman et al. (2018)
Endogenous *Banana streak virus* (eBSV)	*Musa balbisiana*	ORF 1, 2 and 3 (aspartic protease gene)	Transcription or/and translation/post-translational modification of vital viral proteins	*Agrobacterium* mediated transformation of cell suspension culture with CRISPR/Cas9 construct	GM	Tripathi et al. (2019)

Table 23.7 Crop plants harnessing CRISPR/Cas-mediated resistance to bacterial pathogens

Plant species	Disease and causative bacteria	Target gene	Gene function	Strategy	Reference
Oryza sativa	Bacterial blight (*Xanthomonas oryzae* pv. *oryzae*)	SWEET13	Sucrose transporter gene	*Agrobacterium*-mediated transformation of embryogenic callus with Cas9/gRNA expression plasmid vectors and TALEN	Li et al. (2012), Chen et al. (2012), Zhou et al. (2015)
Citrus paradisi	Citrus canker (*Xanthomonas citri* subspecies *citri*)	LOB1	Susceptibility (*S*) gene promoting pathogen growth and pustule formation	*Agrobacterium*-mediated transformation of epicotyl with Cas9/gRNA expression plasmid vectors	Jia et al. (2016)
Citrus sinensis Osbeck	Citrus canker (*Xanthomonas citri* subspecies *citri*)	LOB1	Susceptibility (*S*) gene promoting pathogen growth and pustule formation	*Agrobacterium*-mediated transformation of epicotyl with Cas9/gRNA expression plasmid vectors	Peng et al. (2017)
Malus domestica	Fire blight (*Erwinia amylovora*)	DIPM-1 DIPM-2 DIPM-4	Susceptibility factor involved in fire blight disease	PEG-mediated protoplast transformation with CRISPR ribonucleoproteins	Malnoy et al. (2016)
Solanum lycopersicum	*Pseudomonas syringae* and *Xanthomonas* spp.	DMR6	Susceptibility (*S*) gene involved in downy mildew disease	*Agrobacterium*-mediated transformation of cotyledons with Cas9/gRNA expression vectors	Thomazella et al. (2016)
	Tomato bacterial speck disease (*Pseudomonas syringae* pv. *tomato* (*Pto*))	JAZ2	Regulation of stomatal opening by coronatine	*Agrobacterium*-mediated transformation of cotyledons with Cas9/gRNA expression vectors	Ortigosa et al. (2018)

Table 23.8 Crop plants harnessing CRISPR/Cas-mediated fungal resistance

Plant species	Disease and target species	Target gene	Gene function	Strategy	Reference
Vitis vinifera	Powdery mildew (*Erysiphe necator*)	MLO-7	Susceptibility (*S*) gene involved in powdery mildew disease	PEG-mediated protoplast transformation with CRISPR ribonucleoproteins	Malnoy et al. (2016)
Oryza sativa L. *japonica*	Rice blast disease (*Magnaporthe oryzae*)	ERF922	Transcription factor implicated in multiple stress responses	*Agrobacterium*-mediated transformation of embryogenic calli with Cas9/gRNA expression binary vectors	Wang et al. (2016)
Oryza sativa L. *japonica*	Rice blast disease (*Magnaporthe oryzae*)	SEC3A	Subunit of the exocyst complex	Protoplast transformation with Cas9/gRNA expression binary vectors	Ma et al. (2018)
Solanum lycopersicum	Powdery mildew (*Oidium neolycopersici*)	MLO1, PMR4	Major responsible for powdery mildew vulnerability	*Agrobacterium*-mediated transformation of cotyledons with Cas9/gRNA expression plasmid vectors	Nekrasov et al. (2017), Koseoglou (2017)
Triticum aestivum	Powdery mildew (*Blumeria graminis* f. sp. *tritici*)	MLO-A1	Susceptibility (*S*) gene involved in powdery mildew disease	Particle bombardment of immature wheat embryos with Cas9/gRNA expression plasmid vectors	Wang et al. (2014)
Theobroma cacao	Black pod disease (*Phytophthora tropicalis*)	NPR3	Regulator of the immune system	*Agrobacterium*-mediated transient transformation of stage C leaves with Cas9/gRNA expression binary vectors	Fister et al. (2018)

Loss-of-function mutations created by CRISPR/Cas9 is useful for identifying resistance and susceptibility-determining genes and corresponding regulatory elements like promoters or enhancers. As the NHEJ-induced mutations are stably inherited and the CRISPR/Cas expression cassettes can be eliminated in successive generations, the resulting plant remains transgene-free and thus overcomes the regulatory issues associated with *Agrobacterium* transformed conventional transgenic plants. Delivery of CRISPR/Cas9 constructs as ribonucleoprotein (RNP) complex composed of sgRNA and Cas9 protein into host plant also yields non-transgenic edited plant with disease resistance. Non-transgenic tomato, resistant to powdery mildew, has been created in this manner (Nekrasov et al. 2017). Multiple DNA sequences can be targeted simultaneously by designing gRNA cassettes using golden gate cloning/tRNA: gRNA/gRNA: ribozyme assembly strategy. The ease of multiplexing is highly beneficial for conferring host with sound resistance against single pathogen as well as with broad-spectrum resistance. Multiple regions of pathogen genome can be targeted to alleviate/reduce the possibilities for the evolution of cleavage or recognition resistant variants by overcoming mutations at single site. Although incorporation of resistance gene from foreign species can also be performed by inducing HDR repair pathway of DSB created, efficiency of such events is very less and still needs to be optimized for obtaining high rate of HDR. Cisgenic applications of genome editing can be useful for enhancing disease resistance in species, which are difficult to hybridize by introducing a desirable gene fragment from its natural gene pool using CRISPR/Cas9-induced HDR-based pathway. Genetic engineering approaches manipulating endogenous genetic variations related to specific traits are preferred to exogenous genomic targeting recombinant DNA technology.

Apart from dsDNA targeting Cas9, there are certain CRISPR systems with RNA targeting properties, which can be engineered to confer resistance to RNA viruses or silencing of viral transcripts in host. Class II, type VI Cas effector proteins, Cas13a (C2c2), Cas13b (C2c6), Cas13c (C2c7), Cas13d and Cas9 from *Francisella novicida* (FnCas9), RNA targeting SpCas9 (RCas9), are the RNA-guided RNA targeting Cas effector variants identified in different microbial genomes so far (Abudayyeh et al. 2017). Expression of Fncas9 and sgRNA targeting conserved 3′ UTR of *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) developed stable immunity in *N. benthamiana* against CMV and/or TMV (Zhang et al. 2018). CRISPR-Cas13a was used to confer resistance to *Turnip mosaic virus* (TuMV) in *N. benthamiana* by targeting Hc-Pro and GFR regions of genome (Aman et al. 2018).

A class II, type V CRISPR/Cpf1 system that efficiently target DNA was discovered from *Prevotella* and *Francisella* species and named as Cas12a (Zetsche et al. 2015; Makarova et al. 2015). Cpf1 from *Acidaminococcus sp. BV3L6* Cpf1 (AsCpf1) and *Lachnospiraceae bacterium* Cpf1 (LbCpf1) are other two promising nucleases that can be used for genome editing purposes (Zetsche et al. 2015).

Gene expression regulation and epigenetic modification is also facilitated by mutant Cas9, which lacks cleavage activity. Transcriptional activators or repressors can be recruited to a particular gene of interest by fusing them with dCas9, which then is directed by specific sgRNA to the target site. Similar strategy can be adopted

for editing epigenetic marks using appropriate epigenetic modifiers (Dominguez et al. 2016). This enable fine-tuning of defence regulatory pathways and metabolic pathways involved in immune response. Transcriptional activation of positive regulators of immunity and biomolecules like pathogenesis-related proteins or hydrolytic enzymes, which are directly involved in defence manifestation, repression of negative regulators of immunity, etc., can be achieved using CRISPR/dCas9 system. Resistance traits that are unexpressed due to epigenetic suppression can be activated by reversing the methylation and similarly epigenetically controlled susceptibility also modified. Tissue and developmental stage or stress status specific activity of CRISPR components and target editing events can be achieved by employing tissue-specific/inducible promoters for the expression of Cas effector and guide RNA. This is particularly beneficial for reducing unwanted or off-target editing.

Genome-wide mining of S genes may unravel novel candidate S genes that can confer resistance, durable as well as broad-spectrum, without having any fitness costs. Several trace elements, like Fe, Ca, Cu, K, Mn and Zn, are required by pathogens to meet their nutritional requirements, whereas the same are required in host plants for nutrition as well as to activate/drive certain critical defence responses like Cu-dependent binding of SA binding to its NPR1 receptor (Yuan et al. 2010). Coordinated activities of plant immune signalling pathways to inhibit/suppress bacterial iron acquisition mechanisms substantiated the role of Fe in establishing infection (Nobori et al. 2018). Pathogens compete with host to acquire these mineral nutrients, which is counteracted by plant, by limiting their availability to invading pathogens by a mechanism called as nutritional immunity. Pathogenic mineral transporters strive to acquire Fe, Mn, Zn, K and Cu, which are essential for their virulence/survival, from the host, leaving host deprived of these trace elements and thereby inhibiting immune responses that require these minerals (Ren et al. 2016; Hood and Skaar 2012). Thus, such trace element transporters or their acquisition mechanisms can be potential nutritional immunity-related S gene targets for genome editing (Zaidi et al. 2018).

Complete knockout of S genes mostly has negative fitness effects, like reduced growth, yield and fertility; early senescence; and reduced tolerance to abiotic stress, as most of them being primary in function. Even though fitness cost is not lethal, it can cause phenotypic abnormalities. Alternative strategies, like introduction of S gene variant, creation of intermediate alleles by promoter targeting, transient knockout of S genes by using pathogen-inducible promoter, etc., using CRISPR/Cas9 would efficiently confer resistance, avoiding any cost of fitness. Identification of S gene allelic variants, which can confer resistance, leads to create resistant S gene variant by specifically editing the respective SNP using CRISPR/Cas9 base editors (Rodríguez-Leal et al. 2017; Yan et al. 2018).

Even though the characteristics, like specificity, efficiency, simplicity, flexibility, etc., of this genome editing tool are highlighted, several shortcomings and complications, including off-target effects, low rate of successful transformation events and recalcitrance for regeneration and establishment of edited plants, are associated with CRISPR/Cas. Each stage of experiment, from the selection of target

sequence to stable integration of mutations, needs to be optimized for each and every species for utilizing maximum potential of this genome editing tool for disease resistance.

23.8 Strategies Based on the Mechanism of Plant–Pathogen Interaction

Plants are constantly subjected to stress from various biotic agents/pathogens, like virus, bacteria, fungi, oomycetes, nematodes, insects and parasitic plants, that are causing serious crop loss. Plant–pathogen interaction studies have revealed various components involved in immunity and their mechanism of action during defence response. Interaction is specific characteristic of each group of pathogens. A vast array of well-programmed defensive mechanisms driven by multiple biomolecules which are involved in each stage, viz. invasion of pathogen into host, recognition and initiation of immune response, active deployment of defensive strategies and establishment of resistance, together constitute plant immunity. Pathogen-derived signals during infection process is perceived by specific receptors, mostly located in the cell membrane, which then trigger a cascade of defence activities/pathways making use of a large spectrum of biomolecules and molecular mechanisms (Silva et al. 2018).

Plant innate immunity has organized as a three-layered system with pathogen-triggered immunity (PTI), effector-triggered susceptibility (ETS) and effector-triggered immunity (ETI). The first active line of plant immunity is PTI, which is triggered when pathogen-associated molecular patterns (PAMP), together with this damage-associated molecular patterns (DAMP) released during pathogen invasion and damage, are recognized by transmembrane pattern recognition receptors (PRRs) (Boutrot and Zipfel 2017; Uma et al. 2011). PTI is suppressed by effector-triggered susceptibility (ETS) induced by pathogen-derived susceptibility proteins/effector and results in infection (Jones and Dangl 2006; Chisholm et al. 2006). This activates the second line of defence, effector-triggered immunity induced by recognition of specific effectors/cognate factors, pathogen avirulence (Avr) proteins by another group of receptors encoded by resistance genes (R). A co-evolutionary gene-to-gene molecular arm race occurs between pathogen effectors and host R genes, while pathogen evolves a new effector to restore the compatible interaction to facilitate infection, parallel to which host evolves a new R protein, to strengthen immunity. PTI is conserved over a range of organisms, whereas ETI specific to Avr protein is produced by each organism. ETI mostly/usually activates localized cell death pathway, otherwise known as hypersensitive response (HR), to restrain infection by transmitting defence signals to neighbouring non-infected cells via plasmodesmata and to other systemic organs via phloem, resulting in distal resistance responses, namely, local acquired resistance (LAR) and systemic acquired resistance (SAR), respectively (Coll et al. 2011; Dangl and Jones 2001). Like HR, ETI responses also involve production of salicylic acid (SA), reactive oxygen species (ROS), necrosis and also structural changes, such as lignification and callose deposition. Various

biomolecules, like pathogenesis-related proteins (PR), antimicrobial peptides (AMP), ribosome-inhibiting proteins (RIPs), defensive secondary metabolites, etc., are produced by plants as a part of defence response (Kachroo and Robin 2013; Dempsey and Klessig 2012). Increasing the cytosolic calcium levels in response to exogeneous signals, like H_2O_2/PAMP/DAMPs, etc., produced during infection is sensed by cellular and membrane calcium receptors, which in turn transduce signals to elicit defence responses, like HR, ROs production, transcriptional regulation of stress responsive genes, etc. (Seybold et al. 2014; Silva et al. 2018).

Incompatible interaction between non-host species and pathogen results in immunity against non-host pathogen in non-host plant. Preinvasive NHR is mostly passive mainly based on different physical and chemical barriers to interfere proliferation and accumulation of pathogen. Sometimes, NHR involves active defence response mediated by incompatible R–Avr gene-to-gene interaction result in ETI, and this shows that common mechanisms converge at some points in NHR and HR (Flor 1971; Glazebrook 2005; Gill et al. 2015). Prolonged interaction between host and pathogen occurs, wherein pathogens are under constant dual selection pressures, for increased resistance from host and on pathogen for increased performance of pathogen, which results in co-evolution of pathogen and host with virulence specificities on specific hosts (Allen et al. 2004). Such co-evolved pathogens become unable to infect phylogenetically unrelated hosts, resulting in non-host resistance, which is highly beneficial to control pathogens. NHR is a quantitative trait with multiple genes and pathways, whereas HR has specific R gene (Senthil-Kumar and Mysore 2013). Based on plant response, NHR is considered to be of two types. Type 1 NHR involves no visible symptom as pathogen fails to penetrate tissues. It is manifested by different physical, chemical and metabolical barriers that block pathogen penetration without activating ETI and PTI. Type II NHR involves some degree of pathogen penetration or entry in plant tissue, not as strong as a susceptible host pathogen infection but in a dose sufficient to trigger HR (cell death), and involves ETI. During post-invasive NHR, some pathogens penetrate host by overcoming PTI responses and involve activation of defence responses, like HR, ROS, cell death or the formation of cell wall appositions in infected cells (Collins et al. 2003; Rojas et al. 2012).

23.9 Enhancing Immunity: Transgenic Approaches to Manipulate Plant Innate Immunity

23.9.1 Upregulation of Defence Pathways

Upregulation of molecules involved in defence regulation, signalling and other allied cellular processes can boost hosts' general immune responses, including ROs production, callose deposition, PR proteins and activation of SAR, etc. Resistance to several microbial (bacterial and fungal) pathogens has been achieved by employing this strategy without introducing new metabolic pathway or new gene but exploiting plant's own immune system. Resistance to *Rhizoctonia solani* and

Magnaporthe oryzae (rice blast causative fungi) has been achieved by expressing a native rice gene under the control of a constitutive promotor from maize (Bundó and Coca 2016; Chen et al. 2016; Vincelli 2016). Genome editing techniques, like CRISPR/cas9-mediated transcriptional regulation, can be a potential strategy to serve this purpose.

23.9.2 Production of Antimicrobial Compounds

Microbial pathogens secrete different cell wall degrading enzymes (CWDE) like cellulases, polygalacturonases, xylanases, xyloglucan endoglucanase, chitinases and protease inhibitors to damage cell wall for making their way into host cells. In order to combat CWDEs, plants initiate cell wall strengthening/damage preventing mechanisms, like production of polygalacturonase-inhibiting proteins (PGIPs), xylanase-inhibiting proteins (XIPs) and xyloglucan-specific endoglucanase-inhibiting proteins (XEGIPs) (Schüttelkopf et al. 2010; Xu et al. 2011). As these mechanisms are mostly evolutionarily conserved, their manipulation could confer durable and broad-spectrum resistance in many crop species. Reduced susceptibility to *Phytophthora capsici* by expressing a pepper PGIP in GM tobacco; resistance to *Verticillium* and *Fusarium* wilts by expressing protein GhPGIP1 in *Arabidopsis* and cotton; resistance to *Phytophthora sojae* in soybean by constitutive expression of elevated levels of a *Glycine max XEGIP* (GmGIP1), an inhibitor of a *Phytophthora sojae,* xyloglucan-specific endoglucanase (PsXEG1); etc. have proved the efficacy of enhancing plant's own immune mechanisms by transgenic strategies to confer resistance (Silva et al. 2013; Liu et al. 2017; Wang et al. 2013; Ma et al. 2015).

23.9.3 Enhancing Plant Recognition of Infection

PTI results from perception of self-derived DAMPs and non-self PAMPs signals by PRR comprising receptor like kinases and receptor like proteases, whose active domains include leucine-rich repeats (LRR), lysine M domain (LysM), epidermal growth factor (EGF) like domain and lectin motif (Wu and Zhou 2013; Silva et al. 2018). Transfer and expression of PRRs in heterologous species by transgenic methods broaden the range of pathogens that can be recognized by the host while plant's own defence system or metabolic pathways are left unmodified. Expansion of pathogen recognition window is of prime importance in field conditions, where multiple infections prevail. Even though certain microbes are highly adapted with species-/race-/strain-specific PAMPs and have specific residues/motifs to block perception by PRRs, most of the PAMPs are conserved across microbial species, and thus individual PRR genes can confer broad-spectrum resistance (Zipfel and Felix 2005). Manipulation of PRRs and other receptors or downstream components involved, from a non-host species or unrelated family, is useful for boosting plant immunity as it will be difficult for pathogen to overcome the resulting new PRR/PAMP recognition system and the downstream signalling pathways.

Resistance to *Xanthomonas citri* in transgenic Hamlin sweet orange expressing NbFLS2 and resistance to *Pseudomonas syringae* in *Arabidopsis* expressing *Nicotiana benthamiana* receptor-like protein are required for Csp22 responsiveness (NbCSPR); NbCSPR exemplifies the effectiveness of transgenic expression of PRRs, even in heterologous system (Hao et al. 2016). Similarly, the transgenic expression of AtEFR in tomato (against *P. syringae* pv. *syringae*, *Ralstonia solanacearum* and *Xanthomonas perforans*), tomato PRR Ve1 in *Arabidopsis* and the *Arabidopsis* PRR AtEFR-Tu in wheat and potato against bacterial wilt (BW) causing soil-borne pathogen *Ralstonia solanacearum* is also expected to confer durable and effective resistance to microbial pathogens (Fradin et al. 2011; Lacombe et al. 2010; Schoonbeek et al. 2015). As most of the PAMPs are conserved and metabolically vital molecules of pathogen, it may not evolve quickly along with transferred PRRs in order to widen the host range.

23.9.4 Targeting Susceptible Gene/Protein

PTI is modulated or suppressed by the binding of pathogen effector proteins to host S proteins and thereby triggering ETS, which facilitate pathogen's invasion and establishment. Plant S genes are responsible for susceptibility to pathogen, ETS establishment and supporting compatibility. Molecular mechanisms associated with S genes are exercised in three levels: basic compatibility or early establishment in preinvasive stage when recognition and invasion occurs, sustained compatibility during which proliferation and spread of pathogen occurs and, finally, negative regulation of immune signals via respective signalling during post-penetration stage (van Schie and Takken 2014; Pavan et al. 2010). S proteins can either be positive regulators of immunity by promoting infection and disease development or negative regulators of immunity, and S gene products either facilitate pathogen's growth in host system or negatively regulate the plant immunity (Silva et al. 2018). Disarming/dysfunctioning, such susceptibility genes, by knockout or loss-of-function mutation is an efficient strategy to develop immunity, and the resulting resistance is mostly recessive and may be associated with pleiotropic effects and negative fitness costs, which may be due to constitutive activation of defence response. Durable and broad-spectrum resistance to powdery mildew in different plant species has been conferred in the same manner, by mutating mildew resistance locus O (MLO). Effector targets in host are activated by pathogenic effectors, which then negatively regulate plant immunity. S gene-mediated resistance can be pathogen specific, if the impaired pathway is inevitable for pathogen in any of the pre-penetration/penetration/post-penetration events, and it can be broad-spectrum, if the target S gene has implications in constitutive defence response. Another point to be noted while S proteins are encoded by multiple S genes is that all of them need to be targeted simultaneously; otherwise, the feasibility of same also should be checked. S gene LOF confers plants with broad-spectrum durable resistance than dominant R gene-mediated resistance as this is race/pathovar/strain non-specific. In order to overcome a modified or silenced susceptibility gene conferring host-

pathogen compatibility, pathogen should acquire a new function to replace the host factor it was exploiting, which is a tough task.

Eukaryotic translation initiation factors eIF4e and eIF4g usually interact with the cap structure of transcripts. Upon *Potyviridae* family virus attack, this interaction is disrupted and initiates interaction with Vpg proteins from virus and facilitates translation of viral proteins in host cell, thereby eIF4e and eIF4g act as negative regulators of plant immunity (Zhang et al. 2006; Callot and Gallois 2014). Similar factor rwm1 has been identified in watermelon under *Cucumber mosaic virus* infection (Ouibrahim et al. 2014). Loss-of-function mutation of such factors confers recessive resistance towards respective pathogens and is advantageous as far as it does not cause any serious pleiotropic effect. Sugar efflux from the host cell is activated by TAL activator from *Xanthomonas oryzae*, by binding to promoter region of SWEET14 gene in order to nourish bacterial pathogen (Yuan and Wang 2013; Boch et al. 2009; Chen et al. 2012; Li et al. 2012). Since this may have an adverse impact on plant health and agronomic performance due to altered sugar flux, modification of binding sites or specific residues of SWEET14 gene render is non-accessible or useless for pathogen. This can be accomplished by CRISPR/ Cas9 genome editing tool.

23.9.5 Mining of R Genes

Overexpression or transfer of resistance genes from other related/unrelated species can confer durable broad-spectrum resistance. This is particularly advantageous in some crop species, where natural R gene is lacking or endogenous R genes fail to confer effective broad-spectrum resistance to most dreaded pathogens. In such cases, resistance genes can be introgressed from distantly related or even from unrelated species by genetic engineering, which in any way is impossible with conventional breeding. Conventional breeding itself is a hurdle in the case of polyploid crops, like potato, grape, banana, apple, etc. In such cases, the useful Cis genes also can be introgressed by genetic engineering, by avoiding major drawbacks of breeding techniques, viz, time consumption and linkage drag (Jones et al. 2014). Resistance to potato late blight has been achieved by this strategy (Jo et al. 2014). Though resistance genes efficiently confer resistance, their durability in heterologous hosts, over the course of time in field conditions, is limited. Since R gene action is strain-/ race-specific, it confers species/subspecies level durable, broad-spectrum resistance, particularly in phylogenetically related hosts by heterologous expression of R genes (Eg: Bs2 gene). GM tomato plants expressing Bs2, an R gene from pepper that recognizes AvrBs2 present in some *Xanthomonas campestris* pathovars, were conferred with resistance against *Xanthomonas perforans* (Horvath et al. 2012). The efficacy of R genes in heterologous systems in conferring disease resistance has been demonstrated by similar transgenic plants. Bs2 gene functions as a solid source of resistance to different *Xanthomonas* species and *X. campestris* pathovars (e.g. pv. *vesicatoria* (*Xcv*)) in multiple solanaceous species. Co-transformation of R genes with cognate Avr gene can be followed by stacking of heterologous R genes, once

the R gene becomes able to overcome phytopathogen. Such interspecific R gene transfer confers host with broad-spectrum resistance in unrelated host as well (Gururani et al. 2012; Horvath et al. 2012). Overexpression of certain post-invasion-induced non-host resistance genes (PINGs) and bright trichomes (BRT1) genes that encode UDP glycosyltransferase 84A2, both of which have been found to be involved in NHR, conferred enhanced resistance against *P. pachyrhizi* in soybean (Langenbach et al. 2016). It has been found that expression of translational repressor domain from the TBF1 gene (uORFs), along with the defence-related gene, can circumvent the negative fitness costs associated with expression of defence-related genes in plants. For example, transgenic rice plants overexpressing the AtNPR1 gene and translational suppressor uORFs from the TBF1 gene displayed the expected resistance phenotype towards bacterial and fungal pathogens and reduced negative fitness costs associated with constitutive expression of NPR (Xu et al. 2017).

The principal functional domains of R genes are Toll/interleukin-1 receptor (TIR), nucleotide-binding site (NBS), leucine-rich repeat (LRR), LRR transmembrane domain (TrD), coiled coil (CC), nuclear localization signal (NLS), mitogen-activated protein kinase (MAPK) and MAPK kinase (MAPKK) domains. R proteins are classified into eight groups based on the arrangement of functional elements, and the major subset among them is LRR-NBS protein (LRR interact to Avr proteins with their highly variable, specificity-determining C- terminus. Mostly, R–Avr interactions occur via intermediate molecules regarded as effector targets. Mutation of host receptors mostly by altering or modifying functional domains by transgenic methods would enhance resistance and can be used in agriculture even though it is quite difficult to achieve broad-spectrum resistance by this strategy due to high R–Avr specificities. Mutation of functional domains will alter binding specificities of R proteins, which in turn strengthen the defence response either by blocking perception of specific pathogen by native R gene or by broadening the range of pathogens that can be recognized by R protein. This strategy handles pathogen recognition mechanisms only, while the rest of the defence response is part of the innate immunity only. *Solanum tuberosum* R3a gene (from the CNL group) confers resistance to the *P. infestans* Avr3aKI isolate but not to the Avr3aEM isolate. *N. benthamiana* expressing R3a* mutant variants showed significantly improved recognition of AVR3aEM (Chapman et al. 2014). Such mutated plant R genes enhance possibilities of recognizing pathogens/races that usually escape original R gene perception and thus confer durable resistance in field.

23.9.6 Modifying Pathogen Effector Targets in Host and Targeting Pathogen Effectors

Pathogenic virulence factors involved in virulence usually bind to certain host targets during their activity. Genetic modification of such host molecules targeted by pathogen can efficiently inhibit infection process. Modification/deletion of binding site residues in host target without compromising its other biological functions

can be an alternative strategy to obstruct infection. Pathogen-derived proteins either sustain pathogen growth in susceptible host or induce HR in resistant host, and some of them have proved to be effective in enhancing plant defence response while expressed in host systems and thus can serve as functional genes for creating resistant GM plants (Silva et al. 2018). Expression of such defence promoting effectors from pathogens may confer resistance in host plants. *N. benthamiana* expressing *P. sojae* CRN effector PsCRN115 was conferred with upregulated defence responses, such as ABC transporters, cytochrome P450 and PRRs, and increased the level of plant resistance to *P. capsici* and *P. parasitica* oomycetes (Zhang et al. 2015).

23.9.7 Silencing Pathogen Genes

RNA interference is a potential strategy to control microbial pathogens of all classes – viral, bacterial, fungal (bio-necrotrophic and oomycetes) and nematode – wherein expression of essential genes of pathogen required for proliferation and establishment of infection is silenced post-transcriptionally by siRNAs complementary to pathogen-derived mRNAs. A prerequisite for RNAi is a double-stranded DNA precursor derived from a replicating viral genome or viral RNA secondary structure, which will be cleaved by DICER-like proteins (ribonuclease III – DCL), and the resulting ssRNA is loaded into silencing complex (RISC complex). siRNA-guided activated complex further recognizes and degrades the target mRNA by Argonaut (Ago) protein in cytosol (Seo et al. 2013; Weiberg et al. 2014). Conserved domains of vital genes related to cellular processes, morphogenesis or pathogenesis can be potential target sites for host-induced gene silencing, which may confer broad-spectrum durable resistance. Similarly, host genes, which are inevitable for the infection process, whose silencing does not have any/have minimal negative fitness costs also, can be targeted. Transgenic plants expressing target-specific siRNAs successfully confer resistance by sequence-specific degradation of target mRNA.

23.9.8 Transgenic Expression of Detoxifying Pathogenic Toxins

Several pathogens produce toxins, which adversely affect various metabolic pathways/processes of host system. The expression of detoxifying enzymes, either native or transgenic, specific to each pathogen under native or heterologous promoter would considerably reduce/alleviate the detrimental effects of such pathogenic toxins. For instance, detoxification of phytotoxin oxalic acid from *Cryphonectria parasitica* causing chestnut blight disease was achieved in American chestnut transformed with wheat gene coding for the production of the degradative enzyme, oxalate oxidase, and the resultant GM chestnut plant showed considerable reduction in chestnut blight disease development (Zhang et al. 2013). Similarly, transgenic

wheat expressing a toxin-degrading enzyme from barley showed resistance to *Fusarium* head blight (Li et al. 2015).

23.9.9 Expression of Antimicrobial Compounds

Introduction of genes encoding antimicrobial compounds into host plant can confer efficient resistance to specific pathogens. Defensins, an antimicrobial peptide of plant origin, chitin-degrading enzyme from other microbial species, is a well-established example for this strategy. Resulting transgenic crops must display pathovar-/strain-/race-specific resistance to a respective pathogen in the field, while exhibiting normal growth and development pattern without compromising the yield and their responses to other biotic as well as abiotic stress stimuli. Spatio-temporally regulated expression of these genes using tissue-/development-specific promoters or pathogen-inducible promoter would be advantageous to minimize the negative fitness effects, if any. The effect of transgene encoded antimicrobial compound on animal/mammalian system should be assessed before employing this strategy for transforming edible crops. The manipulation of bacterial cry genes is an efficient strategy for controlling insect pests and thereby prevents vector-borne diseases (Mayee et al. 2003). A detailed understanding of the underlying molecular mechanisms of their mode of action would permit the development of much sophisticated strategies for the regulated expression of appropriate antimicrobial compounds to confer broad-spectrum durable resistance to crop plants.

23.10 Cisgenesis: Alternative to Transgenics

Cisgenesis is emerging as an efficient alternative strategy of crop improvement, which combines the beneficial aspects of classical breeding and modern biotechnology. In cisgenesis, desirable genes are transferred among crossable species/species capable of sexual hybridization (Schouten et al. 2006). In this method, the cisgene to be transferred is same as that in breeding procedure, and the resulting plant also is the same as a classically bred plant. Thus, cisgenesis eliminates biosafety concerns regarding transgenic plants, thereby overcoming the regulatory issues for field cultivation and marketing. As cisgenesis facilitates precise gene transfer, negative effects of linkage drag that associated with conventional breeding can be avoided. Additionally, cisgenesis is not time-consuming as the conventional breeding. Thus, cisgenesis can be adopted for specifically stacking resistance genes from crossable gene pools.

23.11 Conclusion

Genetic engineering technology has made tremendous contributions in developing disease-resistant crop plants. Transgenic technology has evolved multitude of options for precise alteration of crop genome, even at nucleotide level over a very short time span, and crop improvement programmes for disease resistance have taken advantage of these methods in order to circumvent the prevailing as well as expanding challenges/vulnerabilities posed by pathogenic organisms. From the incorporation of resistance genes or pathogen targeting genes and manipulation of plant's biochemistry for harming pathogens to editing of single bases to disrupt the entire plant–pathogen interaction and thereby disease development, genetic engineering techniques have opened up every possible way for crops to combat pathogens. Sequence databases and functional annotation information are expanding on a daily basis with the establishment of high-throughput sequencing and genotyping platforms. This further unravels novel molecular and genomic targets that can be manipulated for conferring robust – broad-spectrum – durable resistance to all crop plant species.

Despite the availability of transgenic technology for developing crops with desirable resistance traits, field-level cultivation and commercialization of produce are still a major challenge in the case of these plants due to stringent regulations. Although some transgenic crops have been commercialized, many are not going for field trials. Stability of resistant phenotypes without any negative fitness effects under field conditions needs to be ensured. Protocols for transformation and regeneration of farmer-preferred cultivars/local cultivars of crop of interest need to be optimized. Stigma associated with transgenic plants is another major issue that restricts the GE crops in laboratories or experimental glasshouses. Even in the case of engineering resistance, GE strategies, which modify or alter the endogenous immune components rather than those adding/introducing novel/foreign gene or sequence or metabolic pathways, are preferred, when such issues are concerned. Ecological and health concerns regarding possibilities of horizontal gene flow, especially the bacterial marker genes used in the transformation process and the loss of endogenous agrobiodiversity, are the prominent underlying causes of such regulations. The evaluation of transgenic crop products to ensure biosafety should be followed before releasing. For transgene-free editing options opened up by third-generation genome editing tool, CRISPR/Cas is a promising strategy to overcome regulatory barriers associated with transgenic crops. Recently, the definitions of genetically modified plants are getting revised, enabling field-level cultivation of many transgenic disease-resistant crops. However, the innovations that render transgenic technology free of any risks need to be emerged for better public acceptance of genetically modified crops. But lastly, the conventional breeding techniques, which created the large pool of disease-resistant cultivars we had all this time, cannot be replaced completely and are powerful to sustain crop production, despite its shortcomings. Genetic engineering can supplement and renovate at points where breeding techniques are struggling to prevail over and that will lead us to the prime goal, sustainable crop/food production.

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RNAi Technology: A Novel Platform in Crop Protection 24

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Abstract

Since long, plant breeding has been the sole option and traditional method for developing resistant cultivar with gene manipulation against different crop pests. Various strategies have been put forward to render plants resistant to fungi, bacteria, viruses, insects and nematodes. In the recent years, RNA interference (RNAi) has become a highly effective and commanding tool of functional genomics for silencing the gene expression for crop enhancement. RNAi-mediated gene suppression approaches have opened up a new path in the development of eco-friendly biotech approaches for crop improvement by knocking-out the specific genes for better stress tolerance and integrating novel traits in various plant species including insect, pest, pathogen resistance and also enhanced nutritional status. RNAi or RNA silencing is a sequence-specific post-transcriptional gene silencing (PTGS) mechanism induced by double-stranded RNA (dsRNA). DsRNA molecules have been shown to play a key role by protecting plants from invasive nucleic acids. The approach could represent a simple and environmentally safe way for controlling plant pathogens and pests. In this chapter, we review RNAi applications in plants to acquire resistance against biotic stress such as viruses, fungi, bacteria and insect pests.

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24.1 Introduction

India is known as a growing economic giant but the benefits of its progress are mostly restricted to urban or semi-urban areas in India. Modern high input monocropping-based intensive agriculture has resulted in loss of biodiversity, outbreak of pests and diseases and degradation of soil and water, which has ultimately led to stagnation of agricultural production and productivity. Climatic changes and plant health management are becoming major factors in the present scenario (Kumar 2013). Effective control of plant pathogens on economically important crop species is the major challenge for sustainable agricultural production in India. Although plant breeding has been the traditional method of manipulating a plant genome to develop a resistant cultivar for controlling plant diseases, the introduction of genetic engineering technology provides an entirely new approach. Presently, the cultivated area of genetically modified crops that are resistant to diseases is less compared with that of crops for tolerance to herbicide, or resistant to insects. Various strategies have been put forward to render plants resistant to fungi, bacteria, viruses and nematodes. In modern-day biotechnology-based approaches in plant disease management, pathogen-derived resistance is considered as one of the most important approach. RNAi-mediated gene suppression approach has opened new avenues in the development of eco-friendly biotech approaches for crop protection by knocking-out the specific genes. RNA interference (RNAi) technology has appeared to be a promising and efficient technology. The advancement of RNAi as a novel non-transgenic gene therapy against fungal, viral and bacterial infection in plants lies in the fact that it controls gene expression via mRNA degradation, repression of translation and by chromatin remodeling through small non-coding RNAs. RNA silencing mechanisms are guided by processing the products of dsRNA degradation by Dicer-like proteins, which are known as small interfering RNAs (siRNAs) and microRNAs (miRNAs). The application of technologies like RNAi to silence several genes simultaneously enhances the researcher's capability to protect the agriculturally important crop varieties against destructive pathogens and pests. The discovery of RNAi has transformed the research areas of plant breeding and serves as a tool to recognize the expression pattern of plant genomes and as a toolbox to control plant gene expression quantitatively and qualitatively (Hirai and Kodama 2008), controlling pathogenicity of plant parasites (Runo 2011) and enhancing resistance against biotic (Wani et al. 2010) and abiotic stresses (Jagtap et al. 2011).

RNAi has revolutionized the chances of making custom "knock-downs" of cistron activity. RNAi operates in each plant and animal and uses double-stranded RNA (dsRNA) as a trigger that targets homologous mRNAs for degradation or inhibition of their transcription or translation, whereby vulnerable genes are often suppressed. This RNA-mediated cistron management technology has provided new technologies for developing eco-friendly molecular tools for crop enhancement by suppressing particular genes responsible for numerous stresses as well as disease resistance. This chapter updates the current state on the use of RNAi, molecular principles underlying the biology of this phenomenon and development of RNAi

technologies in relation to plants and discusses strategies and applications of this technology in plant disease and pest management.

24.2 The Concept of RNAi

'RNA interference' or RNA silencing, is an endogenous machinery, existing in all eukaryotes, that controls gene expression at the post-transcriptional level in several developmental events and contribute to immunity of a plant against invading nucleic acids. It has most likely been evolved as a potent mechanism for cells to suppress foreign genes. The combining features of this phenomena include the production of small RNAs (21–26 nucleotides (nts) that act as sequence-specific determinants for down-regulating gene expression (Waterhouse et al. 2001; Hannon 2002; Pickford and Cogoni 2003) and the requirement of one or additional members of the Argonaute proteins (Hammond et al. 2001). RNAi operates by triggering the action of dsRNA intermediates, which are processed into RNA duplexes of 21–24 nts by a ribonuclease III-like enzyme called Dicer (Fire et al. 1998; Bernstein et al. 2001). The products of dicing are double-stranded small interfering RNAs (siRNAs), which are of 21–24 nt in length, where the guide strand (ssRNA) is subsequently incorporated into the RNA-induced silencing complex (RISC), including Argonaute and other related proteins (RNase H enzymes), guiding the cognate RNA molecules for degradation (Hammond et al. 2000; Tang et al. 2003). The host genome encodes small RNAs called miRNAs that are responsible for endogenous gene silencing. The microRNAs (miRNAs) are naturally existing small non-coding RNA molecules (containing about 22 nucleotides) found in plants, animals and some viruses. The main function of miRNA is to regulate gene expression. MicroRNAs have been part of the organism over evolutionary time, so the organisms have adapted with the presence of the miRNA. miRNAs are products of dsRNAs encoded in genes of the host genome. miRNAs are endogenous. The dsRNAs triggering gene silencing on the other hand can be initiated by several external sources such as invading nucleic acids such as transposons and viruses conferring plant immunity through expression of endogenous or transgenic antisense sequences, expression of inverted repeat sequences or RNA synthesis during viral replication. The cascade leading to the generation of mature siRNA begins with transcription by RNA polymerase II (in animals), RNA polymerase III (from a shRNA template), or RNA polymerase IV (in plants), forming double stranded RNA (dsRNA) (Voinnet 2005). The remarkable feature of RNA silencing in plants is that once it is triggered in a certain cell, a mobile signal is formed and spread through the whole plant causing the entire plant to be silenced (Dunoyer et al. 2007). After triggering the RNA silencing mechanism, the mobile signaling molecules can be spread over or amplified via production of dsRNAs on the primary cleavage of product templates or by their cleavage into secondary siRNAs. Accumulation of siRNAs is considered an indicator or reliable marker of RNAi (Hutvagner and Zamore 2002; Tang et al. 2003). Moreover, production of the secondary siRNAs leads to the increasing activity of silencing via its spread from the first activated cell to the neighbouring cells, and systemically

through the system (Himber et al. 2003). The invention of RNA-binding protein (PSRP1) in the plant phloem and its capability to bind 25 nts sRNA species add further to the argument that siRNAs (24–26 nts) are the main and unique components for the systemic silencing signal (Xie and Guo 2006). The extent of cell-to-cell movements is dependent on the levels of siRNAs produced at the site of silencing initiation but is not dependant on the presence of siRNA target transcripts in either source or recipient cell (Li and Ding 2006).

24.3 RNAi in Plants

RNA-mediated gene control technology has provided new platforms for developing environmentally friendly molecular tools for crop improvement (Umesh et al. 2012). Two main categories of small regulatory RNAs are distinguished in plants, based on their formation and function: (miRNAs) and (siRNAs). MiRNAs and siRNAs have been shown to be highly conserved, important regulators of gene expression in plants (Jones-Rhoades and Bartel 2006; Axtell and Bowman 2008). The modes of action by which small RNAs control gene expression at the transcriptional and post-transcriptional levels are now being evolved into tools for plant molecular biology research. However, consequent work has shown that RNA silencing works on at least three different levels in plants, first is the cytoplasmic silencing by dsRNA that results in cleavage of mRNA and is known as PTGS. Secondly, endogenous mRNAs are silenced by miRNAs, which negatively regulate gene expression by base pairing to specific mRNAs, resulting in either RNA cleavage or arrest of protein translation. Third, RNA silencing is associated with sequence-specific methylation of DNA and the consequent suppression of transcription (TGS) (Mansoor et al. 2006). There are evidences indicating that miRNAs can participate in biotic stress responses in plants. The first such role of miRNAs in plants was described by Jones-Rhoades and Bartel (2006). A number of miRNAs have been linked to biotic stress responses in plants, and the role of these miRNAs in plants infected by pathogenic bacteria, viruses, nematodes and fungi has been reported (Ruiz-Ferrer and Voinnet 2009; Katiyar and Jin 2010). Additionally, miRNAs are also important in regulating plant–microbe interactions during nitrogen (N) fixation by Rhizobium and tumour formation by Agrobacterium species (Katiyar and Jin 2010). Moreover, Mishra et al. (2009) detected a significant increase in the GC content of stress-regulated miRNA sequences, which in turn supports the view that miRNAs act as ubiquitous regulators under stress conditions. The GC content may also be considered a critical parameter for predicting stress-regulated miRNAs in plants. The first plant-endogenous siRNA that was found to be involved in plant biotic stress was *nat-siRNAATGB2*, which regulates R-gene mediated effector triggered immunity (Katiyar et al. 2006). A unique class of endogenous siRNA, the long siRNAs (lsiRNAs), is 30–40 nt long and is prompted by bacterial infection or specific growth conditions, such as cell suspension culture (Katiyar and Jin 2007). However, it may be considered that generation of small RNAs is a mechanism which allows plants to modulate gene expression programmes necessary for adaptation to stressful environments. Small

RNAs may facilitate the flexibility in environmental adaptation. The reason that small RNAs have a high complexity in plants may be justified by the fact that plant growth and reproduction are generally confined to many diverse and extreme habitats.

24.4 Approaches to Induce RNAi in Plants

A major challenge for scientists in RNAi research is to induce/suppress a specific target gene. Genes are induced by various methods. Most successful methods are virus-induced gene silencing (VIGS), agro inoculation and particle bombardment. Fenselau et al. (2012) have reported VIGS as the most successful method for inducing gene activity in plants; different RNA and DNA viruses have been modified to serve as vectors for gene expression. Replication of plant viruses produces dsRNA replication intermediates very effectively as well as efficiently because of a type of RNA silencing gene called VIGS (Senthilkumar and Mysore 2011). When viruses incorporated into plants, they trigger a post transcriptional gene silencing (PTGS) response by generating dsRNAs as replicative intermediates of viral RNAs. Similarly when transgenes are incorporated into plants, they trigger a PTGS response where dsRNAs generated as deviant transgene coded RNAs (Tyagi et al. 2008). Viral RNAs not only trigger PTGS, but they also serve as targets. Cleavage of viral RNA results in reduction of virus titres in local and distant leaves and the plant recovery phenotype (Godge et al. 2008). At the same time, all RNA virus-derived expression vectors will not be useful as silencing vectors because many have potent anti-silencing proteins, which directly interfere with the host silencing machinery (Diaz-pendon and Ding 2008). Similarly, DNA viruses have not been used extensively as expression vectors due to their size constraints for movement (Wani and Sanghera 2010). Another one is agro inoculation; it is a powerful method to study processes connected with RNAi. The injection of agrobacterium carrying similar DNA constructs into the intracellular spaces of leaves for triggering RNA silencing is known as agro inoculation or agro infiltration (Hily and Liu 2007). In most cases, agro inoculation is used to initiate systemic silencing or to monitor the effect of suppressor genes. In plants, cytoplasmic RNAi can be induced efficiently by agro inoculation, similar to a strategy for transient expression of T-DNA vectors after delivery by *Agrobacterium tumefaciens* (Usharani et al. 2005; Karthikeyan et al. 2011). One of the important non-biologistical methods is particle bombardment. As an alternative tool, protoplast transformation was first described as a method for the production of transgenic plants in 1987 (Sanford et al. 1987). Unique advantages of this methodology are discussed in terms of the range of species and genotypes that have been engineered and with high transformation frequencies. In plant research, the major applications of biolistics include transient gene expression studies, production of transgenic plants and inoculation of plants with viral pathogens (Taylor and Faquet 2002). In this method, a linear or circular template is transferred into the nucleus by microbombardment. Synthetic siRNAs are delivered into plants by biolistic pressure

Table 24.1 Exogenous application of naked dsRNA for RNAi-mediated protection against a range of viruses/viroids on different plants

Virus/ viroid	dsRNA target and size	dsRNA expression technique	Host	Virus inoculation	Efficiency	Reference
PMMoV	Replicase gene (977 bp)	In vitro	*N. tabacum* cv. Xanthi, *C. chinense*	Co-inoculation	No lesions observed	Tenllado and Diaz-Ruiz (2001)
PMMoV	Replicase gene (977, 596 and 315 bp)	In vitro	*N. benthamiana*	Co-inoculation	18% infected	Tenllado and Diaz-Ruiz (2001)
AMV	RNA 3 (1124 bp)	In vitro	*N. benthamiana*	Co-inoculation	0% infected	Tenllado and Diaz-Ruiz (2001)
TEV	HC-Pro gene (1483 bp)	In vitro	*N. tabacum* cv. Xanthi	Co-inoculation	0% infected	Tenllado and Diaz-Ruiz (2001)
PMMoV	Replicase gene (977 bp)	Bacterial HT115 expression	*N. benthamiana*	Co-inoculation; sprayed dsRNA and challenged, 3, 5, and 7 days post-spray	Days 1–5: 0% infected Day 7: 80% infected	Tenllado et al. (2003a)
PMMoV	CP gene (1081 bp) HC-Pro gene (1492 bp)	Bacterial HT115 expression	*N. benthamiana*	Co-inoculation; sprayed dsRNA and challenged 5 days post-spray	CP: 27% infected HC-Pro: 17.6% infected	Tenllado et al. (2003b)
CEVd	Less than full-length dsRNA	In vitro	*Gynuraaurantiaca*, tomato	Co-inoculation	50% infected	Carbonell et al. (2008)
PSTVd	180 bp (nucleotide position 1–179)	In vitro	Tomato	Co-inoculation	100% infected, some plants showed delay in symptoms	Carbonell et al. (2008)
CChMVd	Less than full-length dsRNA	In vitro	*Chrysanthemum*	Co-inoculation	50% infected	Carbonell et al. (2008)

TMV	CP gene (480 bp)	Bacterial M-JM109 lacY expression	Tobacco	Co-inoculation	50% infected	Yin et al. (2009)
SCMV	CP gene (CP1: 147 bp, CP2:140 bp)	Bacterial HT115 expression	Maize	Co-inoculation. Sprayed dsRNA and challenged 1, 3, 5, 7 and 9 days post-spray	Co-inoculation CP-1: 20% infected CP-2: 30% infected Day 1: 0% infected Day 3: 4% infected Day 5: 12% infected Day 7: 43.3% infected Day 9: 72% infected	Gan et al. (2010)
PVY	NIb gene (3 different dsRNAs, all 500 bp)	Bacterial M-JM109 lacY expression	Tobacco	Co-inoculation	NIb-1: 34% infected NIb-2: 66% infected NIb-3: 52% infected	Sun et al. (2010a)
PVY	HC-Pro gene, Nibgene CP gene (all 600 bp each)	Bacterial HT115 expression	Tobacco	Co-inoculation	NIb: 28% infected HC-Pro: 54% infected CP: 44% infected	Sun et al. (2010b)
TMV	MP gene, CP gene, RP gene (all 480 bp each)	Bacterial HT115 expression	Tobacco	Co-inoculation	MP: 34% infected CP: 52% infected RP: 66% infected RNA: 60% infected	Sun et al. (2010b)
PRSV	CP gene (279 bp)	Bacterial M-JM109 lacY expression	Papaya	Co-inoculation. Sprayed dsRNA and challenged 1, 2, 3 and 5 days post-spray	35% infected. All others: 100% infected	Shen et al. (2014)
PSbMV	CP gene (500 bp)	In vitro	Pea cv. Raman	dsRNA sprayed and co-inoculated with virus. dsRNA was sprayed after 1, 2 and 21 days post-inoculation.	All 100% infected, reduced viral titre	Safarova et al. (2014)

(continued)

Table 24.1 (continued)

Virus/ viroid	dsRNA target and size	dsRNA expression technique	Host	Virus inoculation	Efficiency	Reference
CymMV	CP gene (237 bp)	Bacterial HT115 expressions	Orchid	Co-inoculation	20% infected	Lau et al. (2014)
TMV	p126 (666 bp), CP gene (480 bp)	In vitro	*N. tabacum* cv. Xanthi	Co-inoculation	p126: 35% infected CP: 50% infected	Konakalla et al. (2016)
ZYMV	HC-Pro, CP gene	In vitro	Cucumber, watermelon and squash plants	Co-inoculation	HC-Pro (cucumber)-82% HC-Pro (watermelon) – 50% HC-Pro (squash) – 18% CP (cucumber) – 70% CP (watermelon) – 43% CP (squash) – 16%	Kaldis et al. (2018)

AMV Alfalfa mosaic virus, *CChMVd* Chrysanthemum chlorotic mottle viroid, *CEVd* Citrus exocortis viroid, *CP* Coat protein, *RP* Replicase protein, *CymMV* Cymbidium mosaic virus, *HC-Pro* Helper component protein, *NIb* Nuclear inclusion b, *MP* Movement Protein, *PMMoV* Pepper mild mottle virus, *PPV* Plum pox virus, *PRSV* Papaya ringspot virus, *PSbMV* Pea seed-borne mosaic virus, *PSTVd* Potato spindle tuber viroid, *PVY* Potato virus Y, *p126* Protein 126, *RP* Replicase protein, *SCMV* Sugarcane mosaic virus, *TEV* Tobacco etch virus, *TMV* Tobacco mosaic virus, *ZYMV* Zucchini yellow mosaic virus

Table 24.2 RNAi against fungal pathogens

Pathogen	Targeted region	Reference
Magnaporthe oryzae	*eGFP*	Kadotani et al. (2003)
Cladosporium falvum	*cgl1 and cgl2*	Segers et al. (1999)
F. oxysporum f. sp. *conglutinans*	*FOW2, FRP1 and OPR*	Zongli et al. (2015)
Blumeriagraminis f.sp. *tritici*	*Rnr*	Dimitar et al. (2014)
Blumeria graminis	*Mlo*	Schweizer et al. (2000)
Venturia inaequalis	*Multiple inverted repeats*	Fitzgerald et al. (2004)

to cause silencing of green florescent protein expression. Bombarding cells with particles coated with dsRNA, siRNA or DNA that encode hairpin constructs as well as sense or antisense RNA activates the RNAi pathway (Shabhir et al. 2010).

24.5 RNA Interference for Engineering Resistance Against Plant Diseases

The effects of gene silencing in plants were used in efforts to develop resistance to diseases caused by viruses, fungi and bacteria. This "pathogen-derived resistance" was achieved by transforming plants with genes, or sequences, derived from the pathogen, with the aim of blocking a specific step in the life or infection cycle of the pathogen.

24.5.1 RNAi Against Plant Viruses

Plant viruses are responsible for a significant proportion of crop diseases and are very difficult to combat due to the scarcity of effective counter measures, placing them among the most important agricultural pathogens. RNAi application has resulted in successful control of many economically important viral diseases in plants (Francisco et al. 2004, Cakir and Tor 2010). The effectiveness of RNAi technology for generating virus resistance in plants was first demonstrated in 1998. VIGS is one of the commonly used RNA silencing methods to control plant viruses (Senthilkumar and Mysore 2011). (Refer Table 24.1).

24.5.2 Application of RNAi for Fungal Resistance Development

RNA interference is a powerful and versatile genetic tool that can be applied to filamentous fungi of agricultural importance. It is shown that gene silencing plays an important role in plant defence against multicellular microbial pathogens, such as vascular fungi belonging to the *Verticillium* genus. Several components of RNA silencing pathways were tested, of which many were found to affect *Verticillium* defence. It is speculated that the gene silencing mechanisms affect regulation of

Verticillium-specific defence responses (Ellendorff et al. 2009). An early successful application of the RNAi system using sense and antisense RNA was reported for the pathogenic fungus *Cryptococcus neoformans* (Liu et al. 2002). The efficacy of RNAi was demonstrated in *Magnaporthe oryzae*, *Venturia inaequalis*, *Phytophthora infestans*, *Histoplasma capsulatum* and *Blastomyces dermatitidis* by expression of the GFP gene in fungus and then silencing by RNAi. Rust fungi cause devastating diseases of wheat and other cereal species globally. Gene fragments from the rust fungus, *Puccinia striiformis* f. sp. tritici or *P. graminis* f. sp. *tritici*, were delivered to plant cells through the Barley stripe mosaic virus (BSMV) system and some reduced the expression of the corresponding genes in the rust fungus. The ability to detect suppression was associated with the expression patterns of the fungal genes because reduction was only detected in transcripts with relatively high levels of expression in fungal haustoria. The results indicate that in plants the RNAi approach can be used in functional genomics research for rust fungi and that it could potentially be used to engineer durable resistance (Yin et al. 2011). The below examples are the RNAi strategies used against different fungal species (Table 24.2).

24.5.3 RNA Silencing-Mediated Resistance to Plant Pathogenic Bacteria

Very few researches have been appeared on the use of gene silencing against plant pathogenic bacteria. Escobar et al. (2001) for the first-time documented RNAi application for engineering resistance in plant against bacterial pathogen triggering crown gall disease. In the particular disease, iaaM and ipt oncogenes were found responsible for gall formation and these genes are pre-requisite for development of galls. Therefore, for management of the disease these oncogenes were targetted. With the help of RNAi technology, they showed that transgenic plants (*Arabidopsis thaliana* and *Lycopersicon esculentum*) containing modified construct of these two bacterial genes (s) showed resistance against crown gall. The transgenic genes shut down the expression of iaaM and ipt oncogenes of the incoming bacterial pathogen, thereby disturbing the hormonal production and ultimately, tumourogenesis or gall formation process after infection. Dunoyer et al. 2007 also reported that plants lacking the modified oncogenes were hyper-susceptible to *A. tumefaciens*. Another example is the RNAi-mediated enhanced resistance to *Xanthomonas oryzae*, the leaf blight bacterium due to successful knockdown of a rice homolog of OsSSI2 (Jiang et al. 2009). Zhai et al. (2011) and Li et al. (2012) studied the function of several miRNA target gene families of plant innate immune receptors (NBS-LRR) in legumes and solanaceae, respectively. They gave a new insight into viral and bacterial infection in plants that suppresses miR482- mediated silencing of R genes. Considering the findings from different researchers (Zhai et al. 2011 and Li et al. 2012), a general understanding can be drawn that miRNA can either act as up- or down-regulators of bacterial invasion. Identification and characterization of pathogen-responsive miRNAs that induced positive regulators of bacterial resistance

will open a flood gate to enhancement of transgenic plants that will involve the constitutive over-expression of miRNA.

24.6 RNAi and Insect Pest Control in Agriculture

RNAi is a powerful tool for gene function studies and control of insect pests. Several research groups have recently explored the possibility of conducting RNAi in insects through different application methods. There is a wide range of target insects from different insect orders, target genes and feeding methods, demonstrating the richness in application of dsRNA and the potential of RNAi. Despite having been considered for many years, application of RNAi technology to give resistance to herbivorous insects has only just been realized. The key to the success of this approach would be (a) insect species and its life stages; (b) the type of exogenous RNA: dsRNA, siRNA, miRNA, etc.; (c) the dose and method of application; (d) the type of target gene and its expression profile; (e) gene function and the type of tissue; (f) nucleotide sequence and length of dsRNA; (g) persistence of the silencing effect and (h) gut physiology.

Several crop insect pests belonging to different orders were tested for their possible control by RNAi. In these insects, RNAi knockdown has been developed for various genes encoding developmental proteins, salivary gland proteins, proteins involved in host–insect interaction, hormone receptors and gut enzymes. Baum et al. (2007) provided evidence for the potential use of RNAi to control insect pests in crop protection and demonstrated the fact that it is possible to silence genes in insects when they consume plant material expressing hairpin dsRNA constructs against well-chosen target genes. They reported the reduction of corn root damage in transgenic maize plants producing vacuolar H + ATPase dsRNA after infestation of the plant with the western corn rootworm. In another report, the model plants *Nicotiana tabacum* and *Arabidopsis thaliana* were modified with the cytochrome P450 gene of *Helicoverpa armigera*. When the cotton bollworm larvae were fed transgenic leaves, the levels of cytochrome P450 mRNA were reduced and larval growth retarded (Mao et al. 2007). Bautista et al. (2009) studied the influence of silencing the cytochrome P450 gene CYP6BG1 that is over-expressed in a permethrin-resistant diamondback moth (*Plutella xylostella*) strain. When the gene was silenced after consumption of a droplet of dsRNA solution, the moths became significantly more sensitive to the pyrethroid insecticide. Another significant development employing RNAi is that the susceptibility of insect pests to Bt toxins could be enhanced by silencing the genes involved in Bt resistance development.

24.7 Conclusion

Application of RNAi in management of biotic stress will prove to be an incredible revolution in the field of functional genomics and a breakthrough in plant molecular genetics. If RNAi technology is developed successfully and employed for the

management of major diseases on a commercial scale, it can prove to be an eco-friendly and biologically safe technology. Moreover, this technique eliminates the risk associated with development of transgenics, and it will also have enormous potential for engineering control of gene expression. An agronomically superior cultivar can be engineered for additional plant fitness by using RNAi technology. However, selection of targeting sequence and delivery of siRNA are major challenges for plant molecular biologists. More understanding and exploration in the field of RNAi promoting resistance is needed. Therefore, further molecular research is needed to unfurl the factors affecting RNAi-mediated resistance and solve all the challenges in delivering siRNA to the host system and identifying the targeted region to effectively overcome the pathogen and promote crop improvement.

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Genome Editing for Plant Disease Resistance

25

Rajeev Singh

Abstract

Plant diseases severely affect crop yield and quality, and this poses a huge threat to global food security. Plant pathogens are a hazard for agriculture. Mostly phytopathogens are known to misuse the dominantly inherited genes, called susceptibility (S) genes, to facilitate their proliferation. Genetic disruption of these genes has been one of the successful ways to combat the pathogens and induce a durable disease resistance. Novel genome-editing technologies offer opportunities to control viral, bacterial, fungal pathogens, etc., and implement a pathogen resistance in plants. Site-directed mutagenesis, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) are some of the most important genetic tools witnessed in the recent years. CRISPR/Cas9 has been reported as an effective tool since it is versatile, less expensive, easier to design and implement, and has a higher success rate. In this chapter, we focus on the use of the genome-editing techniques for the development of transgene-free and durable disease-resistant crop varieties.

Keywords

Gene editing · CRISPR/Cas9 · Plant pathology · Plant diseases · Agriculture

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25.1 Introduction

Agriculture is one of the prime sources of survival and development of the society. Due to the increasing population and requirement of high crop yield, we aim at sustainable agriculture, which provides food security, whilst reducing the environmental pressure. Plant breeding has been the most successful approach for developing new crop varieties and hence coping up with the increasing demand for food. Crops are prone to pathogens (fungi, bacteria, viruses), which greatly affects the crop yield (20–40% loss) and hence the intensifying the economic losses. The resulting plant diseases adversely affect plant growth, impeding the quality and also affecting the long-term storage of crops, which leads to a slow agricultural development (Borrelli et al. 2018; Yin and Qiu 2019). In most parts of the world, the use of chemical pesticides to curb plant diseases is one of the most common methods. Although, the reports suggest that these pesticides pose a greater threat to the environment, as they are not specific, and they may or may not be harmful to the humans in the longer run. But, the high evolutionary potential of various pathogens leads to different kinds of mutations and recombination, hence developing resistance against different pesticides due to natural selection. This can have a greater geographical impact, as it leads to an increase in the development of resistant genotypes and can easily spread to other locations (Damalas and Eleftherohorinos 2011). New fumigation methods, improved diagnostic protocols, improved trade standards among countries, and high throughput screening technologies are some of the many initiatives being taken but looking at the current situation, we need a more stagnant solution.

Conventional resistance breeding was based on the incorporation of the identified natural and induced mutant allele in a preferred genotype through breeding techniques. Although the different traditional genetic approaches to disease control have mostly yielded positive results for decades, they have several limitations too. These approaches can only be performed within the plants having enough genetic variation and can mate with each other. These approaches can also be imprecise and uncertain as they can transfer many different traits (large genome regions) instead of just the resistance trait (single gene insertion). Also, genetic crossing and progeny selection can be impeding resources like time and labor. Therefore, in the current world, conventional techniques have to keep pace with the evident availability of genome and transcriptome sequences, changing pathogens, and increasing global food demand particularly during an era of global climate change (Gao 2018).

25.2 Genome Editing Technology

Genetic association studies, utilizing single nucleotide polymorphisms (SNPs) and different other molecular markers identified from linkage studies, are becoming increasingly helpful in determining the identification of quantitative trait loci (QTL). These QTLs are becoming a part of the new breeding programs, as they provide quantitative resistance to pathogens by using resistance (R) genes introduced into varieties with agricultural characteristics.

Genome editing (also known as gene editing) is a group of technologies that allows genetic material to be added, removed, or altered at specific locations in the genome of a living organism. New breeding techniques (NBT) encompass current and precise molecular approaches for genetic modification of single and multiple gene targets (Nelson et al. 2018). The core processing of genome editing is based on DNA double-stranded break (DSB) repair mechanics. DSB breaks are produced using sequence-specific nucleases for recognizing specific DNA sequences. Two major pathways repair DSBs: nonhomologous end joining (NHEJ) pathway and homologous recombination (HR) pathway (Voytas and Gao 2014). NHEJ uses different enzymes to repair the DSBs, whereas HR is more specific and uses a homologous sequence as a template to regenerate the missing DNA sequences at the breakpoint. Although the NHEJ pathway is error-prone and can result in insertion or deletion mutations, most cells use this pathway to repair DSBs. However, in the presence of a donor DNA template, the HR pathway is mostly used, which results in precise and specific changes. Four different types of sequence-specific nucleases are used in genome editing: meganucleases (MNs), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas9). In recent years, CRISPR/Cas9 editing is very effective and useful for improving the agronomic traits in crops (Fig. 25.1) (Mohanta et al. 2017). CRISPR/Cas9, unlike another gene editing (GE) technologies, is independent of protein

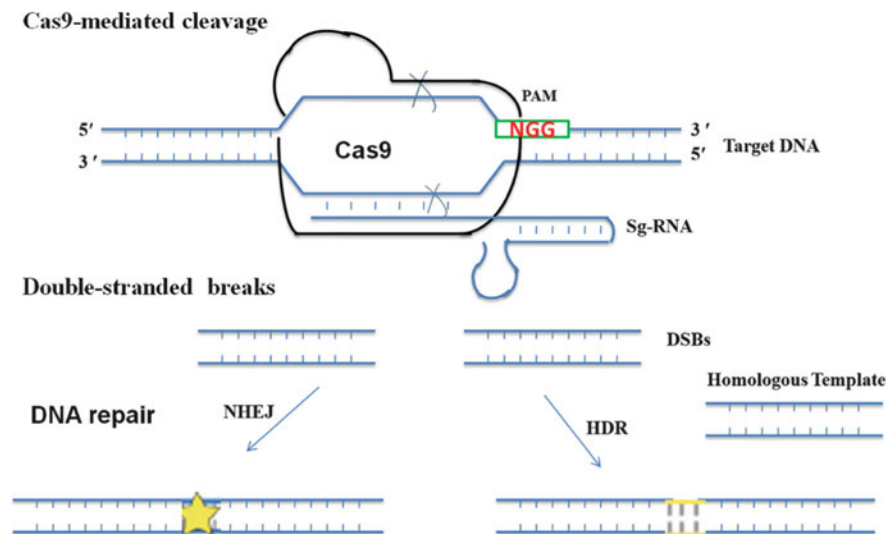

Fig. 25.1 A brief overview of the CRISPR/Cas9 system. Engineered CRISPR/Cas9 system depends on RNA-guided nuclease, Cas9 to introduce double-stranded breaks in target DNA. A single guide RNA, whose 20 nucleotides match the target DNA and a PAM (NGG or NAG, where N is any nucleotide) are essentially required for cleavage of the DNA in a sequence-dependent manner. Cas9 cleavage generates DSBs, which can be repaired through NHEJ or the HR pathway. (Source: Mushtaq et al., Frontiers in Plant Science 2019)

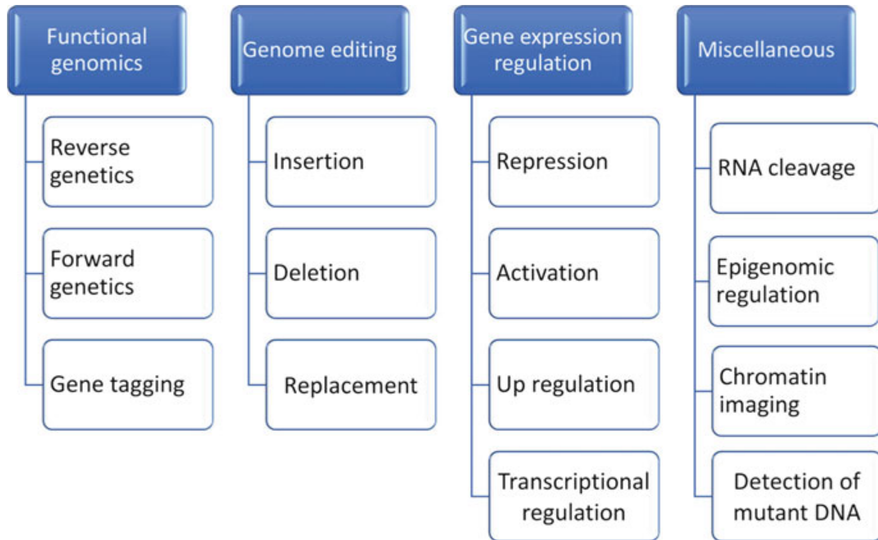

Fig. 25.2 Different applications of CRISPR technology. (Source: Ahmad et al., J Cell Physiol. 2019: 1–17)

engineering steps and sequence alteration of the single-guide RNA is enough for generate new DNA sequences. This technique requires a duplex-RNA structure which includes CRISPR RNA (crRNA): trans-activating crRNA (tracrRNA), and this guides the Cas9 nucleases to target DNA. For efficient usage of this technology, the dual crRNA: tracrRNA structure is modified/engineered into single-guide RNA (sgRNA) and is targeted to specific genomic loci (Fig. 25.2) (Mushtaq et al. 2018; Ahmad et al. 2020).

25.3 Genome Editing for Resistance Against Bacterial Pathogens

The diversity of bacterial pathogens, high multiplication rate, and increase in the epidemics have led to an overall increase in bacterial diseases. Phytopathogenic bacteria can spread in a lot of different ways and are difficult to control due to undetected asymptomatic infections and paucity of specific agrochemicals. To target the bacterial diseases more specifically, a lot of research has been conducted in elucidating the molecular pathways in connection with various host–bacterial pathogen interactions. Many host plant genes have been identified, which also includes some S genes which participate in this complex process. S genes have become one of the popular targets for breeding crops that are resistant to bacterial diseases via genome editing.

Phytopathogenic bacteria can be grouped as crop specific, such as *Clavibacter michiganensis*, causal agent of tomato bacterial ring rot; polyphagous specific, such

Table 25.1 CRISPR/Cas9 applications for bacterial resistance

Plant species	Fungus	Target gene	Gene function	Strategy
Oryza sativa	Bacterial blight (*Xanthomonas oryzae pv. oryzae*)	SWEET13	Sucrose transporter gene	*Agrobacterium*-mediated transformation of embryogenic callus with Cas9/gRNA expression plasmid vectors and TALEN
Citrus paradisi	Citrus canker (*Xanthomonas citri* subspecies *citric*)	LOB1	Susceptibility (*S*) gene-promoting pathogen growth and pustule formation	*Agrobacterium*-mediated transformation of epicotyl with Cas9/gRNA expression plasmid vectors
Citrus sinensis Osbeck	Citrus canker (*Xanthomonas citri* subspecies *citric*)	LOB1	Susceptibility (*S*) gene-promoting pathogen growth and pustule formation	*Agrobacterium*-mediated transformation of epicotyl with Cas9/gRNA expression plasmid vectors
Malus domestica	Fire blight (*Erwinia amylovora*)	DIPM-1 DIPM-2 DIPM-4	Susceptibility factor involved in fire blight disease	PEG-mediated protoplast transformation with CRISPR ribonucleoproteins

Source: Borelli et al., Frontiers in Plant Science (2019)
CsLOB1 Lateral Organ Boundaries 1, *DIPM* DspE-interacting proteins of Malus

as *Ralstonia solanacearum*, which causes disease in multiple monocot and dicot species; and “kingdom crosser,” such as *Dickeya dadantii*, which is an entomo-phytopathogen and can affect plants and animals.

Few studies have been published where the application of the CRISPR/Cas system to counteract crop bacterial diseases has been discussed (Table 25.1). CRISPR/Cas9 mutagenesis of *OsSWEET13* in rice has been performed to achieve resistance against the bacterial blight disease caused by γ-proteobacterium *Xanthomonas oryzae* pv. *oryzae* (Zhou et al. 2015). *OsSWEET13* is an *S* gene that encodes a sucrose transporter involved in plant–pathogen interaction. *X. oryzae* produces an effector protein, PthXo2, inducing *OsSWEET13* expression in the host and the consequent condition of susceptibility. In a previous work which concerns *OsSWEET14* promoter mutagenesis adopting a TALEN approach, the disruption of this gene rendered the *X. oryzae* effector unable to bind *OsSWEET14* and it ultimately resulted in disease resistance (Li et al. 2012). Similarly, Zhou et al. (2015) obtained a null mutation in *OsSWEET13* for exploring PthXo2-dependent disease susceptibility, and thereby the resultant mutants were resistant to bacterial blight. In the future, genome-editing strategies for multiplexed recessive resistance, using a combination of the major effectors and other resistance (*R*) genes, would be the next step toward achieving bacterial blight resistance.

Citrus canker is another devastating disease that is caused by the bacterium *Xanthomonas citri* ssp. *citri* (*Xcc*). *CsLOB1* is a member of the lateral organ

boundaries domain (LBD) family of transcription factors, which was previously identified as an S gene for *Xcc* (Hu et al. 2014). The*CsLOB1* promoter contains an effector-binding element (EBE), which is recognized by the *Xcc* effector PthA4, which activates *CsLOB1* expression to facilitate canker advancement. In one study, the *CsLOB1* promoter EBE was targeted, whereas another study targeted the coding region of *CsLOB1* using CRISPR/Cas9. Both studies showed that editing*CsLOB1*provided resistance to *Xcc* (Jia et al. 2017; Peng et al. 2017). Although the potential negative effect of mutating*CsLOB1* on plant growth has yet to be determined, the growth status of *CsLOB1* null mutant was similar to the wild-type plants (Jia et al. 2017), suggesting that*CsLOB1* is an ideal promoter for engineering canker resistance in elite citrus varieties.

Although tomato is one of the most economically important crops throughout the world, the crop yield and quality are still limited by several major pathogens, including *Pseudomonas syringae*, *Phytophthora* spp., and *Xanthomonas* spp. (Schwartz et al. 2015). A recent study has shown that mutation of a single gene in *Arabidopsis*, *DMR6* (downy mildew resistance 6), led to increased salicylic acid levels and resistance to some plant pathogens, which includes bacteria and oomycetes (Zeilmaker et al. 2015). The tomato orthologue *SlDMR6–1* is also upregulated in response to infection by *P. syringae* pv. tomato and *Phytophthora capsici*. Null mutants of*SlDMR6–1*, generated using the CRISPR/Cas9 system showed resistance to *P. syringae*, *P. capsica*, and *Xanthomonas* spp. without detrimental effects on tomato growth and development. Taken together, these results suggest that knocking out *DMR6* might be a good strategy to establish broad-spectrum disease resistance to plants.

25.4 Genome Editing Resistance Against Fungal Pathogens

Fungal pathogens are prominently responsible for various plant diseases such as mildew smut, rust, and others. These diseases have a drastic effect on the quality of the crop yield and thereby greater economic losses. Due to increased genetic diversity, these pathogens are steady with invading new hosts and compromising the R gene-mediated resistance and thereby assuring the resistance to fungicides (Doehlemann et al. 2017). Also due to the production of secondary metabolites such as mycotoxins via mycotoxigenic fungi, these pathogens pose a greater threat to humans and the livestock, which are exposed to contaminated feed. The evolving knowledge of molecular mechanisms in the field of plant–pathogen interaction has led to different strategies in the area of disease control. Modification of potential host S genes is the prime target for editing via CRISPR/Cas9 technology.

Powdery mildew is one of the most common fungal diseases that affect a wide variety of plants. Developing resistant varieties is one of the most effective approaches to combat the disease. Hybridization of the resistance-induced R genes from foreign species into the elite species was the traditional approach used against this disease. With evolving generations of wheat powdery mildew, the resistance

genes are slowly lost and thereby we need more broad-spectrum and resistant varieties.

The discovery of barley *mildew resistance locus o (MLO)* mutants was a significant step in generation disease-resistant varieties (Büschges et al. 1997). The *MLO* gene was cloned in 1997 and it encodes a protein with seven transmembrane domains localized in the plasma membrane and is evolutionarily conserved in monocots and dicots. It has been reported that *MLO* were *S* genes and homozygous loss-of-function mutants had shown increased resistance against powdery mildew in barley, *Arabidopsis*, and tomato (Piffanelli et al. 2004; Consonni et al. 2006; Bai et al. 2008). Bread wheat is an allohexaploid that has three orthologues of barley *MLO* (*TaMlo-A1, B1,* and *D1*). Using CRISPR/Cas9 technology the MLO genes were modified and it showed improved resistance against *Blumeria graminis* f. sp. *tritici* (*Bgt*) infection, demonstrating an important role of *TaMlo* genes in powdery mildew disease (Wang et al. 2014). This shows that genome editing plays a greater role in modifying targets within polyploidy genomes. In tomato, *MLO* knockout mutants are generated by targeting *SlMlo1*, which is identified as one of the most important of 16 *SlMlo* genes. *SlMlo* was targeted at two sites and a 48 bp deletion was obtained, which generated plants that were self-pollinated to obtain CRISPR/Cas cassette-free individuals. As a result, the new non-transgenic variety, "Tomello" was fully resistant to *Oidium neolycopersici,* a tomato powdery mildew fungus (Nekrasov et al. 2017).

Enhanced disease resistance 1 (EDR1) in *Arabidopsis* is highly conserved across plant species and is also known to negatively regulate the resistance against *Erysiphe cichoracearum* and thereby it becomes an important target for improving powdery mildew. Wheat *EDR1* has three homologs that were targeted by the CRISPR/Cas9 system leading to the generation of *Taedr1* wheat plants. *Taedr1* mutant plants have shown to have resistance against *Bgt*, but without mildew-induced cell death (Zhang et al. 2017).

Rice blast is one of the most devastating diseases caused by *Magnaporthe oryzae*, and it affects the rice production drastically worldwide. To improve the adaptation of rice during biotic or abiotic stresses, ethylene responsive factors (ERFs) play a major role and these factors belong to APETELA2/ERF (AP2/ERF) superfamily. *M. oryzae* is known to induce the expression of *OsERF922*and the plants resistant to rice blast disease are generated by disrupting *OsERF922*and *OsSEC3A* genes in rice using CRISPR/Cas9 technology (Wang et al. 2016). Overall, these examples put forward the positive implications of the CRISPR/Cas9 system for crop improvement as regards fungal disease resistance (Table 25.2).

25.5 Genome-Editing Resistance Against Viral Pathogens

Plant viruses are a serious threat to a lot of economically important crops and this is because of the rapid evolvement of the viruses and involvement of the insect vectors. Plant viruses are classified according to their genome structure into six categories: (1) double-stranded DNA (dsDNA) viruses, (2) single-stranded DNA (ssDNA)

Table 25.2 CRISPR/Cas9 applications for fungal resistance

Plant species	Fungus	Target gene	Gene function	Strategy
Triticum aestivum	Powdery mildew (*Blumeria graminis* f. sp. *tritici*)	MLO-A1	Susceptibility (*S*) gene involved in powdery mildew disease	Particle bombardment of immature wheat embryos with Cas9/gRNA expression plasmid vectors
Solanum lycopersicum	Powdery mildew (*Oidium neolycopersici*)	MLO1	Major responsible for powdery mildew vulnerability	*Agrobacterium*-mediated transformation of cotyledons with Cas9/gRNA expression plasmid vectors
Vitis vinifera	Powdery mildew (*Erysiphe necator*)	MLO-7	Susceptibility (*S*) gene involved in powdery mildew disease	PEG-mediated protoplast transformation with CRISPR ribonucleoproteins
Vitis vinifera	Gray mold (*Botrytis cinerea*)	WRKY52	Transcription factor involved in response to biotic stress	*Agrobacterium*-mediated transformation of proembryonal masses with Cas9/gRNA expression binary vectors
Theobroma cacao	Black pod disease (*Phytophthora tropicalis*)	NPR3	Regulator of the immune system	*Agrobacterium*-mediated transient transformation of stage C leaves with Cas9/gRNA expression binary vectors
Oryza sativa L. japonica	Rice blast disease (*Magnaporthe oryzae*)	SEC3A	Subunit of the exocyst complex	Protoplast transformation with Cas9/gRNA expression binary vectors
Oryza sativa L. *japonica*	Rice blast disease (*Magnaporthe oryzae*)	ERF922	Transcription factor implicated in multiple stress responses	*Agrobacterium*-mediated transformation of embryogenic calli with Cas9/gRNA expression binary vectors

Source: Borelli et al., Frontiers in Plant Science (2019)
MLO, MILDEW RESISTANT LOCUS; NPR3, non-expressor of pathogenesis-related 3; ERF922, ethylene responsive factor

viruses, (3) reverse-transcribing viruses, (4) double-stranded RNA (dsRNA) viruses, (5) negative-sense single-stranded RNA (ssRNA–) viruses, and (6) positive-sense single-stranded RNA (ssRNA+) viruses (Roossinck, Martin and Roumagnac 2015). Remedies to combat the plant viral diseases have been growing and various novel methods targeting the pathogen-derived resistance have been implemented. Genome editing adds to novel tools that are being used against viral pathogens (Table 25.3).

Geminiviruses are a large group of plant DNA viruses which comprise of 360 species or more. They are a huge threat to plant families such as Cucurbitaceae,

Table 25.3 CRISPR/Cas9 applications for viral resistance

Plant species	Virus	Target gene	Gene function	Strategy
Nicotiana benthamiana and *Arabidopsis thaliana*	BeYDV	CP, rep, and IR	RCA mechanism	*Agrobacterium*-mediated transformation of leaves with Cas9/gRNA expression plasmid vectors
Nicotiana benthamiana	BSCTV	LIR and rep/RepA	RCA mechanism	*Agrobacterium*-mediated transformation of leaves with Cas9/gRNA expression plasmid vectors
Nicotiana benthamiana	TYLCV BCTV MeMV	CP, rep, and IR	RCA mechanism	*Agrobacterium*-mediated transformation of leaves with a TRV vector in Cas9 overexpressing plants
Nicotiana benthamiana	TuMV	GFP1, GFP2, HC-Pro, CP	Replication mechanism	*Agrobacterium*-mediated transformation of leaves with a TRV vector in Cas13a overexpressing plants
Nicotiana benthamiana and *Arabidopsis thaliana*	CMV TMV	ORF1, 2, 3, CP and 3′UTR	Replication mechanism	*Agrobacterium*-mediated transformation of leaves with FnCas9/gRNA expression binary vectors floral dipping for *Arabidopsis*
Cucumis sativus	CVYV ZYMV PRSV-W	eIF4E	Host factor for RNA viruses translation	*Agrobacterium*-mediated transformation of cut cotyledons (without embryo) with Cas9/gRNA binary vectors
Arabidopsis thaliana	TuMV	eIF(iso)4E	Host factor for RNA viruses translation	*Agrobacterium*-mediated transformation with Cas9/gRNA recombinant plasmid binary vectors (floral dipping)
Oryza sativa L. *japonica*	RTSV	eIF4G	Host factor for RNA viruses translation	*Agrobacterium*-mediated transformation of immature embryos with Cas9/gRNA expression plasmid vectors

Source: Borelli et al., Frontiers in Plant Science (2019)

Euphorbiaceae, Solanaceae, Malvaceae, and Fabaceae (Zaidi et al. 2016). These viruses have a circular single-stranded DNA which is replicated through a rolling-circle amplification mechanism via a double-stranded DNA (dsDNA) or by recombination-mediated replication (Hanley-Bowdoin et al. 2013). The first two genome-editing studies involving CRISPR/Cas9 approach on developing resistance against geminiviruses focused on beet severe curly top virus (BSCTV) and bean yellow dwarf virus (BeYDV) in *N. benthamiana* and *Arabidopsis* (Baltes et al. 2015) (Ji et al. 2015). Begomoviruses which are a genus of geminiviruses are known to infect dicotyledonous plants. Their genome is either monopartite or bipartite with a common region of 220 bp (Gilbertson et al. 2015). To develop resistance against begomoviruses, CRISPR/Cas9 system was expressed in the host cell nucleus to

target and cleave the virus during replication. This system was also tested against the monopartite beet curly top virus (BCTV) and bipartite Merremia mosaic virus (MeMV) geminiviruses. The results showed attenuated symptoms of both the viruses (Ali et al. 2015, 2016).

Host-translation machinery is responsible for the synthesis of viral proteins. For the translation of the viral proteins, a multicomponent translation complex consisting of eukaryotic translation initiation factor 4E (eIF4E) and its isoforms, recruits ribosomes to the 5′ untranslated regions (UTRs) of mRNAs, because viruses do not harbor ribosomes (Sanfaçon 2015). Loss of function mutation in *the eIF(iso)4E* gene has shown to confer resistance against Turnip mosaic virus (TuMV) in *Arabidopsis* mutants and does not affect the plant vigor. This makes eIF4E genes an ideal target for generating broad-spectrum virus resistance (Lellis et al. 2002). CRISPR/Cas9-generated mutations in *eIF4E* genes in cucumber led to resistance against cucumber vein yellowing virus (CVYV), Zucchini yellow mosaic virus (ZYMV), and papaya ring spot mosaic virus-W (PRSV-W) (Chandrasekaran et al. 2016). Also, in cassava, where only two (novel cap-binding protein-1 (nCBP-1) and nCBP-2) out five genes encoding eIF4E proteins can associate with viral genome-linked proteins (VPgs), CRISPR/Cas9-generated *ncbp-1/ncbp-2* double mutants showed attenuated symptoms after infection with Cassava brown streak virus (CBSV) (Gomez et al. 2019). With the advancement of the CRISPR/Cas9 system, it is possible to target the plant viruses with RNA genomes eventually leading to the production of RNA virus-resistant plants.

BeYDV, bean yellow dwarf virus; BSCTV, beet severe curly top virus; TYLCV, tomato yellow leaf curl virus; BCTV, beet curly top virus; MeMV, Merremia mosaic virus; TRV, tobacco rattle virus; CLCuKoV, cotton leaf curl Kokhran virus; TuMV, turnip mosaic virus; CMV, cucumber mosaic virus; TMV, tobacco mosaic virus; CVYV, cucumber vein yellowing virus; ZYMV, zucchini yellow mosaic virus; PRSV-W, papaya ring spot mosaic virus-W; PVX, potato virus X; TCV, turnip crinkle virus; CMV, cucumber mosaic virus; RTSV, rice tungro spherical virus; CP, coat protein; Rep, replication association protein; IR, intergenic region; RCA, rolling-circle amplification; LIR, long intergenic region; GFP1, green fluorescent protein 1; GFP2, green fluorescent protein 2; HC-Pro, helper component proteinase silencing suppressor; ORF, open reading frame; UTR, untranslated terminal repeat; eIF4E, eukaryotic translation initiation factor 4E; eIF4G, eukaryotic translation initiation factor 4G.

25.6 Future Perspectives

The present situation demands the reduction in the use of chemicals in pesticides, improvement in crop yield, and development of disease-resistant varieties to ensure food security around the globe. Genome editing could be one of the most useful tools to target the upcoming challenges in agriculture. With the help of genome editing, we can also overcome the limitations of conventional breeding for disease resistance. Genome editing is specific and does not introduce any further changes than the target

site and it also bypasses genetic crosses and progeny selection. The use of the CRISPR/Cas9 system is more and more justified as the knowledge of the metabolic pathways has increased considerably and mostly disease resistance can be obtained by modification of a single gene using this system. Also with the help of targeted mutagenesis using the CRISPR system, susceptibility genes can be inactivated which would lead to disease resistance. Although it is expected that additional S genes will be discovered, hence proving more targets for editing. Nonetheless, as S genes are responsible for plant growth and development, generating S gene mutants can also have some detrimental effects. Before implementing the editing technologies, we need to take into account the challenges that we would face.

We need to look into the feasible implementation of the concept from regulated environments to the crop field conditions, which are certainly more dynamic. Due to variable conditions, with time it is difficult to devise a stable method. We would also need to test the fitness of the edited crop in the field, and thereby regular field tests become a necessity. As discussed before, the downside of editing a gene could be a disrupted physiological effect. For example, the triple knockouts of wheat *TaMLO* were resistant to powdery mildew but also showed leaf chlorosis (Wang et al. 2014). On the contrary, ethyl methanesulfonate (EMS)-induced triple mutants with non-conservative point mutations did not show any phenotypic effects (Acevedo-Garcia et al. 2017). Thereby to rule out the negative effects, regular checks of the agronomic varieties which are generated in labs or greenhouses should be done and all the parameters should be measured and statistically recorded to find if there exists a discrepancy.

Secondly, we need to take into account the durability of the conferred disease resistance. It is an important point if we have to maintain the resistance such that the public use of the crop variety is sustained. To upscale the durability of the disease resistance, we can stack up to different resistance genes via different modes of action, we can focus on more stable systems, and we can perform good agricultural practices, such as crop rotations and use of biocontrol agents. With the help of CRISPR technology, we have created the knockout of *TaMLO* (Wang et al. 2014) and *TaEDR1* (Zhang et al. 2017) against the same disease, powdery mildew. In this way, we can stack the resistance genes and can also create multiple resistances in a single generation by multiplexing.

Thirdly, we need to overcome the limitations generated by targeted mutagenesis. True genome editing of crop plants would introduce predetermined base changes at one or several specific positions in a gene, whereas in targeted mutagenesis, random mutations are introduced.

There are three major parts of plant pathology – pathogen, host, and a favorable environment. Plant diseases occur with the presence of all three parts. By using genome editing we could constrain any one part of the system and achieve interrupted plant-pathogen interaction and thereby control the disease. To attain a long-term success with CRISPR/Cas9 technology, we need to increase our knowledge of molecular pathways, to know more about the specific genes, signaling

molecules, and receptor proteins that can be targeted for disease resistance. Altogether, genome editing has become an important tool for molecular plant–microbe interactions and disease-resistance breeding. With the ongoing development and research in this area, environmentally sustainable agriculture would improve and thereby accelerate the quality and quantity of the crop yield.

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Green Nanotechnology and Its Application in Plant Disease Management

26

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Abstract

Nanotechnology is a new approach for the management of plant diseases. Varieties of microorganisms and plant extracts have been used for the effective synthesis of green metal nanoparticles. Thus, green synthesis of nanoparticles is the most convenient, simple, and environmentally friendly way, and by avoiding the use of toxic chemicals and the generation of harmful/dangerous by-products it minimizes the side effects of chemical and physical processes. The use of nanoparticles due to their superior properties is common and has been intensively studied in recent years. The physical and in vitro effects of antimicrobial, antioxidative and non-toxic nanoparticles obtained by green synthesis are becoming increasingly important. Metal nanoparticles have both antifungal and antibacterial activity, hence these are future weapons to combat plant diseases. For effective disease management, nanoparticles must be incorporated as one of the components in integrated disease management. Future studies will probably focus on obtaining nanoparticles with antimicrobial effects at the maximum level and toxicity at the minimum level. Because of this reason, synthesizing metallic nanoparticles, especially by non-toxic green synthesis methods, which are used in many application fields.

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26.1 Introduction

Plant pathogens cause significant crop loss as observed in several crop species. In agriculture, annual crop losses due to pre- and post-harvest fungal diseases exceed 200 billion euros (Fernandez et al. 2010). Nearly one by fourth of food crops worldwide are affected by mycotoxins such as aflatoxins, ergot toxins, *Fusarium* toxins, patulin and tenuazonic acid (Schneider and Ullrich 1994). Horticultural product waste is estimated around 20–30% in developing countries, so even if we manage to reduce this amount by 5–10%, huge saves will be obtained. Reducing these losses can not only improve farmers' incomes but could also encourage more consumption of this highly nutritious fruit in a region where per capita consumption is only half of the recommended level. Now, increasing production efficiency and decreasing post-harvest wastage of products by using novel sciences such as biotechnology and nanotechnology could be counted as the best solution to this problem.

Nano is a metric measure of one billionth of a meter and covers a width of 10 atoms. In terms of comparison with real objects, an example that hair is 150,000 nanometres may be given. The term "nano" has found, in the last decade, a wider application in different fields of knowledge and sciences. Nano-science, nanotechnology, nano-particles and nano-chemistry are only a few of the new nano-containing terms that occur frequently in scientific reports, popular books and newspapers. The word nano comes from the ancient Greek through the Latin word *nanus*, which means literally *dwarf* or *very small*. According to the convention of International System of Units (SI), nano is used to indicate a reduction factor of 10^{-9} times. Hence, the word nano is typically used in nanometers (1 nm is equal to 10^{-9} m), and it encompasses systems whose size is greater than molecular dimensions and below macroscopic sizes (generally >1 nm and <100 nm). Nanotechnology is the science of the small and the very small. It is the application and manipulation of matter at a nanoscale. At this size, atoms and molecules act differently and provide a variety of novel and interesting properties and uses. The rapidly developing nanotechnology is the inter-disciplinary research and development in the field of biology, chemistry, physics, food, medicine, electronics, aerospace, agriculture and so on, which examines the synthesis or manufacture, assembly, characterization of materials smaller than 100 nanometres in scale and the application of miniature functional systems derived from these materials (Pearce 2012).

26.2 Nanoparticles and Their Properties

The process of removing toxic and waste metals from the environment through microorganisms, plants and other biological structures is achieved by means of oxidation, reduction or catalysis of metals with metallic nanoparticles. Metallic nanoparticles produced by biological or green methods are used in the biomedical field for purposes such as protection from harmful microorganisms, bio-imaging,

drug transport, cancer treatment, medical diagnosis and sensor construction because of their unique properties such as insulating, optical, antimicrobial, antioxidant and anti-metastasis properties and biocompatibility, stability and manipulability. Metallic nanoparticles can be used in the industrial field due to their catalytic activity (Singh et al. 2016). The reason for the great interest of scientists nowadays in nanotechnology is that nanoparticles can exhibit different novel properties and functions compared to normal bulk materials. The most important factors that enable production of nanostructures are the desired size, shape and properties. These enable their usage in various fields of bio-medical, agricultural and other life sciences. Other reasons for the different behaviour of nanoparticles in physical, chemical, optical, electrical and magnetic properties include the limitation of load carriers, size-dependent electronic structures, increased surface/volume ratios, and other factors incurred by the unique properties of atoms (Shah et al. 2015).

26.3 Methods of Nanoparticle Synthesis

The synthesis of nanoparticles can be natural or synthetic in origin, and they exhibit unique properties at the nanoscale. Two basic approaches that include various preparation methods are known from the past research. The first approach is "top-down" which means breaking down of solid materials into small pieces by applying external force/pressure. In this approach, many physical, chemical and thermal techniques are used to provide the necessary energy for nanoparticle formation. The second approach, known as "bottom-up", is based on gathering and combining atoms or molecules. These two approaches have advantages and disadvantages relative to each other. The top-down approach is costlier to implement, and it is impossible to obtain perfect surfaces and edges due to cavities and roughness that can occur in nanoparticles in this method. Excellent nanoparticle synthesis results can be obtained by the bottom-up approach. In the bottom-up approach, there are no waste materials that need to be removed and nanoparticles having a smaller size can be obtained with better control of their sizes.

26.4 Green Synthesis of Nanoparticles

The biological or green method of nanoparticle synthesis is represented as an alternative to chemical and physical methods because it provides an environmentally friendly way of synthesizing nanoparticles. Moreover, green synthesis does not require expensive, harmful and toxic chemicals. Metallic nanoparticles with various shapes, sizes, contents and physicochemical properties can be synthesized through the biological methods which are actively used in recent years. Green synthesis can be done using biological organisms such as fungi, bacteria, actinobacteria, yeasts, molds and algae and plants and their products. Molecules in plants or microorganisms such as proteins, enzymes, phenolic compounds, amines, alkaloids and pigments are used as reducing agents in nanoparticle synthesis (Nadaroglu et al.

2017). In traditional chemical and physical methods, reducing agents involved in the reduction of metal ions and stabilizing agents used to prevent undesired agglomeration of the produced nanoparticles carry a risk of toxicity to the environment and to the cell. Besides, the contents of the produced nanoparticles are thought to be toxic in terms of the shape, size and surface chemistry. In the green synthesis method in which nanoparticles with biocompatibility are produced, these agents are naturally present in the employed biological organisms (Hussain et al. 2016). Because of rapid development, affordable culturing costs and easy control and manipulation of the growth environment, bacteria are clearly targets in the production of nanoparticles. At the same time, it is known that some species of bacteria have special mechanisms to suppress the toxicity of metals or heavy metals. Bacteria preferred for these properties can perform nanoparticle synthesis in-situ and ex-situ. Through the use of biochemical pathways and reducing agents such as proteins, enzymes, etc., which present in the bacteria, metal ions can be reduced and precipitated for nanoparticle production (Korbekandi et al. 2009).

Plants have great potential for detoxification, reduction and accumulation of metals. These are promising, fast and economical in removing metal-borne pollutants. Metallic nanoparticles having various morphological characteristics can be produced intra-cellularly and extra-cellularly. The synthesis process is initiated by addition of extracts obtained from plant parts such as leaves, roots and fruits into the aqueous solution of metal ions. The materials present in plant extracts viz., sugar, flavonoid, protein, enzyme, polymer and organic acid, which act as reducing agents, result in bio-induction of metal ions into nanoparticles (Park et al. 2016). Synthesis of nanoparticles can be done extra-cellularly or intra-cellularly with enzymes by simply employing cultured and fast-breeding eukaryotic yeasts and molds. The incubation conditions and the metallic ion solutions used in the synthesis influence the size and distribution of the nanoparticles produced (Moghaddam et al. 2015). The shape and size of the nanoparticles mainly depend on the variation in the composition and concentration of active biomolecules in plants or microorganisms and their interaction with the metal ion aqueous solution of the precursor. In case of chemical and biological synthesis of nanoparticles, the aqueous metal ions from metal salt precursor aqueous solutions are reduced, which leads to a change in the colour of the reaction mixture and which provides qualitative indication of nanoparticle formation. The nanoparticles synthesized from green reducing agents may exhibit general toxicity, engendering serious concern for developing eco-friendly processes. The process of the formation of nanoparticles begins by mixing a metal–salt aqueous solution with plant or microbial extracts. During the synthesis of nanoparticles, biochemical reduction of the metallic salt solution starts immediately after reaction with green reducing agents and the change in the colour of the reaction mixture indicates the preliminary confirmation of the formation of nanoparticles. During synthesis, initially there is an activation period process, and in this period metal ions are converted to zero-valent state from their mono or divalent oxidation states, and hence the nucleation of reduced metal atoms occurs (Yu et al. 2016). Further, the process of nanoparticle synthesis is followed by the integration of smaller adjacent particles to form larger nanoparticles, which are thermodynamically

stable. Finally, the metal ions are reduced biologically. In this manner, growth progression and nanoparticle aggregation occur to form a variety of shapes of nanoparticles, such as spheres, cubes, triangles, rods, wires, hexagons and pentagons. In the final stage of the process, the ability of plant or microbial extracts to stabilize the nanoparticles determines the stable morphology of synthesized nanoparticles. Significantly, the size and morphology of the nanoparticles are influenced by properties of plant or microbial extracts (Niederberger and Garnweitner 2006).

Different kinds of copper (Cu) and copper oxide (CuO) nanoparticles have been synthesized from plant and microbial extracts. For example, Cu nanoparticles were biologically synthesized using magnolia leaf extracts as the reducing agent and stable nanoparticles sized from 40 to 100 nm were developed. Further, Cu nanoparticles have shown potential antibacterial activity against *Escherichia coli* (Harikumar and Aravind 2016). *Syzygium aromaticum* (clove) extracts were used in the synthesis of Cu nanoparticles. Synthesized nanoparticles were spherical to granular in morphology and their mean particle size was 40 nm (Tran et al. 2013). Cu nanoparticles were synthesized by using the stem latex of *Euphorbia nivulia* (common milk hedge). These nanoparticles were stabilized by peptides and terpenoids which were present in latex. Also *Procumbens, Calotropis procera, Tinospora cordifolia* and *Euphorbia milii* extracts were used to synthesize the smallest spherical Cu nanoparticles with the size ranging from 43 to 342 nm (Rai et al. 2009). Salem et al. (2016a, b) synthesized sulphur nanoparticles (SNPs) from sodium thiosulphate in the presence of *Punica granatum* peel aqueous extracts at room temperature. Sodium thiosulphate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) was dissolved in *Punica granatum* peel extracts under mild stirring for 10 min. at room temperature and then diluted with deionized water. Then the precipitation was centrifuged to obtain suspended sulphur nanoparticles. Awwad et al. (2015) synthesized SNPs from sodium thiosulphate in the presence of *Albizia julibrissin* fruit extracts at room temperature. Sodium thiosulphate was dissolved in *Albizia julibrissin* fruit extracts under stirring for 5 minutes at room temperature and then diluted with sterile distilled water. Further, the precipitation was centrifuged and nano pellets were dried.

Wei et al. (2009) synthesized AgNPs by reducing silver nitrate salts using non-toxic and biodegradable chitosan as a green reducing agent. Green synthesis of AgNPs was done by stirring and heating $AgNO_3$ in chitosan solution. A change in the colour from colourless to yellowish brown gave the preliminary confirmation of AgNPs. Ponarulselvum et al. (2012) synthesized AgNPs by the reaction of silver nitrate with the leaf extracts of *Catheranthus roseus.* Nanoparticle formation was confirmed by change in the colour. Banerjee et al. (2014) synthesized AgNPs by using *M. balbisiana*, *Azadirachta indica* and *Ocimum tenuiflorum* leaf extracts. Similarly, Jalaluddin et al. (2016) synthesized AgNPs (~30.5 nm) using *Aloe vera* leaf extract. *Aloe vera* leaf extract was added into the aqueous solution of silver nitrate and incubated in the dark overnight at room temperature. Complete reduction of $AgNO_3$ to Ag^+ ions was confirmed by the change in colour from colourless to colloidal brownish yellow.

26.5 Characterization of Nanoparticles

Nanoparticles are characterized by their size, morphology and surface charge using advanced microscopy techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The average particle diameter and their size distribution and charge affect the stability and distribution of the nanoparticles. Electron microscopy techniques are very useful in retrieving the shape of polymeric nanoparticles. The surface charge of the nanoparticles affects the physical stability and dispensability of the polymer dispersion and their performance.

Particle size, distribution and morphology are the most important parameters of characterization of nanoparticles. The morphology and size are measured by electron microscopy. Recently, the fastest and most popular method of determining the particle size has been dynamic light scattering (DLS). DLS is widely used to determine the size of Brownian nanoparticles in colloidal suspensions in nano and submicron ranges. Passing of monochromatic light (laser) into a solution of nanoparticles causes a Doppler shift when the light hits the moving particle and changes the wavelength of the incoming light. Scanning electron microscopy (SEM) is a revealing morphological examination with direct visualization. The electron microscopy techniques have several advantages in morphological and sizing analysis. However, they provide limited information about the size distribution and true population average. For SEM characterization, nanoparticle solutions should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold using a sputter coater. The sample is then scanned with a focused fine beam of electrons (Jores et al. 2004). The surface characteristics of the sample are obtained from the secondary electrons emitted from the sample surface. The average size obtained by SEM is comparable with results obtained by dynamic light scattering. Moreover, these techniques are time consuming, costly and frequently need complementary information about sizing distribution (Molpeceres et al. 2000).

TEM operates on a different principle than SEM. The sample preparation for TEM is complex and time consuming because of its requirement to be ultra thin for electron transmittance. The nanoparticles are deposited onto support grids or films. To make nanoparticles withstand the instrument vacuum and facilitate handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, uranyl acetate, etc., or by plastic embedding. The surface characteristics of the sample are obtained when a beam of electrons is transmitted through an ultra-thin sample, interacting with the sample as it passes through (Molpeceres et al. 2000). Atomic force microscopy (AFM) offers ultra-high resolution in particle size measurement and is based on a physical scanning of samples at the sub-micron level using a probe tip of atomic scale (Muhlen et al. 1996). The instrument provides a topographical map of the sample based on forces between the tip and the sample surface. Samples are usually scanned in contact or non-contact mode depending on their properties. In contact mode, the topographical map is generated by tapping the probe onto the surface across the sample and the probe

hovers over the conducting surface in non-contact mode. The prime advantage of AFM is its ability to image non-conducting samples without any specific treatment, thus allowing imaging of delicate biological and polymeric nano and microstructures. AFM provides the most accurate description of size. Moreover, the particle size obtained by the AFM technique provides a real picture, which helps understand the effect of various biological conditions (Polakovic et al. 1999).

Green-synthesized (*Catharanthus roseus*) silver nanoparticles were characterized using UV-Vis spectrophotometry and XRD. The result confirmed that silver nanoparticles were successfully synthesized with an average size of 35–55 nm and were crystalline in nature with face-centred cubic structure (Ponarulselvum et al. 2012). Banerjee et al. (2014) characterized the green-synthesized silver nanoparticles by SEM, which showed an average size of 35–55 nm and XRD showed that the particles were crystalline in nature. AgNPs synthesized using aqueous leaf extracts of *Urtica dioica* (Linn.) were characterized by UV-vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The results of characterization revealed that AgNPs were in the size range of 20–30 nm and crystallized in face-centred cubic structure (Jyoti et al. 2016). The synthesized sulphur nanoparticles were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The average particle diameter was found to be 20 $\pm$ 4 nm. SEM analysis showed that the nanoparticles are crystalline in nature with a spherical shape (Salem et al. 2016a, b). The list of published research papers on synthesis and characterization of nanoparticles by various instruments is depicted in the below table (Table 26.1).

26.6 Evaluation of Nanoparticles Against Plant Pathogens

Plants in nature are attacked by various kinds of pathogens which suppress their growth and productivity. In view of the huge crop losses inflicted by pathogens, various methods are used by farmers to check the pathogen attack, but none of them offer perfect holistic control of the disease. Innovative and advanced methods are needed to be integrated with conventional methods to enhance the efficiency of disease management modules. Hence, nanoparticles have great scope in the management of plant diseases in future. Singh et al. (2013) reported that among the tested 15 nano-micronutrients, $CuSO_4$ and $Na_2B_4O_7$ were found to be most effective in controlling rust disease of field peas. El-Hai et al. (2009) reported that the nano form of manganese and zinc suppressed spread of damping-off and charcoal rot diseases in sunflower. Metallic nanoparticles provide protection to plants through different mechanisms. The simplest and most obvious treatment of nanoparticles is direct application into the soil, on the seeds or foliage to protect plants from pathogen invasion. Nanoparticles such as carbon tubes, cups, rods and clays can also be used as carriers of some highly reactive chemicals such as pheromones, systemic acquired resistance (SAR) inducing chemicals, polyamine synthesis inhibitors or even concentrated active ingredients of pesticides for their controlled release, especially

Table 26.1 Synthesis and characterization of NPs by various instruments

Sl. No.	Precursor	Reducing agent	Instruments							References
			UV-Vis (λ) nm	PSA nm	XRD nm	FTIR	AFM	SEM/ FESEM	TEM/ HR-TEM	
1	$ZnCl_2$	Chitosan and NaOH	200–400	–	Crystalline ZnO Size: 30–60 nm	√	–	Shape: hexagonal Size: 100–200 nm	–	Vaseeharan et al. (2015)
2	$Zn(NO_3)_2{\cdot}6H_2O$	Chitosan	360	–	–	–	Shape: spherical to irregular	–	–	Vinay et al. (2016)
3	Zn $(O_2CCH_3)_2(H_2O)_2$	*Chlamydomonas reinhardtii* extract	–	–	√	√	–	Size: 55–80 nm Shape: Nanorod	–	Rao and Gautam (2016)
4	Zn $(O_2CCH_3)_2(H_2O)_2$	*Passiflora caerulea* extract	380	–	Zn–75.36% O–22.36% Size: 37.67 nm	√	√	Shape: spherical Size: 70 nm	–	Santhoshkumar et al. (2017)
5	ZnO	*Pseudomonas fluorescens* extract	365	67	–	–	Shape: spherical to irregular	–	–	Vinay et al. (2017)
6	$ZnSO_4$	*Fusarium* sp. extract	261	–	√	–	Size: 20–60 nm	–	–	Harishkumar and Savalgi (2017)
7	Zn $(O_2CCH_3)_2(H_2O)_2$	*Glycosmis pentaphylla*	351	–	Crystalline ZnO	√	–	Shape: spherical	Size: 36 nm	Vijayakumar et al. (2018)

					Size: 30 nm			Size: 32–40 nm		
8	$CuSO_4.5H_2O$	*Phyllanthus amarus* leaf	285	–	5–10		–	–	Size: 50 nm	Acharyulu et al. (2014)
9	$CuSO_4.5H_2O$	*Vitis vinifera* leaf	384	–	–	–	–	–	–	Angrasan and Subbaiya (2014)
10	$CuSO_4.5H_2O$	*Nerium oleander* leaf	325–370	–	–	–	–	–	–	Gopinath et al. (2014)
11	$CuSO_4.5H_2O$	*Penicillium aurantiogriseum, P. citrinum* and *P. waksmanii*	265	80–179	–	–	–	Shape: spherical	Shape: spherical	Honary et al. (2012)
12	$Na_2S_2O_3$	*Melia azedarach* leaf	–		–	–	–	Shape: spherical Size: 5–80 nm	–	Salem et al. (2016a, b)
13	$FeCl_3{\cdot}6H_2O$	*Padina pavonica* extract	–	√	√	–	–	Size: 10–27.4 nm Shape: spherical	–	El-Kassas et al. (2017)
14	$Na_2S_2O_3$	*Sophora japonica* pods	–	–	√	√	–	Shape: spherical Size: 80 nm	–	Awwad et al. (2014)
15	$Na_2S_2O_3$	*Albizia julibrissin* pods	–	–	√	√	–	Shape: spherical Size: 10–100 nm	–	Awwad et al. (2015)

under flooded conditions. Hence, to reveal the potentiality of nanotechnology in plant disease management, the effects can be discussed through two major goals and perceptions. The goals are understanding the direct effect of nanoparticles (NPs) on microorganisms/pathogens and the use of nanomaterials in formulating the pesticides, i.e. nanopesticides.

Jo et al. (2009) reported that various forms of silver ions and nanoparticles inhibited the plant pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea*. Effective concentrations of AgNPs in inhibiting the colonization by 50% (EC50) were higher for *B. sorokiniana* than for *M. grisea.* Inoculation assays in growth chamber again confirmed that both ionic and nanoparticles of silver significantly reduced the diseases caused by the above two fungi on perennial rye grass (*Lolium perenne*). Kasprowicz et al. (2010) recorded a significant reduction in the mycelial growth of *Fusarium culmorum* when spores were incubated with silver nanoparticles. Safavi et al. (2011) reported that nano silver had good antibacterial potential for removing bacterial contaminants in the tobacco plant tissue culturing process.

The effect of silver nanoparticles at different concentrations was evaluated against *Golovinomyces cichoracearum* or *Sphaerotheca fusca* on cucumber, melon and pumpkin under field conditions. The silver nanoparticle treatment (100 ppm) resulted in greater suppression of the fungal diseases when applied on the initiation of the cucurbit powdery mildew disease. The treatment also resulted in maximum growth inhibition of fungal hyphae and conidial germination in vivo (Lamsal et al. 2010). Krishnaraj et al. (2012) studied the effect of silver nanoparticles (AgNPs) on various plant pathogenic fungi viz., *Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea* and *Curvularia lunata*. The result revealed that 15 mg/L concentration of AgNPs greatly inhibited the growth of all the tested plant pathogenic fungi. Kim et al. (2012) showed the antifungal effect of AgNPs against various plant pathogenic fungi viz., *Alternaria alternata, A. brassicicola, Botrytis cinerea, Cladosporium cucumerinum, Corynespora cassiicola, Cylindrocarpon destructans, Didymella bryoniae, Fusarium oxysporum* f. sp. *cucumerinum, F. oxysporum* f. sp. *lycopersici, F. oxysporum, F. solani, Glomerella cingulata, Monosporascus cannonballus, Pythium aphanidermatum, Pythium spinosum* and *Stemphylium lycopersici* under in vitro conditions.

Al-Zubaidi et al. (2019) reported the antifungal activity of AgNPs against various pathogenic fungi. The biosynthesized AgNPs inhibited the growth of three different pathogenic fungi, including *Fusarium oxysporum, Aspergillus flavus* and *Penicillium digitatum*. Hence, AgNPs could be considered as excellent broad-spectrum antifungal agents. The minimum inhibitory concentration (MIC) of AgNPs was estimated to be 0.5–10.0 μg/mL, which was lower than that of the standard antibiotic Nystatin. Arciniegas-Grijalba et al. (2017) reported the antifungal efficacy of ZnONPs on pink disease of coffee caused by *Erythricium salmonicolor*. The inhibitory effect on the growth and morphological change includes thinning of the fibres of the hyphae and a clumping tendency of the fungus. TEM analysis noticed the changes in the ultra structure of pathogen, which include liquefaction of the

cytoplasmic content, making it less electron-dense, with the presence of a number of vacuoles and significant detachment of the cell wall.

Zinc oxide nanoparticles (ZnONPs) showed antifungal activity against *Botrytis cinerea* and *Penicillium expansum* at 12 mmol l^{-1}. ZnONPs significantly inhibited the growth of *B. cinerea* and *P. expansum.* SEM images and Raman spectra indicated that ZnONPs caused deformation in fungal hyphae and restricted the development of conidiophores and conidia (He et al. 2011). Rao and Paria (2013) reported the fungicidal efficacy of sulphur nanoparticles (SNPs) against two phytopathogens, *Fusarium solani* (causing early blight and wilt diseases) and *Venturia inaequalis* (causing apple scab disease). SNPs (35 nm) were found to be more effective compared to the bigger-sized particles in preventing fungal growth. Microscopic study confirmed that the fungicidal effect was mainly because of the deposition of particles on the cell wall, resulting in subsequent damage. The NP deposition caused an imbalance in the cell wall structure as supported by a Biuret assay test. Hence, nano sulphur fungicides can effectively control the fungal diseases of crops.

For the management of plant pathogens, copper in several formulations has been used since ancient times. Copper sulphate is a compound which has antifungal and antibacterial properties and is a key ingredient in most of the commercially available fungicides for disease management in agricultural crops. Copper nanoparticles (CuNPs) inhibit the invasion or colonization of plant pathogenic fungi viz., *Alternaria alternata*, *Fusarium oxysporum*, *Curvularia lunata* and *Phoma destructiva* (Kanhed et al. 2014). Bramhanwade et al. (2016) also reported the antifungal effect of CuNPs on the colonization of *Fusarium culmorum, F. graminearum* and *F. oxysporum* under in vitro conditions. Treatment with CuNPs caused suppression of colonization of *F. culmorum*, *F. equiseti* and *F. oxysporum*. Giannousi et al. (2013) reported that foliar application of Cu nanoparticles (11–25 nm) on tomato effectively inhibited the infection of *Phytophthora infestans* at a concentration much lower than the commercial fungicidal formulations. Similarly copper-chitosan complex nano-gels effectively inhibited *F. graminearum* (Brunel et al. 2013). Salem et al. (2019) evaluated silver nanoparticles (AgNPs) for their antifungal effects on the incidence and severity of gray mold in tomato fruits caused by *Botrytis cinerea*. The results revealed that there is no incidence of grey mold in case of tomato fruits with silver nanoparticles for forty days. Finally the lowest incidence (0.3%) and severity (1.5%) of diseases were after thirty days of cold storage in case tomato fruits were treated with Nano–Silver + Inoculated. However, the highest incidence and severity (7.5%) of grey mold was noticed after ten days of cold storage in case of treatment of tomato fruits with *Botrytis cinerea*, in comparison with the incidence and severity (1%) of the disease percentage after twenty days of cold storage in case of untreated tomato fruits (control).

Lamsal et al. (2011a, b) evaluated silver nanoparticles (AgNPs) against powdery mildew of cucumber and pumpkin under in vitro and in vivo conditions at various concentrations to determine antifungal activities. In both fields and in vitro evaluations, the application of 100 ppm AgNPs showed the highest inhibition of

Table 26.2 Evaluation of metal nanoparticles against major fungal and bacterial plant pathogens

Sl. no.	Nanoparticles	Size (nm)	Plant pathogens	Tested concentration	Effective concentrations/zone of inhibition/per cent inhibition	References
1.	Silica-silver	Silica = 20 Silver = 100	*Pythium ultimum, Magnaporthe grisea, Colletotrichum gloeosporioides, Botrytis cinerea, Rhizoctonia solani Bacillus subtilis. Azotobacter chrococuum, Pseudomonas syringae* and *Xanthomonas compestris pv. vesicatoria*	0.3, 3, 10 and 100 ppm	*P. ultimum, M. grisea, C. gloeosporioides and B. cinerea, R. solani* = 10 ppm *B. subtilis. A. chrococuum, R. tropici, P. syringae* and *X. c. pv. vesicatoria* = 100 ppm	Park et al. (2006)
2.	Silica-silver	Silica = 20 Silver = 100	*Sphaerotheca fuliginea*	0.3 ppm	Squash powdery mildew = 100% disease control	Park et al. (2006)
3.	Sulphur	50	*Aspergillus niger*	125, 500, 1000 and 2000 ppm	24.33 mm = 2000 ppm Control = 20.33 mm	Choudhurya et al. (2009)
4.	Silver	25	*Bipolaris sorokiniana* and *Magnaporthe grisea*	25, 50 and 200 ppm	50 and 100 ppm = most effectively inhibited the spore germination	Jo et al. (2009)
5.	Nano-silver liquid	7–25	*Sclerotium cepivorum*	1, 3, 5, 7, 10, 25, 50 and 100 ppm	90% inhibition of *S. cepivorum* = 7 ppm	Jung et al. (2010)
6.	Silver	4–8	*Colletotrichum gloeosporioides*	10, 30, 50 and 100 ppm	90% inhibition of fungal mycelium under in vitro = 100 ppm lowest disease incidence (9.7%), over the control (84.1%) at 50 ppm	Lamsal et al. (2011a, b)
7.	Zinc oxide and magnesium oxide	30 & 50	*Alternaria alternate, Fusarium oxysporum* and *Rhizopus stolonifer*	0.3, 0.2 and 0.1 ml	Spore germination inhibition *A. alternata* = 44.94 to 9.80% *F. oxysoprum* = 66.28 to 12.96% *M. plumbeus* = 41.54 to 6.40%	Wani and Shah (2012)

8.	Sulphur		*Erysiphe cichoracearum* of okra	100 ppm	*E. cichoracearum* = least conidial germination (4.56%)	Gogoi et al. (2013)
9.	Copper	3–10	*Phoma destructiva* (DBT-66), *Curvularia lunata* (MTCC no. 2030), *Alternaria alternate* (MTCC no. 6572) *Fusarium oxysporum* (MTCC no. 1755)	20 μg/disc	*P. destructiva* = 22 ± 1 mm *C. lunata* = 21 ± 0.5 mm *A. alternate* = 18 ± 1.1 mm *F. oxysporum* = 24 ± 0.5 mm	Kanhed et al. (2014)
10.	Copper	32	*Exserohilum turcicum*	1–2000 ppm	250 ppm = 100% spores inhibited	Chikkanna et al. (2016a)
11.	Copper	58–100	*Exserohilum turcicum*	1800 ppm	No inhibition	Nargund et al. (2016c)
12.	Silver	50–200	*Curvularia lunata, Xanthomonas axonopodis* pv. *punicae*	169 ppm	*C. lunata* = 95% spores inhibited *Xap* = 9.25 mm	Nargund et al. (2016a)
13.	Copper, sulphur	30–80 <50	*CuNPs* = *Xap* and *Xac* *SNPs* = cucumber powdery mildew	1–2000 ppm 500 ppm	CuNPs = *Xap* & *Xac* = no inhibition SNPs = recorded only 20% PDI	Nargund et al. (2016b)
14.	Zinc oxide	<100	*Pectobacterium carotovorum* subsp. *wasabiae*	1–30 mg/ml	Effective conc. = 30 mg/ml Zone of inhibition = 32 mm	Hafez et al. (2014)
15	Iron oxide	17–42	*Xanthomonas* sp. *Proteus vulgaris*	10–50 mg/ml	Effective conc. = 50 mg/ml *Xanthomonas* sp. = 14 mm *P. vulgaris* = 22 mm	Prabhu et al. (2015)
16	Zinc oxide	<100	*Xanthomonas axonopodis* pv. *citri* *Aspergillus* sp. *Penicillium* sp.	1–50 μg/ml	Effective conc. = 30 μg/ml *Xac* = 16 mm Spore germination and mycelial inhibition in *Aspergillus sp.* and *Penicillium sp.*@ 30 μg/ml	Poovizhi and Krishnaveni (2015)
17.	Zinc oxide	<100	*Aspergillus fumigatus* *Aspergillus flavus*	20, 40, 60 and 80 μg/ml	Mycelia inhibition of both fungi @ 80 μg/ml = 75%	Navale et al. (2015)

(continued)

Table 26.2 (continued)

Sl. no.	Nanoparticles	Size (nm)	Plant pathogens	Tested concentration	Effective concentrations/zone of inhibition/per cent inhibition	References
18.	Zinc	<100	*Peronospora tabacina*	1–10 mg/l	Inhibited leaf infection @ 10 mg/l Reduction of germ tube elongation @ 5 mg/l = >60%	Wagner et al. (2016)
19.	Silver	50–200	*Curvularia lunata* *Xanthomonas axonopodis* pv. *punicae*	169 ppm	*C. lunata* spore germination inhibited = 95% *Xap* = 9.25 mm	Nargund et al. (2016)
20.	Copper	32	*Exserohilum turcicum*	1–2000 ppm	Inhibition of spore germination @ 250 ppm = 100%	Chikkanna et al. (2016b)
21.	Iron oxide	35	*Escherichia coli* *Staphylococus aureus*	10–50 mg/ml	Effective conc. = 50 mg/ml *E. coli* = 36 mm *S. aureus* = 30 mm	Manyasree et al. (2016)

disease in field and maximum inhibition of growth of fungal hyphae and conidial germination in vitro. The foliar spray technique was used to apply silver nanoparticles on pumpkin plants 3–4 weeks before the outbreak of the disease and after disease occurrence, and distilled water was used as control. Gogoi et al. (2013) evaluated the one Sulphur NPs ,and three commercial products viz., commercial sulphur (Merck), commercial nano-sulphur (MK Impex, Canada) and Sulphur 80 WP (Corel Insecticide) were evaluated in vitro for their fungicidal efficacy at 1000 ppm against *Erysiphe cichoracearum* of okra. All the sulphur fungicides significantly inhibited the germination of conidia of *E. cichoracearum* as compared to control. The lowest conidial germination was noticed in synthesized nano-sulphur treatment (4.56%) followed by Canadian nano-sulphur (14.17%), sulphur 80 WP (15.97%) and control (23.09%). Cleistothecial appendages were also disrupted in contact with nano-sulphur and the cleistothecia became sterile. Miguel et al. (2011) evaluated the antifungal activity of AgNPs against *Colletotrichum gloeosporioides*, which causes anthracnose of papaya fruits. Two different mean nanoparticle sizes (5 and 24 nm) and various concentrations (13, 26 and 52 μg silver/mL PDA) of AgNPs were tested against anthracnose of papaya in vitro. The inhibition of the fungus was maximum (90%) at 52 μg/mL. The various nanoparticles that were tested against different plant pathogens are presented in Table 26.2.

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Plant Disease Management in Organic Farming System: Strategies and Challenges

27

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Abstract

Organic farming can be stated as an ecologically, economically, and socially dependable way of farming that may provide continuous supply of safe and healthy food and fibres, with the least probable loss of nutrients and energy, and the least negative impacts on the environment. Though the use of chemical inputs in agriculture is inevitable to combat dreaded pests and meet the growing demand for food in a populous nation like India, there are opportunities where organic production can be encouraged to meet the domestic and export demand for fresh fruits and vegetables. Organic agriculture can be seen as a pioneering effort to create sustainable development based on different principles compared to mainstream agriculture. Modification in cultural practices, mechanical destruction of the source of inoculum, clean cultivation, use of organic amendments, developing pesticides of organic origin, encouraging biocontrol agents, use of cover and trap crops and use of heat treatment, cold temperature, solar energy, etc. can be conveniently used to manage disease incidence below the economic injury level under organic farming. This chapter provides an overview of the potential role and challenges of organic agriculture in this global perspective where organic farming servs as an alternative example for the broader implementation of ecological justice in agriculture and society.

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27.1 Introduction

The human civilization from "hunting and gathering" to present day agriculture has underwent several changes. The fast changes witnessed depletion of production bases in many fertile grounds coupled with contamination of natural resources and human health hazards, which warranted a change in the direction of agriculture for sustainable development. Modern agriculture and food systems, including organic agriculture, are undergoing a technological and structural modernization with growing globalization. Organic agriculture can be seen as a pioneering effort to create sustainable development based on different principles compared to mainstream agriculture (Kristensen 2005). Mainstream agriculture is conventional agriculture where externally industry-produced inputs such as fertilizers and pesticides are used. The indiscriminate use of fertilizers and plant protection chemicals to increase the yield potential and save the crops from insect pests and diseases respectively, no doubt, has doubled or tripled our total food production that has helped us to sustain low cost along with more food supply, but has also created a number of health hazards and deteriorated the agro-ecosystem badly. Their negative impacts far outweigh their social benefits. In recent years, more ecological approaches are now being researched and there has been a world wide swing to the use of eco-friendly methods for protecting the crops from pest and disease. The challenge today is how to achieve not only food security but also food safety (Rawat 2011). This situation has compelled us to switch over to organic agriculture to cultivate valuable crops and safer foods for health and at the same time to safeguard our environment.

27.2 Concepts and Definitions of Organic Agriculture

The concept of organic agriculture has been perceived differently by different people. To most of them, organic agriculture is a way of agriculture that relies on ecosystem management rather than external agriculture inputs. This approach excludes the use of synthetic inputs, such as synthetic fertilizers and pesticides, and genetically modified organisms and usually subscribes to the principles of sustainable agriculture. Organic agriculture has been defined by Lampkin (1990) as a production system which avoids or largely excludes the use of synthetic compounded fertilizers, pesticides, growth regulators and livestock feed additives. Palaniappan and Annadurai (1999) stated that the organic agriculture system depends on different approaches and their integrations, which include crop rotation, crop residues, animal manures, legumes, green manures, off-farming wastes and the aspect of biological pest and disease control, to maintain soil productivity and tilth to

supply plant nutrients, and to control insects, pests and weeds. Later on the Food and Agriculture Organization (1999) defined organic agriculture as a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity. This definition aims at three strategies viz., (i) the use of management practices in preference, (ii) the use of off-–farm inputs and (iii) taking into account the regional conditions that require locally adapted systems. Therefore, the basic concept of these definitions is to maintain the soil as a living system that develops the activities of beneficial micro-organisms present in the soil.

Organic agriculture is not the simple replacement of chemical fertilizers, herbicides, fungicides and insecticides with biologically active formulations and other organic inputs; instead it adopts broad management practices to improve the soil productivity by enhancing soil health, soil life and mineral particles. Soil air and water exist in a stage of dynamic equilibrium and regulate the ecosystem processes in mutual harmony by complementing and supplementing each other. In healthy soil, the population of soil microfauna and microflora multiplies rapidly, which in turn sustains the biochemical process of dissolution and synthesis at a high rate, and as a result, the regeneration capacity of soil will enhance making it resilient to absorb the effects of climate variability and occasional failures in agronomic management. The principal elements to be considered while practicing organic agriculture are: (i) maintaining a living soil, (ii) making all the essential nutrients available, (iii) organic mulching for conservation and (iv) attaining a sustainable high yield (Palaniappan and Annadurai 1999).

In traditional agriculture, generally, we use chemical inputs (synthetic pesticides and water-soluble synthetically purified fertilizers) while in organic agriculture these inputs are replaced with natural inputs (bio pesticides and bio fertilizers). Therefore, organic agriculture integrates the scientific knowledge of ecology, developed modern technologies and conventional agricultural tactics based on natural biological processes. The motive of organic farming is to raise the crops in such a way that it keeps the soil alive and healthy by incorporating organic wastes and bio-fertilizers in the soil and releases nutrients to the crops for sustainable production in an eco-friendly pollution-free environmental condition. The basic rules of organic agriculture are based on crop rotation, incorporation of green manures in soil, biological control of diseases and insect pests along with mechanical cultivation of crops. The enhancement of crop productivity depends on the integration of these basic rules, because crop rotations renew the soil and confuse pests, incorporation of legume crops in rotation fix the atmosphere-free nitrogen in the soil, while mulches in the crops are used to conserve soil moisture and control diseases and weeds. Fi (2006) argued that some of the methods developed for organic agriculture have been borrowed by more conventional agriculture such as integrated pest management, which is a multifaceted strategy that uses various organic methods of pest control whenever possible, but could include synthetic pesticides only as a last resort.

27.2.1 Why Organic Agriculture?

- To produce food of high nutritional quality in sufficient quantity
- To work with natural systems rather than seeking to dominate them
- To encourage and enhance the biological cycles within the farming system involving microorganisms, soil flora and fauna and plants and animals
- To maintain and increase the long-term fertility of soil
- To use, as far as possible, renewable resources in a locally organized agriculture system
- To give better conditions of life to all livestock that allow them to perform all aspects of their innate behaviour
- To avoid all forms of pollution that may result from agricultural techniques
- To maintain the genetic diversity of the agricultural system and its surroundings, including protection of both plant and wildlife habitats
- To consider the wider social and ecological impact of the farming system

27.3 Components of Organic Agriculture

The ideal organic agriculture approach is that where the conservation of biological potential of soil and other natural resources is maintained by adopting the integrated crop models (Fig. 27.1). These models are devoid of chemical inputs, by which the interaction among beneficial soil microorganisms is stimulated and sustained and the soil life and health are also improved. Crop production and health in organic farming systems are attained through a combination of structural factors and tactical management components to ensure products of sufficient quality and quantity for human and livestock consumption. Following are the important components of organic agriculture:

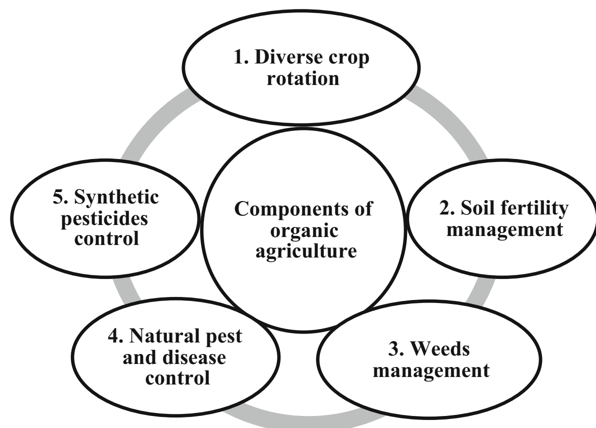

Fig. 27.1 Components of organic agriculture

27.3.1 Diverse Crop Rotation

Diversification of crops helps to maintain soil health and enhances its efficiency to produce more food by reducing disease and pest incidence enhancing the soil rhizosphere system and soil fertility and renewing the root zone soil. Divers crop rotation systems include many cropping systems such as rotational cropping, sequential cropping, intercropping, multistoried cropping system, etc. These cropping systems can be adopted in organic agriculture to reduce soil erosion and soil-borne diseases and improve weed control and soil water holding capacity. The success of crop rotation depends on the choice of crops and their varieties and spatial and temporal design. According to Stockdale et al. (2001), development and implementation of well-designed crop rotations is central to the success of organic agriculture. So crop rotation can be designed in such a way as to improve soil health and minimize the spread of weeds, diseases and pests as stated by Altieri (1995). Herridge et al. (2008) observed that the inclusion of pulse crops in the farming system can enhance the availability of nitrogen in the soil. Enhancement of soil nitrogen level is due to the ability of many pulse crops to fix atmospheric nitrogen through symbiosis with *Rhizobium*, as stated by Peoples et al. (1995). Pulse crops in the rotation not only enhance the crop yield and nitrogen use efficiency but also decrease inputs of inorganic fertilizers, as argued by Gan et al. (2009). Kirkegaard and Ryan (2014) reported that dry pea and lentil crops use 15–35% less water than cereal and oilseed crops, thereby enhancing water-use efficiency in the semiarid northern regions.

27.3.2 Soil Fertility Management

Management of soil fertility in organic agriculture is a core component, because efficient management of nutrients and soil structure ensure good yield of crops, while poor management can result in poor yield, poor animal health and increased environmental pollution. In organic agriculture, soil fertility can be improved by inclusion of organic manures, crop residues, dung and urine from domesticated animals, wastes from slaughter houses, human excreta and sewage, biomass of weeds and organic wastes from fruit and vegetable production and processing units. For the good physical conditions of soil viz., soil structure, aeration and water holding capacity, it is essential to have high amounts of organic matter. Organic farming uses a variety of methods to improve soil fertility, including crop rotation, cover cropping, reduced tillage and application of compost. Due to zero tillage or less tillage, soil is not much disturbed, less exposed to air and the loss of soil carbon to the atmosphere is minimum, resulting in higher organic carbon depositions into the soil, which can reduce the effect of greenhouse gases and help fight changing environmental conditions. The other tactics to enhance soil fertility through biological nitrogen fixation can be achieved by using *Azolla*, *Blue green algae* for rice, *Rhizobium* for pulses and *Azatobactor* and *Azospirillum* for other crops. Different crops can fix different amounts of nitrogen into the soil. For

example, white clover can fix up to 250 kg N ha^{-1} year^{-1}, as reported by Kristensen et al. (1995), red clover up to 240 kg N ha^{-1} year^{-1} (Schmidt et al. 1999) and lucerne fix up to 500 kg N ha^{-1} year^{-1}, as recorded by Spiertz and Sibma (1986), while Van Kessel and Hartley (2000) estimated up to 200 kg N ha^{-1} year^{-1} fixed by field beans.

Plants require a number of nutrients in varying quantities for growth and development. Supplying enough nitrogen, and particularly synchronization, so that plants can get enough nitrogen at the time when they need it most is a challenging task for organic farmers, as stated by Watson et al. (2002). They also observed that crop rotation, green manure and intercropping fix nitrogen into the soil from the atmosphere through symbiosis with rhizobial bacteria, the crop residues can be ploughed back into the soil, and different plants leave different amounts of nitrogen, potentially aiding synchronization, but the competition between legumes and crops can be problematic and thus wider spacing between crop rows is required. Organic farmers also use animal manure, certain processed fertilizers such as seed meal and various mineral powders such as rock phosphate and green sand, a naturally occurring form of potash that provides potassium. Gillman (2008) stated that in some cases pH may need to be amended through natural pH agents, i.e. lime and sulfur, but in the United States some compounds such as iron sulfate, aluminum sulfate, magnesium sulfate and soluble boron products are allowed in organic farming. Biological research of soil and soil organisms has proven beneficial to organic farming because these soil microorganisms (bacteria and fungi) break down chemicals, plant matter and animal waste into productive soil nutrients, resulting in healthier yield and more productive soil for future crops, as argued by Ingram (2007), while fields with less or no manure display significantly lower yield due to decreased soil microbe community. Similarly, Fliebbach (2006) observed that increased manure improves biological activity, providing a healthier, more arable soil system and higher yields. Soil microbial biomass gets benefitted by the introduction of organic amendments because microorganisms use the available C more efficiently and also contribute to nutrient mineralization (Fliebbach and Mader 2000).

27.3.3 Weed Management

Weeds are identified as a major problem in organic farming, so an appropriate integrated strategy is necessary to control weeds in the organic agriculture system. Experimentally it has been proved that deep summer ploughing, inter-cultivation tactics, field operation time, timely sowing, line sowing, mulching and soil solarization suppress weed germination and favour crop growth and development. Kathleen and Robert (2003) stated that organic weed management promotes weed suppression, rather than weed elimination, by enhancing crop competition and phytotoxic effects on weeds of cover crops and crops with dissimilar life cycles to discourage weeds associated with a particular crop. They also suggested other cultural practices to enhance crop competitiveness resulting in reduced weed pressure viz., selection of competitive crop varieties, high-density planting, tight row spacing and late planting

into warm soil to encourage rapid crop germination. Szykitka (2004) classified the mechanical and physical weed control practices into different groups such as: *tillage* – turning the soil between crops to incorporate crop residues and soil amendments; removing existing weed growth and preparing a seedbed for planting; turning soil after seeding to kill weeds, including cultivation of row crops; *mowing and cutting* – removing top growth of weeds; flame weeding and thermal weeding – using heat to kill weeds; and *mulching*– blocking weed emergence with organic materials, plastic films, or landscape fabric. However, still researches are required to develop organic methods that suppress the growth or germination of common weeds through promoting the growth of natural microorganisms.

27.3.4 Natural Pest and Disease Control

Pest and disease management without the use of synthetic plant protection chemicals is an important feature of organic agriculture. Plant protection in organic agriculture is based on the maintenance of on-farm diversity, improvement of soil and plant health through crop diversification and the use of bio-agents and plant-based biopesticides. Organic agriculture, which is free from the indiscriminate and irrelevant use of synthetic chemicals, results in an increase in the population of naturally occurring beneficial insects and micro-organisms that are known as bio-control agents, having potential to control insects and diseases in organic farming. In India, organic farmers predominantly use neem oil, fermented butter milk, cow urine, panchgavya and baking soda for the control of pests as well as diseases in organic farming. Butter milk and panchgavya have potential to control foliage diseases in plants. Organic farmers also use baking soda for the control of mildew and rust diseases on plants, while they use butter milk against blight, mildew, mosaic virus and other fungal and viral diseases. Amrit paani, which is a fermented product of cow dung and urine, is used by Indian farmers to enhance crop growth and disease management. Such fermented solutions are known to have high bacterial population of cellulose degraders, nitrogen fixers, P-solubilizers, plant growth promoters and antagonists of disease-causing fungi, as reported by Venkateswarlu et al. (2008).

The use of essential oils extracted from aromatic plants as insecticides has increased considerably owing to their popularity with organic growers and environmentally conscious consumers, as stated by Hikal et al. (2017). According to Shelton et al. (2002), these plant-based products have repellent, insecticidal, antifeedant, growth inhibitory, oviposition inhibitory, ovicidal and growth-reducing effects on a variety of insects and pathogens. Organic farmers are allowed to use naturally occurring insecticides such as *Bacillus thuringiensis* (a bacterial toxin), pyrethrum (a chrysanthemum extract), spinosad (a bacterial metabolite), neem (a tree extract) and rotenone (a legume root extract) in organic farming. But only 10% organic farmers use these naturally derived insecticides regularly, according to the survey carried by Lotter (2003). These pesticides are not always safe or environmentally friendly than synthetic pesticides and can cause harm, as stated by Gillman (2008). Marking and Bills (1976) said that rotenone and pyrethrum are particularly

controversial because they work by attacking the nervous system, like most conventional insecticides, while rotenone is extremely toxic to fish and can induce symptoms resembling Parkinson's disease in mammals, as argued by Panov et al. (2005). Pyrethrum (natural pyrethrins) is more effective against insects when used with piperonyl butoxide (which retards degradation of the pyrethrins), as reported by Jones (1998). Scheuerell and Mahaffee (2004) said that the compost tea contains a mix of beneficial microbes, which may attack or out-compete certain plant pathogens but variability among formulations and preparation methods may contribute to inconsistent results or even dangerous growth of toxic microbes in compost teas, as stated by Brinton et al. (2004). They also said that some naturally derived pesticides like nicotine sulfate, arsenic and strychnine are not allowed for use in organic farms.

27.3.5 Synthetic Pesticide Control

The oldest synthetic fungicide is Bordeaux mixture which is prepared by reaction of copper sulphate and calcium hydroxide in water to manage a number of diseases in agricultural and horticultural crops. Gayon and Sauvageau (1903) stated that the first copper-based antimicrobial compound used in agriculture was Bordeaux mixture, which was accidentally discovered in 1885 by a French scientist, Pierre-Marie Alexis Millardet. Copper acts in two ways, (i) as a co-factor for several enzymes involved in respiration and electron transport proteins in all living organisms including plants (Sommer 1931) and (ii) as a broad-spectrum fungicide at higher concentrations due to its interaction with nucleic acids, disruption of enzyme active sites, interference with the energy transport system and finally the disruption of the integrity of cell membranes, as argued by Fleming and Trevors (1989). A wide range of copper-based antimicrobial compounds are formulated for the use of foliar disease management in annual and perennial crops, especially in organic agriculture since the application of conventional fungicides is prohibited in this system.

Diseases caused by oomycetes viz., downy mildew of grapevine and late blight of potato are mostly managed by using copper-based antimicrobial compounds in organic farming (Tamm et al. 2015; Finckh et al. 2015a, b). However, apple scab and various coffee diseases which are difficult to manage without fungicides also benefit from the use of copper-based antimicrobial compounds, as reported by Holb et al. (2003) and Souza et al. (2015). The other diseases, i.e. tomato spot (Roberts et al. 2008), citrus canker (Behlau et al. 2017), fire blight of pome fruits (Elkins et al. 2015), walnut blight (Ninot et al. 2002), stone fruit canker (Sayler and Kirkpatrick 2003), mango apical necrosis (Cazorla et al. 2006) and olive knot (Teviotdale and Krueger 2004) are controlled by using copper-based antimicrobial compounds in organic farming. However, copper sulfate and Bordeaux mixture (copper sulfate plus lime) approved for organic use in various jurisdictions can be more environmentally problematic than some synthetic fungicides disallowed in organic farming, as stated by Edwards-Jones and Howells (2001). Application of copper-based formulations like copper sulfate and copper nitrate in agricultural soil either in too high dose or too

frequently may cause plant stress and reduce soil fertility, having adverse effects on the crop yield and quality (Dumestre et al. 1993), because these formulations release Cu ions when they are dissolved in water and thus an excessive uptake of Cu ions by plants at any time may lead to damage, also known as phytotoxicity. Lamichhane et al. (2018) observed that the prolonged application of copper-based antimicrobial compounds for over a century has resulted in accumulation of this heavy metal in the soil particularly at upper 15 cm top soil. However, the potential toxicity of Cu varies from one soil to another independently of the concentration of Cu accumulated in the soil, for example, alkaline soils with increased calcium availability ameliorate the effects of Cu phytotoxicity, and downward movement of copper through the soil profile is greater in sandy soils than soils rich in clay or organic matter, as reported by Alva et al. (1993 and 1995). Furthermore, copper availability and toxicity in the soil is greatly increased as the soil pH decreases below 5.5, as observed by Fan et al. (2011). Therefore, research is also needed to seek alternatives for replacement of copper-based fungicides in organic agriculture.

27.4 Principles of Organic Agriculture

Organic farming has set out to be an alternative to conventional agriculture, and Luttikholt (2007) stated that it is based on principles and values. The four basic principles which are the roots from which organic agriculture develops are described below:

1. Health

The first principle of organic agriculture is health, which is sustained and enhances the health of soil, plant, animal and human as one and indivisible, as suggested by IFOAM (2006). According to this principle, there is a relationship between healthy soil and human and animal health, because healthy crop produced from healthy soil fosters human and animal health. Balfour (1943) argued that the living soil is a necessary condition for healthy plant growth and human being. The key characteristics of health in organic farming are immunity, resilience, regeneration and maintenance of social, ecological, mental and physical well-being. Therefore, the goal of organic agriculture is to produce healthy and quality food by avoiding chemicals like fertilizers and pesticides that contributes to preventive health care of humans, animals and ecosystem.

2. Ecology

Organic agriculture should be based on living ecological systems and cycles, work with them, emulate them and help sustain them, as suggested by IFOAM (2006). According to this principle, the living ecosystem is the heart of organic agriculture because nutritious food can be achieved through ecology of specific production environment, which is based on ecological process and recycling. Darnhofer et al. (2010) elaborated the meaning of ecology in organic management, to build up resilience of the agro-ecological system, attain ecological balance through

cropping systems, inputs reduced by reuse, recycling, efficient management and must be adapted to local conditions, ecology, culture and scale.

3. Care

Organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment as suggested by IFOAM (2006). Kirchmann et al. (2008) argued that the caring for the environment is a basic principle necessary for its sustainability in order to provide humans with well-being, food and other essentials. Scientific knowledge alone is not sufficient, practical experience, accumulated wisdom and traditional and indigenous knowledge offer valid solutions, tested by time as reported by Kirchmann et al. (2008). Therefore, the key concern in organic management, development and choices of technology to organic agriculture should be precaution and responsibility.

4. Fairness

Organic agriculture should be built on relationships that ensure fairness with regard to the common environment and life opportunities, as suggested by IFOAM (2006). The principle of fairness adds new aims to organic agriculture, not explicitly addressed by the pioneers, such as equity, respect, justice, eradication of poverty, animal welfare, equitable systems for distribution and trade as well as social costs as stated by Kirchmann et al. (2008) This principle insists that organic agriculture should prevent significant risks by adopting appropriate technologies and rejecting unpredictable ones. Therefore, fairness requires systems of production, distribution and trade that are open and equitable and account for real environmental and social costs (IFOAM, 2006).

27.5 Strategies for Plant Disease Management in Organic Farming System

Occurrence of a disease depends on the balanced interaction among host, pathogen and environment. Disease management practices (Fig. 27.2) under organic agriculture aim to disrupt this balance and disallow the pathogen to cause disease beyond the economic injury level. Pathogens need suitable environmental conditions like humidity, temperature, moisture, host exudates, etc. to germinate, survive and infect. In the absence of these, pathogens cannot survive and perish. Most of the strategies under agronomic, physical, biological and genetic methods described below interfere with the micro-environmental conditions to make them uncongenial for pathogen propagation, multiplication and initiating infection. Further, a majority of these strategies are specific to a particular disease in a crop and hence a combination of strategies based on the crop growth stages and disease cycle need to be integrated as a module for a crop in a particular agro-climatic region. The following methods are used for plant disease management under organic agriculture:

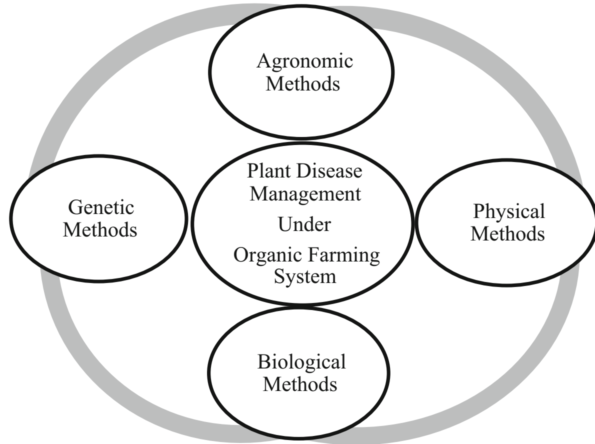

Fig. 27.2 Methods of plant disease management in organic farming system

27.5.1 Agronomic Methods

The crop productivity potential depends on its production environment and farmers expertise to identify and overcome the factors that minimize the production potential. Modification in crop management tactics to disallow disease development is one of the oldest and mostly accepted methods in plant disease management. The following agronomic practices can be helpful for management of plant diseases under the organic agriculture system.

(i) Sanitation

The aim of field sanitation is to completely or partially destroy the source of infection present in the soil. The periodic clean off of diseased plants from a population is a basic sanitary precaution of organic agriculture. For the viral disease control, field sanitation is one of the effective recommendations. It includes removal of diseased plants, pruning of infected parts of plants, removing or effectively treating plant material, burning infected crop stubble, etc. and preventing the inoculum from finding suitable infection courts, by preventing wounding, establishing barriers and defoliation (Zentmyer and Bald 1977). Sanitation particularly is applicable to pathogens that do not spread from plant to plant in the field and that require a large amount of inoculum to develop an epiphytotic condition when crops are grown in the same field for several years. As an example, a sanitation practice for late blight of potato is to eliminate the refuse piles where infected tubers give an early start for the disease (Finckh et al. 2006). In vineyards and orchards, diseased branches are pruned away and plant residues are removed from greenhouses (Finckh et al. 2015a, b).

(ii) Crop Residue Management

Crop residues are non-economic plant parts that are left in the field after harvesting. The organic carbon in the soil can be enhanced through decomposition of crop residues that foster soil health and disease control. Generally, the growth of

pathogen spores, their sporulation and survival depend on the crop residue amount and quality that is left in the field after harvesting of host and non-host plants. After decomposition of the crop residues through the release of fungicidal and fungistatic compounds, it provides food to facultative pathogens for feed on. The factors that determine the rate of decomposition are depth of placement, type of crop, quantity of residues, allelopathic interactions among existing soil biota and time as stated by Bailey and Lazarovits (2003). They also reported that the partial disease control can be attained using residue management methods, such as tillage and crop rotation that lower the pathogen's inoculum density in the soil, reduce its ability to survive, deprive the pathogen of its host, and create conditions that favour the growth of other microorganisms at the expense of the pathogen. Palaniappan and Annadurai (1999) suggested two principle methods of residue decompositions, (i) thermo-chemical including direct combustion, pyrolysis, liquefaction and gasification with air or oxygen and (ii) biological including anaerobic digestion and hydrolysis followed by fermentation.

(iii) Tillage

The basic objective of tillage is to prepare seed-bed for seeding by sizeable disturbance of the soil, while zero tillage or single tillage involves minimum amount of soil disturbance. Lupwayi et al. (1999) stated that the organic matter accumulation through sequestering carbon in the soil can be achieved by minimizing tillage, and as a result the rate of decomposition increases by microflora and microfauna, as reported by Kennedy and Smith (1995). Soil having higher organic matter showed their potential to prevent spore germination of *Cannabis sativa* and the total population of soil microbes enhances with increasing soil organic matter as observed by Chinn (1967). Plough practices directly or indirectly displace the spore of pathogens through the crop residue placement in the soil, which fosters the activity and competition among soil microbes (Cook 1990). Therefore, reduce tillage practices play an important role for disease management, because they minimize the potential of disease-causing agents by removing the primary source of inoculums.

(iv) Crop Rotation

Crop rotation is a long established practice to reduce the activity, pathogenesis and survival of soil-borne fungi, nematodes or other pathogens (Baker and Cook 1974). Typical examples of soil invaders that can be controlled in 3–4 year rotation with non-host crops include the organisms causing cabbage black rot, bacterial blight of bean, cabbage blackleg and bean anthracnose. Sequeira (1958) stated that the bacterial wilt of banana can be controlled by disking the surface 24 cm of soil during the dry season followed by 9 months fallow taking advantage of the sensitivity of the causal organism to desiccation. Long periods of rotation are often necessary particularly for soil inhabitants and pathogens that survive by means of sclerotia or thick-walled resting spores. The development and implementation of well-designed crop rotations is central to the success of organic production systems (Stockdale et al. 2001). However, crop rotation can be ineffective if the pathogen is long-lived in the soil with a wide host range. For

the formation and maintenance of healthy soil through organic crop rotation, it should comprise multi-year grass ley or grass-legume ley or an alfalfa crop, as stated by Van Bruggen and Termorshuizen (2003). The effectiveness of crop rotation in disease management depends on (i) wide host range, (ii) mechanisms of pathogen survival, (iii) amount of inoculums, (iv) crop susceptibility, (v) types of crops that stimulate the formation of resting structure, (vi) poor crop residue management, (vii) residue management techniques, (viii) frequency of soil infestation with pathogens from external sources and (ix) type of soil conducive to diseases.

(v) Soil Disinfestations

Soil disinfestations play an important role for reducing the initial inoculum. The following methods can be used for soil disinfestations under organic agriculture.

(a) Flooding

This is a pre-planting practice which can be regarded as soil disinfestation treatment. The harmful effect of flooding on soil-borne pathogens may be related to lack of oxygen, increased CO_2 or various microbial interactions, e.g. production of substances that are toxic to the pathogen upon anaerobic processes (Bruehl 1987). A classic case of management on a large scale was demonstrated with the panama wilt disease of banana caused by *Fusarium oxysporum* f.sp. *cubense* (Stover 1962). The soil is flooded for 3–4 months or more with a minimum of 30 cm of water. Flooding is not effective when large populations of the pathogen are present, or in soil which contain unknown factors which may favour the pathogen. However, flooding can only be used as a cultural practice for disease management only in countries where large resources of water are available (Patil 1981).

(b) Soil Solarization

In soil solarization, moist soil is covered with transparent, UV-resistant plastic and exposed to sunlight for a few weeks (Gamliel and Katan 2012). Most plant-pathogenic fungi, bacteria and nematodes, except for some heat-tolerant fungi and viruses, are quite sensitive to increased temperatures, i.e. 45–55 °C (Klein et al. 2011). The solarization effect can be enhanced by incorporation of isothiocyanate producing residues from brassica crops into soil before covering with plastic (Klein et al. 2012). Along with the direct heat effects on pathogens, soil solarization can also enhance plant growth by increasing the availability of mineral nutrients and improving soil tilth (Gamliel and Katan 2012). Fungal diseases such as damping-off, root rots, stem rots, fruit rots, wilts and blights caused by *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., *Sclerotium rolfsii, Rhizoctonia solani, Sclerotinia sclerotiorum,* and *Verticillium* spp. can easily be managed by soil solarization. Similarly, a number of bacterial diseases like bacterial canker of tomato and nematode diseases such as *Ditylenchus dipsaci, Globodera rostochiensis, Heterodera* spp., and *Meloidogyne* spp. have been successfully managed in fields by soil solarization (Akhtar et al. 2008).

(c) Anaerobic Soil Disinfestations

In anaerobic soil disinfestations, fresh organic material is incorporated into soil, and the soil is moistened and covered by airtight plastic for 3–6 weeks (Momma 2008). Proliferating bacteria deplete the available oxygen until anaerobic bacteria continue to decompose the carbon source. Toxic products, including alcohols, aldehydes, organic acids and other volatile compounds accumulate and soil pH is reduced, affecting the survival of soil-borne pathogens (Huang et al. 2015). Anaerobic bacteria such as *Bacillus* and *Clostridium* spp. may also contribute to pathogen inactivation. Anaerobic soil disinfestation results in the control of many soil-borne plant-pathogenic fungi, bacteria, and nematodes, viz., *Rhizoctonia, Fusarium, Verticillium, Sclerotinia, Phytophthora, Ralstonia, Meloidogyne* and *Globodera* spp., as well as most weeds (Butler et al. 2012). The changes in microbial communities' characteristics for anaerobic soil disinfestation often result in general disease suppression that can remain active for several years (Goud et al. 2004).

(d) Biofumigation

It involves the addition of organic amendments to soil, generating biologically derived volatile compounds that are toxic to soil microorganisms. Green manure crops that contain glucosinolates, mainly *Brassica* spp., are most commonly used (Cohen et al. 2005). After tissue decomposition, hydrolysis results in the release of various toxic compounds, such as organic cyanides, nitriles, and thiocyanates, which have fungistatic or biocidal properties (Finckh et al. 2015a, b). Application of animal-derived residues that are high in nitrogen, such as manure or compost, can result in the production of ammonia gas, which is toxic to a wide range of pathogens and nematode pests (Lazarovits et al. 2001).

(vi) Application of Organic Amendments

Soils with low microbial diversity promote establishment of plant pathogenic organisms. Healthy soil is the mainstay of organic agriculture. Improved soil biological activity is known to play a key role in suppressing weeds, pests and diseases (IFOAM 1998). Improving soil health through use of cover crops, green manures and animal manures to fertilize the soil not only helps in restricting soil-borne pathogens but also maximizes biological activity and maintains long-term soil health. Application of composts and organic amendments tends to increase the quantity and diversity of soil microbial diversity and consecutive disease suppressiveness. Organic amendments are biodegradable and are generally available on the farmer's fields. Neem cake used for soil amendment @ 0.25 to 0.5 t/ha contributes significantly in control of nematodes and soil-borne pathogens. Soils rich in organic matter are high in soil biodiversity with abundance of beneficial soil microorganisms (Singh 2003).

(vii) Cultural Control

Cultural control is more like habit of good agricultural practices, which promote healthy soils and healthy plants. From choosing the date of planting to field sanitation and weed management, the specific cultural measures reduce the initial

load of inoculum and favourable conditions for growth of pathogens. Litterick et al. (2002) opined that pest control strategies in organic farming systems are mainly preventive rather than curative. The management of cropped and un-cropped areas, crop species and variety choice and the temporal and spatial pattern of the crop rotations are actually aimed to reduce interaction between susceptible host and virulent pathogen, while maintaining a diverse population of beneficial organisms in the field. Ensuring good drainage is essential for disease management. Poor drainage in the fields not only reduces general health of the plant but also allows the pathogen to multiply rapidly. Many pathogens can survive on debris and weeds. Tilling and cleaning of plant residue at the end of the season allows the break down of the organic matter, leaving potential pathogens without a host. Moderate fertilization induces steady growth and makes a plant less vulnerable to infection.

(viii) Management of Environment

The changing climatic conditions pose a potential threat to agricultural production and productivity throughout the world and this might affect the crop yields, incidence of weeds, pests and plant diseases and the economic costs of agricultural production. This changing climatic condition has a twin effect in front of agricultural production. First, the frequent outbursts of diseases are obviously due to break of resistance chain of the respective crops and fast acclimatization of disease causing agent, i.e. causal organism under changing climate conditions (IPCC 2007). Modification of the environment may play an important role in resistance of plant to disease. Walker (1969) stated that the polygenic resistance is less stable than monogenic resistance in relation to temperature change. Monogenic resistance to fusarium wilt of cabbage is stable up to 26 °C while polygenic resistance breaks down at 24 °C temperature. Therefore, the microclimate can be modified by using the following methods for management of diseases under the organic agriculture system.

(a) Date of Seeding

Some diseases are very destructive when susceptible age of the host and optimum soil and atmospheric conditions for aggressiveness of the pathogen coincide. Alteration of the date of sowing in such a way that the susceptible stage of the plant growth does not coincide with the favourable environment for the pathogens helps in reducing losses from such diseases. Delayed emergence of seedlings above the soil surface gives the pathogen more time for causing infection. Regulation of the depth of sowing in such cases helps young plants escape infection. Dickson (1923) reported that the wheat, a low-temperature crop, grows well and escapes invasion by *Fusarium roseum* f. sp. *cerealis* in early spring when the soil temperature is in the range of 8–12 °C. Planting times can be adjusted to avoid heavy aphid flights or periods when other diseases will surge by planting crops at the proper time of the year or by ensuring enough crop growth before the onset of an epidemic (Finckh et al. 2015a, b).

(b) Spacing of Crop

Growth habit and density of planting can be significant factors in increasing microclimate near the leaf surface and thus increasing some foliage diseases. Therefore, spacing between plants can help reduce disease incidence because of better ventilation, sunlight and lesser humidity in the crop and those organisms that flourish in high humidity such as downy mildews are discouraged. Steadman et al. (1973) found that the wider spacing provided satisfactory control of white mold disease in bean. Less disease incidence by *Cercospora apii* on celery due to wider space was reported by Berger (1975) who stated that the less disease incidence was due to modified micro-climatological factors rather than less inoculum of *Cercospora apii*.

(c) Mixed Cropping System

Mixed cropping system is defined as the cultivation of a mixture of two crops together in the same field. Mixed cropping system can be characterized according to the degree to which roots of different crop species interact, which is determined not only by the mixed cropping system but also by the root architecture of each of the crops in the mixture (De Kroon 2007). Pigeonpea mixture with sorghum reduces the wilt disease incidence probably by increasing the distance between host plants and between infected and healthy roots by creating root barriers between the roots of diseased and healthy plants, and through toxic root exudates (Singh, 2007). The following mechanisms are reported to reduce diseases under mixed cropping system:

(i) Host Dilution

Host dilution plays an important role in reduction of soil-borne diseases or pathogens in mixed cropping systems. Mundt (2002) found host dilution to be a dominant factor for disease-reducing mechanism for air-borne pathogens in mixed cropping systems. While, the effect of host dilution likely is a reduction in disease incidence rather than disease severity on infected plants, as stated by Burdon and Chilvers (1982). Host dilution might have a direct effect on the pathogen itself as well as indirect effects on other factors than the pathogen on disease suppression in mixed crops. Otten et al. (2005) reported the reduced incidence of *Rhizoctonia* damping-off in radish–mustard mixtures because of increasing densities of the non-host mustard plants and spread halted at host densities below a threshold density. The intensity of root intermingling in mixed cropping may be an important determinant for the interference processes (Kroon 2007) and the level of disease suppression may therefore be determined by the crops or cultivars grown and their root architectures. The non-host crop simply acts as a physical barrier, thus reducing disease spread (Vilich-Meller 1992). The barrier function can reduce the impact of raindrops thus reducing dispersal, and it can intercept splashing spores that would reach a host plant under conditions of monoculture (Soleimani et al. 1996).

(ii) Allelopathy

Allelopathy is defined as any biochemical interaction among plants, including those mediated by microorganisms, resulting in either detrimental or beneficial effects on the interacting plants (Wu et al. 2001). When watermelon was intercropped with rice, allelopathic substances from rice roots reduced production and germination of conidia of *Fusarium oxysporum* f.sp. *melonis*, leading to a 67% reduction in wilt (Ren et al. 2007). The allelopathic exudates only reduced *Fusarium* conidial density in the rhizosphere and not in bulk soil indicating a limited diffusion. Natarajan et al. (1985) found delayed germination of spores of *F. udum*, causing wilt in pigeonpea because of allelopathic substances exuding from sorghum roots. To be effective in inhibiting rhizosphere-inhabiting pathogens, allelopathic substances should be present at sufficiently high concentrations in the micro sites where the pathogen is located and roots of mixed crops should be in close proximity. Roots of non-hosts can sometimes stimulate the germination of the survival propagules of the pathogen leading to a decline in the inoculum density (Mol and Van Riessen 1995). In relay mixed crops, this premature germination might have a disease-suppressive effect, especially in combination with inoculum burial and enhanced microbial antagonism.

(iii) Microbial Antagonists

Enhanced antagonistic populations are main mechanisms for disease reduction in mixed-cropping. In mixed crops, increased plant diversity leads to more diverse root exudates and consequently to a more diverse rhizosphere-inhabiting microbial community (Kowalchuk et al. 2002). Rhizospheres of mixed crops support different bacterial and fungal microbial communities compared to the corresponding single-crop rhizospheres (Song et al. 2007). Wheat root infection by *G. graminis* var. *tritici* was reduced by 25% in wheat-trefoil mixed cropping system (Lennartsson, 1988) while 75% reduction in Fusarium wilt was reached when bottle gourd was mixed with Chinese chive because of stimulation of *Pseudomonas gladioli* populations on the Chinese chive roots (Arie et al. 1987). Also, increased occupation of available niches by non-pathogenic fusaria was held responsible for increased disease suppression in oil-palm–legume mixed cropping (Abadie et al. 1998). Rhizosphere microbial communities, including pathogens, antagonists and plant-growth-promoting bacteria are crop- and cultivar-specific (Germida and Siciliano 2001). Cultivar-specific resistance against races of pathogens is widely known and often applied in mixed crops. Mazzola (2004) used wheat to stimulate the natural antagonistic populations of fluorescent pseudomonads, which led to control of apple replant disease.

27.5.2 Physical Methods

Heat therapy of seeds is commonly used to control certain seed-borne pathogens while leaving the host tissue viable. Many media have been used for heat treatment including water, steam, air and microwave radiation.

27.5.2.1 Concept of Heat Therapy

The concept of heat therapy is that microorganisms are killed or viruses are destroyed at temperatures not injurious to seed (Baker 1962). However, it is universally accepted that heat cause inactivation and immobilization of pathogens. There are two schools of thoughts regarding inactivation of pathogens (viruses) by heat. One holds the opinion that the heat treatment stimulates enzymes that cause the degradation of virus, though according to Benda (1972) this has not been established. The others pursue the idea that heat causes loosening of bonds both in nucleic acid and the protein components of the virus. In the nucleic acid, when the bonds are disrupted the linear arrangement of nucleotides is disrupted and thus the virus loses infectivity. In proteins, the bonds holding the chains of amino acids may be destroyed. Disruption of bonds causes denaturing of protein molecules, which become less soluble in water, and finally leads to coagulation. The rate at which the pathogen is inactivated is determined by temperature and the duration of treatment (Table 27.1). Seeds with low moisture content are ideal for heat therapy, while seeds with high moisture content are killed during heat therapy because of denaturation of protein, lipid liberation, hormone destruction, tissue asphyxiation, depletion of food reserves and metabolic injury with or without accumulation of toxic intermediates, as stated by Baker (1962).

Table 27.1 Details of temperature and exposure time for control of plant pathogens by heat therapy methods

Crop	Disease	Temperature	Duration
Brassica spp.	Black rot	50 °C	20–30 min.
Cluster bean	Bacterial blight	50 °C	10 min
Cucumber	Seeding blight	50 °C and 75% relative humidity	3 days
Lettuce	Leaf spot	70 °C	1–4 days
Groundnut	Testa nematode	60 °C	5 min after soaking for 15 min in cool water
Pearl millet	Downy mildew	55 °C	10 min
Potato	Potato phyllody	50 °C	10 min
Rice	White tip	51 °C to 53 °C	15 min after soaking for 1 day in cool water
Safflower	Leaf spots	50 ° C	30 min
Tobacco	Hollow stalk	50 ° C	12 min
Tomato	Bacterial black speck	52 °C	1 hours

Source: Chaube and Singh (1990)

(i) Hot Water

Hot water is used widely to control the pathogens especially bacteria and viruses. The following steps are included in hot water heat therapy:

(a) Selection of Seeds

Seeds that can withstand hot water therapy are preferred. Hot water therapy is recommended for seeds with deep-seated infection.

(b) Pre-soaking the Seeds

Pre-soaking is done to replace the air between the embryo and seed coat with water, which is a better heat conductor. The water soak may stimulate pathogen growth, by which the pathogen becomes more heat susceptible.

(c) Pre-heating

Pre-heating is done to counter the cooling effects of seeds soaked in cool water. In pre-heating, seeds are heated for 1–2 min at 9–10 °C below the temperature of the final treatment.

(d) Hot Water Soak

The temperature and time required varies with the pathogen and crop. Time must be precise otherwise seed viability is lost. A large volume of water helps maintain a constant temperature. Seeds should be packed loosely in porous bags, screen boxes or frames with sufficient provision for an ample water flow.

(e) Cooling

Treated seeds are spread out for cooling and drying immediately after treatment.

(f) Drying

Seeds are dried quickly to prevent sprouting.

Hot water therapy is an eco-friendly technique for controlling the pathogens and economically viable, but because the seed temperature must be raised quickly, only small quantities of seeds can be treated at one time and germination particularly of older seeds may be reduced.

(ii) Hot Air Therapy

Hot air treatment is less injurious to seed and easy to operate but also less effective than hot water treatment. It has been used against several diseases of sugarcane. Red rot of sugarcane is completely controlled by hot air treatment of 54 °C for 8 h (Singh 1973). Similarly, grassy shoot disease of sugarcane has been controlled by hot air at 54 °C for 8 h (Singh 1968). Drying tomato seeds in an oven for 6 h at 29.5–37.5 °C eliminated *Phytophthora infestans* from discoloured seeds of infected fruits (Vartanian and Endo 1985). Dry heat treatment of tomato seeds for 2 days at 78 °C temperature reduced tomato mosaic virus without an effect on germination. Zeigler and Alvarez (1988) found dry heat treatment effective for controlling the *Pseudomonas avenae* and *Pseudomonas glumae* in rice. Similarly, dry heat treatment of capsicum seeds for 7 days at 70 °C gave control of capsicum mosaic virus (Stijger and Rast 1988).

(iii) Aerated Steam Treatment

The use of aerated steam is safer than hot water and more effective than hot air in controlling seed-borne infections. The heating capacity of water vapour is about half that of water and 2.5 times that of temperature control and no damage to seed coat of legumes (Agarwal and Sinclair 1996). Most frequent application of steam and aerated steam has been in greenhouses where steam also provides heat during cold seasons. As gas, it moves readily through soil, in contrast to the slow, inefficient movement of water. Aerated steam provides an opportunity to treat soil at temperatures lower than those possible with pure steam. Navaratnam et al. (1980) obtained complete eradication of *Septoria apiicola* from celery seeds after treatment for 30 min at 56 °C and *X. campestris* pv. *campestris* in cabbage seeds after treatment for 30 min at 54 °C. ¬The use of aerated steam to disinfect seeds is an easy-to-handle, inexpensive method that was already shown to be effective in eliminating seed-borne plant-pathogenic bacteria and fungi (Heller and Zoller 2010).

(iv) Moist Hot Air Therapy

The moist hot air therapy has been proposed by Singh (1973) to eliminate the grassy shoot disease of sugarcane. Under this technique, the setts of sugarcane are initially exposed to hot air for 8 h at 54 °C, after that these setts are exposed to aerated steam at 50 °C for 1 h and finally to moist hot air at 54 °C for 2 h.

(v) Solar Heat Therapy

Solar heat therapy is one of the simplest and oldest techniques used in India for elimination of seed-borne pathogen of loose smut disease. This technique devised by Landen (1939) to eliminate the deep-seated infection of *Ustilago nuda*. Previously, the hot water treatment was followed to eliminate loose smut. In this technique, seeds are soaked in cold water for 4 h in the forenoon on a bright summer day followed by spreading and drying the seeds in hot sun for 4 h in the afternoon. But extensive care is necessary of the embryo to avoid injuries from thermal death point because it is very close to pathogen and embryo.

27.5.3 Biological Methods

The use of bio-agents puts forward a practical and economical alternative for plant disease management in organic agriculture, in which the ecological community of available microorganisms is maintained that enhance plant immunity by suppressing the pathogens. Disease suppression by using bio-agents is a result of interaction between plant, pathogen, bio-agent and microbial community on and surrounding the plant and their physical environment. There are two basic strategies of biological control (a) enhanced biological control via endemic natural enemies, including competitors, antagonists, predators or parasites, by means of habitat management and (b) inundative biological control via the release of specific competitors, antagonists, predators or parasites. Srinivas and Ramakrishna (2005) stated that the microbial bio-control agents isolated from native environments are relatively

safe, host specific and do not disturb other biotic systems. Commercial bio-fungicides contain beneficial living organisms and used for disease management in organic agriculture system. These are available in different forms viz., as powders for seed treatments, as granulars for soil application and as suspensions for soil drenches and foliar sprays.

27.5.3.1 Biocontrol Agents in Plant Disease Management

Biological control agents like *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp. have proven their worth in managing a range of plant diseases. Handelsman and Stabb (1996) observed that bacteria like *Bacillus, Pseudomonas, Serratia* and *Arthrobacter* have shown efficacy to manage numerous fungal diseases. Bacterium such as *Pseudomonas fluorescens*, which produces the antimicrobial polyketides, has the ability to control several fungal pathogens, including *Phythium* spp. (Girlanda et al. 2001), while Bacillus sp. accelerates plant growth by suppressing soil-borne diseases (Utkhede and Smith 1992). Deka Boruah and Dileep kumar, (2003) reported that *Rhizoctonia solani, Fusarium solani, Fusarium semitectum* and other fungal diseases have been controlled by *Bacillus* sp., when it is used as a seed coating agent. A combination of *Pseudomonas* strains is effective in siderophore-mediated competition for iron and induction of systemic plant resistance to improve control of Fusarium wilt of radish (De Boer et al. 2003). Seed treatment with *Trichoderma harzianum* or *Pseudomonas fluorescens* @ 6 g/kg coupled with two sprays of *Pseudomonas fluorescens* @ 0.3% at the time of flowering and second after 10 days can control leaf, neck and finger blasts very effectively in small millets (Patro et al. 2008), while Nagaraja et al. (2012) stated that the blast can be managed by seed dressing using bioformulation of *Pseudomonas fluorescens*.

27.5.3.2 Biocontrol Agents as a Major Component of Integrated Disease Management System

An integrated pest management (IPM) system entails simultaneous or sequential use of several methods of control. Biological control is of particular interest as a component of, and can best be exploited within the framework of an IPM system (Barzman et al. 2015). Biocontrol agents (BCAs) have distinct advantages in being compatible with most of the agricultural practices and hence can be successfully utilized as a part of total crop management practices or broadly, as a component of the agro-ecosystem management (Rawat et al. 2012). Different biocontrol agents have been integrated with cultural practices, soil solarization, fungicides and disease-resistant varieties for managing different crop diseases. Combination of the seed/root application of *Trichoderma harzianum* or *Pseudomonas fluorescens* with soil solarization was very effective in management of seed and seedling diseases of tomato, brinjal and capsicum in nursery at farmers field (Singh 2003). Wilt and root-rot complex of chickpea, lentil and pigeon pea were successfully managed by integration of *Trichoderma harzianum* or *Trichoderma virens* with carboxin (Mukhopadhyay and Mukherjee 1996). Integration of fertilizers or herbicides with biocontrol agents to control plant diseases has also been attempted. Bacterial biocontrol agents are compatible with most of the fungicides. Even fungal

biocontrol agents like *Trichoderma* spp. are insensitive to fungicides like carboxins, oxycarboxins, metalaxyl, tricyclazoles, carpropamid host defense inducers (e.g. benzothiadiazole), etc. Therefore, they could be integrated easily. Integrating biocontrol agents with reduced doses of fungicides seems to be an effective way of controlling pathogens with less interference with biological equilibrium (Patibanda and Ranganathswamy 2018). This would not only reduce the use of fungicides but also improve the efficacy of a biocontrol system with reduced cost and lessen the chances of development of fungicide-resistant strains of the pathogen. Integration of biocontrol agents with compatible fungicides for seed treatment is very effective even against high population of fast-growing pathogens like *Rhizoctonia solani* in soil. Under such conditions, a biocontrol agent alone may not be very effective as by the time it gets activated in soil, the pathogen is able to penetrate the host. Integration of fungicide with biocontrol agents helps in early protection by fungicide and the later protection by introduced biocontrol agents. A fungicide also provides a congenial environment to compatible biocontrol agents to multiply and colonize spermosphere and rhizosphere by suppressing other microbes sensitive to the fungicide (Singh et al. 2003; Rawat et al. 2013)**.**

27.5.3.3 Mechanisms of Biological Disease Control

The mechanisms underlying successful biological control vary from niche competition and antagonism to parasitism and predation. Van Bruggen and Termorshuizen (2003) stated that the biocontrol mechanisms for soil-borne diseases are much effective than aerial diseases because aerial diseases are influenced by microclimatic conditions.

In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. The different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions (Table 27.2). Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the biocontrol agent(s). In such a scheme, hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect. In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). Stimulation of plant host defense pathways by non-pathogenic BCAs is the most indirect form of antagonism.

However, when the microbial products are applied on seeds or soil, they may also induce systemic resistance to foliar diseases as stated by Vallad and Goodman (2004). The mechanisms reveal by BCAs are a result of direct antagonism from physical contact and/or a high-degree of selectivity for the pathogen. In such a scheme, hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect. In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the biological control agents. Stimulation of plant host defense pathways by non-pathogenic biological control agents is the most indirect form of antagonism.

Table 27.2 Types of interspecies antagonisms resulting in biological control of plant pathogens

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism/ predation	Lytic/some non-lytic mycoviruses *Ampelomyces quisqualis, Lysobacter enzymogenes, Pasteuria penetrans, Trichoderma virens*
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol, Phenazines, Cyclic lipopeptides
	Lytic enzymes	Chitinases, Glucanases, Proteases
	Unregulated waste products	Ammonia, Carbon dioxide, Hydrogen cyanide
	Physical/ chemical interference	Blockage of soil pores, Germination signal consumption, Molecular cross-talk confused
Indirect antagonism	Competition	Exudates/leachate consumption, Siderophore scavenging, Physical niche occupation
	Induction of host resistance	Contact with fungal cell walls, Detection of pathogen-associated, molecular patterns, Phytohormone-mediated induction

Source: Pal, K. K. and B. McSpadden Gardener (2006)

For instance, Pseudomonads known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defenses, as reported by Iavicoli et al. (2003). Additionally, DAPG-producers can aggressively colonize roots, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of wheat through competition for organic nutrients, as stated by Raaijmakers and Weller (2001). Chandrashekara et al. (2012) reported direct- (hyperparasitsm, predation), indirect- (induce host resistance and competition) and mixed (antibiotic, lytic enzymes production, interference)-type antagonism.

In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause. Some examples of antibiotics reported to be involved in plant pathogen suppression are listed in Table 27.3.

27.5.3.4 Future Prospects for Large-Scale Commercialization

Microbial BCAs are ideal for both short- and long-term pest suppression and are also compatible with most other control methods. Their mechanisms of action include competition, antagonism, antibiosis, enhanced nutrient uptake, induction of host resistance, plant growth promotion (Kloepper et al. 1997; Singh et al. 2003; Rawat et al. 2011), etc. and unlike chemical pesticides, they are harmless to humans and other non-target organisms, they do not leave chemical residues on crops, are easy and safe to dispose of and do not contaminate water systems. A number of commercial formulations of bio-control agents are available in the market for use as seed treatment, soil application and foliar sprays. If a BCA is having high antagonistic

Table 27.3 List of some of the antibiotics produced by biocontrol agents

Antibiotic	Source	Target pathogen	Disease
2, 4-diacetyl-phloroglucinol	*Pseudomonas fluorescens* F113	*Pythium* spp.	Damping off
Agrocin 84	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*	Crown gall
Bacillomycin D	*Bacillus subtilis* AU195	*Aspergillus flavus*	Aflatoxin contamination
Bacillomycin, fengycin	*Bacillus amyloliquefaciens* FZB42	*Fusarium oxysporum*	Wilt
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomyces cochlioides*	Damping off
Gliotoxin	*Trichoderma virens*	*Rhizoctonia solani*	Root rots
Herbicolin	*Pantoea agglomerans* C9–1	*Erwinia amylovora*	Fire blight
Iturin A	*B. subtilis* QST713	*Botrytis cinerea* and *R. solani*	Damping off
Mycosubtilin	*B. subtilis* BBG100	*Pythium aphanidermatum*	Damping off
Phenazines	*P. fluorescens* 2–79 and 30–84	*Gaeumannomyces graminis* var. *tritici*	Take-all
Pyoluteorin, pyrrolnitrin	*P. fluorescens* Pf-5	*Pythium ultimum* and *R. solani*	Damping off
Pyrrolnitrin, pseudane	*Burkholderia cepacia*	*R. solani* and *Pyricularia oryzae*	Damping off and rice blast
Zwittermicin A	*Bacillus cereus* UW85	*Phytophthora medicaginis* and *P. aphanidermatum*	Damping off

Source: Pal, K. K. and B. McSpadden Gardener (2006)

activity, competent saprophytic ability and besides this, if it is having plant growth promotion activity, induce resistance in host, longer shelf life, tolerance to biotic and abiotic stress and broad host range and is successfully commercialized, the specific BCA will be a boon to the industry associated with its commercialization. The broad-action spectrum of a BCA is a guarantee of its wide acceptability in the market.

However, the following are the associated limitations of the BCAs:

- Inconsistence field performance
- Too specific or slow acting
- Poor shelf life of formulations
- Subject to environmental influences, i.e. effect of temperature, pH, salinity and water stress.

These problems can be overcome by isolating or developing broad-action spectrum BCAs, which could be used under heterogeneous adverse environmental conditions and these BCAs can be further improved for better stress tolerance by using advanced techniques like mutagenesis, genetic transformation and protoplast

fusion. But the problems associated with these techniques are high cost, sophisticated and required broader setup. Therefore, it is more feasible to isolate and screen the BCAs for stress tolerance by testing them in vitro and natural field conditions, where such adverse conditions prevail. Strains obtained by conventional methods can be registered without any protest from environmental protection agencies. In this regard, the first requirement of biological control is the identification and deployment of highly effective strains controlling several biotic stresses and at the same time, it is important to have information about the effects of various environmental factors on the biocontrol efficiency of BCAs in designing effective and safe biocontrol strategies (Rawat 2011). Biocontrol agents may be used in rotation or in integration with compatible pesticides and or synthetic substances. There are some synthetic substances such as copper hydroxide, copper oxychloride, copper oxide, copper sulphate, hydrated lime, potassium bicarbonate and lime sulphur that are accepted in organic farming provided that their use should be judicious and the number of applications should be moderated.

There is a need for investigations on compatibility of BCAs with other agents, cultural practices and agrochemicals. This will help in the development of more effective and efficient formulations of BCAs. Industries should work closely with researchers and extension service workers in resolving these matters before the products reach the market.

27.5.4 Genetic Methods

Resistance of diseases and pests is a naturally occurring phenomenon in plants. That is how they have been facing the attack of enemies probably since their evaluation. Nature and pathogens have been eliminating the weak and susceptible ones from the population while the farmers selected the best yielders from the survivors. These surviving populations carried different sets of major or minor genes for resistance. By using these surviving populations, numerous varieties or cultivars have been developed for the cultivation. There are two approaches under genetic method for managing diseases in the organic farming system.

27.5.4.1 Use of Disease-Resistant Varieties

The use of a resistant variety is an important low external input alternative to chemical pesticides in organic agriculture. Exploiting the diversity and variability in the host genetic constitution for resistance against a pathogen in a crop is the best strategy for disease management without application of hazardous pesticides. It can individually restrict the incidence of a particular disease in a crop. Successful disease establishment depends on the compatible gene for gene interaction between a host and a pathogen. Resistant varieties tend to remain disease free for a long period of time owing to morphological manifestation of their genetic constitution in the form of leaf and stem toughness, time of maturity, nutrient content, plant architecture and growth habit which can deter growth of pathogen, their reproduction and host preference. Care should be taken to include more than one resistant variety in a

region to dissipate selection pressure on the pathogen. However, this strategy is very specific and tends to tackle only one or two diseases at a time owing to its resistance (Srinivas 2017).

27.5.4.2 Use of Multiline Varieties

Multiline varieties and variety mixtures can also provide functional diversity that limits pathogen and pest expansion cropping system (Finckh et al. 2000). These approaches also reduce the risk of resistance breakdown, which are due to a range of mechanisms including barrier and frequency effects as well as induced resistance. Also, differential adaptation, i.e. adaptation within races to specific host genotypic backgrounds, may prevent the rapid evolution of complex patho-types in mixtures (Finckh et al. 2000). Therefore, yield stability is commonly greater in mixtures than in pure stands (Finckh 2008). The wider application of variety mixtures in organic farming is constrained by the concern of farmers and processors about the anticipated negative effect on the homogeneity of the quality. However, if the mixture components are carefully designed and selected for desired traits in the breeding progress, product quality may be equal to or higher than that obtained in pure stands (Finckh 2008). Nevertheless, there is still a risk that due to genotype × environment interaction, unacceptable heterogeneity may occur under different environments.

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Organic Agriculture for Plant Disease Management

28

D. K. Shahi, Sweta Kachhap, Arvind Kumar, and B. K. Agarwal

Abstract

Green Revolution ushered the nation into a millennium of self-sufficiency, but we have again been presented with the threat of diminishing agricultural production due to deterioration in soil health and, ultimately, risking the sustenance of the human race. Indiscriminate and reckless use of chemicals has fuelled the concerns of environmental pollution, pesticide toxicity and soil health even further. Amidst this, organic agriculture has come out to be a holistic paradigm for sustaining life as we know it. Though diseases are not a threat in organically managed farms, still in order to prevent disease incidence from reaching economically damaging levels, organic agriculture uses the natural process and fundamental components of the ecosystem, such as nutrient cycling and microorganism population as soil management tools. Disease control through soil management can be accomplished either through modifying nutrient availability or by modifying its uptake. Organic agriculture follows composting, manuring, crop rotation and reduced tillage systems for suppression of pathogens and control of disease.

Keywords

Organic farming · Holistic approach · Soil management · Disease management · Soil fertility · Biodiversity · Crop rotation · Compost · Organic matter

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28.1 Introduction

Agriculture has changed a lot since the evolution of mankind, but the world witnessed a dramatic change in the agriculture system especially since the end of World War II. With the challenge to meet the growing demands of the burgeoning population and the "Green Revolution" in full flight, the government policies favoured unchecked use of mechanization, fertilizers and pesticides. And, though the food and fibre production soared and food surplus could be attained as Green Revolution ushered the nation into a millennium of self-sufficiency, sadly, today, even with high inputs, agricultural production has realized a plateau which is sustained with diminishing returns of falling dividends. Moreover, the reckless and indiscriminate use of chemicals has contributed to concerns of environmental pollution, pesticide toxicity and soil health. And the world has again been presented with the threat of diminishing agricultural production due to deterioration in soil health and, ultimately, risking the sustenance of the human race.

So, embracing a holistic notion that the health of any agriculture-based nation is dependent on the long-term vitality of its soil, and motivated by a desire to reverse these agricultural problems the concept of organic agriculture was born in the early twentieth century. But plant diseases continue to create challenging problems and pose real economic threats in agricultural ecosystems. Despite a wide use of chemical pesticides for crop produce, it has been manifested that the losses due to diseases are significant, apart from causing toxicity to the soil and environment and entering the food chain through contaminated food.

While there's still a debate if organic agriculture can sustain the growing demands of the ever-growing population, a more compelling question is whether organic agriculture has feasible options for disease management. Our objective here is to describe the potential of soil management practices in organic agriculture for disease management as an alternative way for conventional agricultural practices, which can lead to a sustainable resource utilization and contribute in mitigating global soil health problems. This chapter makes an attempt to highlight the strategies of soil management for crop protection in organic farming to suppress disease-causing pathogens and decrease losses due to crop failure.

28.2 Organic Agriculture – Concept, Principles and Components

28.2.1 Definitions of Organic Agriculture

Organic agriculture is one of the several approaches to sustainable agriculture. It has many definitions and has been described differently by different people, but Lampkin (1990) provides the most apt and comprehensive definition of organic agriculture. He defines organic agriculture as a production system, which exclusively avoids the use of synthetic compounded chemical fertilizers, growth regulators, livestock feed additives and pesticides, and instead uses only organic-based

fertilizers, such as compost, farmyard manure, biofertilizers, green manures, vermicompost and natural pesticides and biocontrol agents.

The Food and Agriculture Organization (FAO) suggests organic agriculture to be *"a unique production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles and soil biological activity which is accomplished by excluding all synthetic off-farm inputs and instead making use of on-farm agronomic, biological and mechanical methods"* (FAO/WHO Codex Alimentarius Commission 1999).

Over the years, organic agriculture has emerged as an ecologically, economically and socially dependable way of production which provides a continuing supply of healthy and safe food and fibre, with minimal probable nutrients and energy losses, and the least negative forces on the environment, as regulated by certification agencies which scientists are looking at as a viable alternative way of restoring the lost production and soil health equilibrium (Finckh et al. 2015).

28.2.2 Principles of Organic Agriculture

The International Federation of Organic Agriculture Movements (IFOAM) goes further beyond biophysical aspects to define organic agriculture as *"a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved."* IFOAM also gives four principles which are the roots from which organic agriculture grows and develops (IFOAM 2020).

Organic agriculture, according to IFOAM, is guided by four principles that include how people tend plants, animals, water and soil for production of food and its distribution, and can be applied to agriculture in the broadest sense (Fig. 28.1). These principles which concern the manner in which people relate to each other interact with living landscapes and influence the future generations to come and are as follows:

Fig. 28.1 Principles of organic agriculture

1. **The Principle of Health –** Health means the integrity and wholeness of living systems. The principle of health points out that organic agriculture should be able to sustain as well as enhance not only human and animal health but also the health of soil and plant including the planet as one and indivisible. It not only means avoiding illness but also to maintain physical, social, mental and ecological well-being. In the same context, it in turn means largely excluding the use of fertilizers, pesticides, food additives and animal drugs that can cause adverse health effects.
2. **The Principle of Ecology –** It points out that the basis of production in organic agriculture should be ecological processes including efficient management of materials and recycling, working with them, emulating them and helping in sustenance and should be aimed at achieving ecological balance, improving the environmental quality and conserving resources.
3. **The Principle of Fairness –** Fairness encompasses equity, justice, respect and stewardship, and this principle emphasizes that organic agriculture should be directed towards building a good quality of life by ensuring fairness at all levels of food production and consumption in a manner that is socially just and environmentally viable too.
4. **The Principle of Care –** Organic agriculture should be directed towards responsibly managing the supply to the demands and conditions (both external and internal) in a manner that not only ensures health, safety and ecological soundness of the present generation but also discards risks that jeopardize the health and well-being of the future generations. Organic agriculture should make use of science and technology to increase productivity and enhance efficiency, but with care and precaution so as to prevent significant risks.

28.2.3 Components of Organic Agriculture

Organic agriculture has come out to be a holistic paradigm for sustaining life as we know it on earth and hence is so much more than just a way to naturally treat soil, plants and animals. Organic agriculture involves:

1. Reducing soil erosion and improving and maintaining soil fertility, soil structure and soil biodiversity.
2. Reducing risks of exposure of toxic materials to humans, animals and environment alike.
3. Fine-tuning farming practices to meet production and distribution and attaining sustainable yield.

For these, organic agriculture must employ components (Fig. 28.2) that envisage a comprehensive management approach to enhance and maintain soil productivity without compromising the soil fertility and soil health and regulate the ecological processes working together in synergy and maintain a stage of dynamic equilibrium.

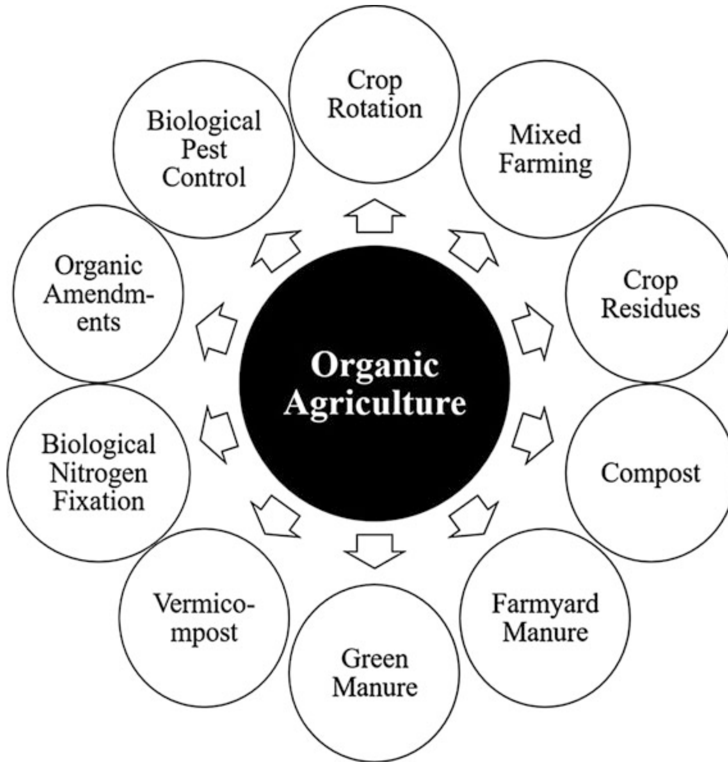

Fig. 28.2 Components of organic agriculture

28.2.4 Organic Agriculture Vs. Conventional Agriculture

Organic agriculture does not merely imply a production system that works on simple replacement of synthetic fertilizers and other chemical pesticides, but there's much more to it besides restricting and eliminating the entry of chemicals into the environment and there are a lot of fundamental differences between organic agriculture and conventional agriculture (Table 28.1). When we compare organic agriculture to conventional farming, organic agriculture production systems by and large: (a) have higher plant diversity, both in space and time; (b) have enhanced soil organic matter content by means of cover crops and crop rotation; (c) have a higher diversity of soil flora and fauna; (d) have enhanced water-holding capacity with enhanced water-use efficiency and (e) have increased cycling, improved cation exchange capacity (CEC) and reduced loss of nutrients (Aparna et al. 2014; Gomiero et al. 2011; Tuck et al. 2014).

Table 28.1 Fundamental differences between organic and conventional farming

	Organic agriculture	Conventional agriculture
1	Synthetic fertilizers and synthetic pesticides are not permitted	Synthetic fertilizers and synthetic pesticides are allowed
2	Genetically modified organisms (GMOs) are not allowed	GMOs can be used
3	Soils have higher water holding capacity	Soils have lower water holding capacity
4	Has larger floral and faunal biodiversity (complex crop pattern)	Has smaller biodiversity (simple crop pattern)
5	The agricultural landscape is characterized by heterogeneity (multicultural system)	The agricultural landscape is characterized by homogeneity (monocultural system)
6	Minimizing the use of non-renewable resources by recycling plant and animal waste into the soils (on-farm inputs)	Depends largely on non-renewable resources (off-farm inputs)
7	More sustainable	Less sustainable
8	Strictly regulated by international and national institutional bodies such as Codex Alimentarius and IFOAM	Not strictly regulated
9	Crop protection depends mainly on natural processes such as soil fertility, crop cycle, and biodiversity (more preventive)	Crop protection relies mainly on human intervention with synthetic chemicals (more curative)
10	Optimum input: output ratio	Low input: output ratio
11	No pollution	Considerable pollution
12	Stability due to diversification	Market based programme
13	Ecological orientation and holistic approach	Economical orientation and economical approach

28.3 Disease Incidence and Disease Management in Organic Agriculture

There are, basically, three conditions for a plant disease to happen. Plant diseases occur only when a disease-causing pathogen comes in contact with a susceptible host in a conducive environment (Fig. 28.3). And if any of these three conditions are not satisfied, disease incidence will fail to happen. Hence, the disease management strategies in organic agriculture, which are mainly of preventive nature rather than being curative, are aimed at creating the environments less favourable and the hosts less susceptible for disease invasion.

Successful disease management in organic agriculture demands an exhaustive understanding of life cycles of the crop and the disease-causing pathogens and their interactions with themselves, soil, climate and other factors of the production system. To achieve this, organic agriculture relies heavily on the concept that within an agro-ecosystem, all natural processes are reciprocally dependent on one another, and its management should be aimed at supporting and maintaining self-regulation through these natural processes (Birkhofer et al. 2008). Therefore, to replenish nutrients taken from the soil, organic agriculture largely depends on the natural

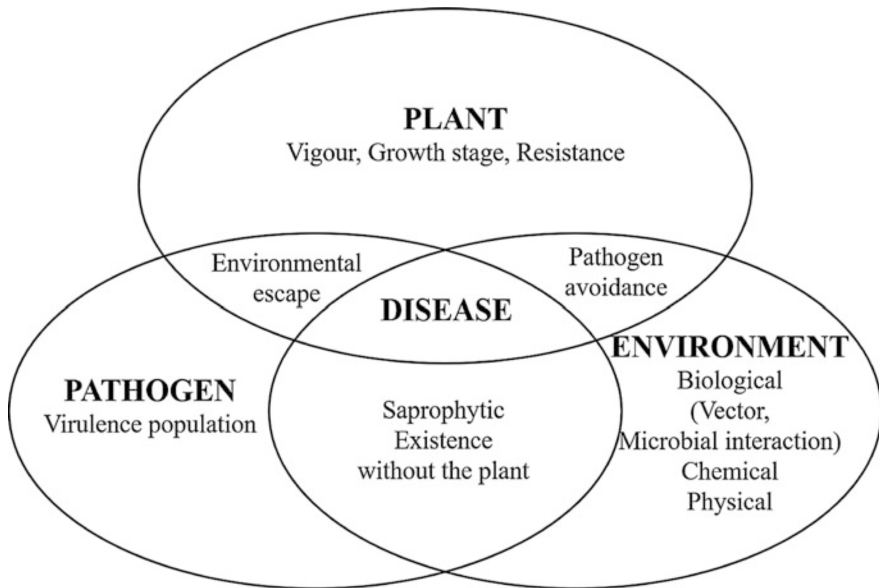

Fig. 28.3 A schematic representation of the interacting components involved in plant disease (Huber and Haneklaus 2007)

breakdown of organic matter by means of agricultural practices like composting and green manuring, which are driven by beneficial soil microorganisms and allow natural cycling of the nutrients in the soil.

28.4 Soil – A Resource of Infinite Possibilities

Soil, a complex mix of organic and inorganic matter, is the fundamental medium for crop growth in all production systems where plant roots along with living microorganisms bind organic matter and mineral particles into a dynamic structure that governs air, water and nutrient flow. A healthy and fertile soil is the basis of a productive, profitable and environmentally sound agro-ecosystem. A healthy soil allows for so many functions that support crop growth by means of nutrient cycling, regulation of water and air supply and also by way of biological control of plant pests. And in context to the agricultural production system, soil health pertains to the ability of the soil to sustain crop productivity simultaneously protecting the environment.

Soil being a critical resource can improve or degrade the quality of that resource owing to the ways in which it is managed. And, the success of this dynamic living resource, to a great extent, depends on characteristics which affect the rooting of crops, such as structural characteristics and nutrient supply. However, soil condition is one of the factors that can influence plant growth and development via occurrence

and severity of disease incidence. And so by understanding the physical, chemical and biological components of a healthy soil and by understanding how the soil processes work and how they support plant growth, it is possible to design a soil management system that maintains and improves soil health.

28.4.1 Disease-Suppressive Soils

Disease-suppressive soils are defined as the kind of soils in which either the pathogen is unable to establish or persist, the pathogen though gets established but is unable to cause damage or though the pathogen is able to cause some damage, the disease fails to progress in severity despite the pathogen persisting in the soil (Cook and Baker 1983). In other words, a soil is referred to as disease-suppressive when, despite a conducive environment for disease incidence, a disease-causing organism does not establish; it gets established but is unable to produce disease, or even if it gets established and produce disease it is only for a short time after which it declines (Schneider 1982).

The concept of disease-suppressive soils is not new and has been getting acknowledged since several decades now and involves two types of disease suppression strategies which are as follows:

(1) **General Suppression** – of a disease-causing organism is a consequence of high microbial biodiversity and microbial activity in the soil or the plant at a vital time in the life cycle of the pathogen that create an unfavourable environment for the disease to develop. General suppression operates against most pathogens and is mostly non-specific.
(2) **Specific Suppression** – of a pathogen is a result of one organism targeting and operating against a certain type of known pathogen to suppress it and reduce disease incidence. Specific suppressiveness has been described for *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani*, Fusarium wilts, *Gaeumannomyces graminis* var. *tritici* and *Thielaviopsis basicola* (Table 28.2).

Table 28.2 Examples of different plant pathogens controlled by disease-suppressive soils (Chandrashekara et al. 2012)

	Pathogen involved	Reference
1	*Phytophthora cinnamomi*	Broadbent and Baker (1974)
2	*Pythium* spp.	Hancock (1977)
3	*Rhizoctonia solani*	Henis et al. (1978, 1979)
4	*Fusarium oxysporum*	Stotzky and Martin (1963) and Scher and Baker (1980)
5	*Gaeumannomyces graminis* var. *tritici*	Cook and Rovira (1976)
6	*Plasmodiophora brassicae*	Murakami et al. (2000)
7	*Streptomyces scabies*	Menzies (1959)
8	Cyst nematode *Heterodera* spp.	Kerry (1988) and Westphal and Becker (1999)

While the degree of suppresiveness is associated with the physical, chemical and biological attributes of the soil including soil pH, fertility level, the type and population of soil organisms, nature of the soil and soil management (Sullivan 2001), the mechanisms employed in disease suppression include predation, parasitism, antibiosis, induced resistance and nutrient competition by beneficial soil organisms. But, although abiotic properties and characteristics of soil can help in disease suppression, the level of disease suppression is typically a function of microbial activity and microbial metabolites in a soil. So, the higher the microbial mass and microbial activity in the soil, the higher is its capacity to utilize carbon, nutrients and energy to suppress pathogens.

28.5 Soil Management for Disease Management

The soil is home to many living organisms since it is the ultimate source of their mineral nutrients. A soil takes a period of time to develop suppression to pathogens, and while natural suppressiveness is often related to the natural properties of the soil independent of the crop history, we are more interested in induced suppressiveness, which is completely dependent on soil management and agricultural practices. Hence, soil management is important to crop productivity, human and animal health and environmental sustainability, both directly and indirectly. Good soil management ensures that appropriate mineral elements enter the food chain and that mineral elements do not become deficient or toxic to plants and humans. All these functions are governed by the physical, chemical and biological properties of soil, which in turn are influenced by the soil management practices. In order to prevent disease incidence from reaching economically damaging levels, organic agriculture uses the natural process and fundamental components of the ecosystem such as nutrient cycling and microorganism population as soil management tools, both directly and indirectly. Some key soil management strategies and tools that are employed under organic agriculture for successful disease control have been discussed below under different subheads.

28.5.1 Soil Fertility

A fertile soil is the one which is abundant in essential and beneficial plant nutrients and rich in organic matter. Soil fertility is referred to as the inherent capacity of the soil to provide plants with essential nutrients in adequate quantities and proportions at the optimum time for desirable plant growth. Soil fertility is a major factor in disease management since nutrients are not only crucial for growth and development of plants and microorganisms but their availability in the soil also plays a key role in disease control. The nutrient status of the soil and the use of a specific fertilizer and amendment may have substantial impacts on the environment of the disease-causing pathogen (Agrios 2005).

28.5.1.1 Soil Nutrients

The availability of a particular nutrient in the soil can affect the severity of a disease both positively as well as negatively by way of decreasing or increasing disease incidence, respectively, under different environmental conditions (Marschner 1995; Graham and Webb 1991; Huber 1980). Adequate crop nutrition makes plants more tolerant of or resistant to a disease-causing organism since nutrients can govern disease control through influencing disease tolerance or resistance. While disease tolerance is the ability of the plant to maintain its growth and development despite the disease infection, disease resistance refers to the ability of the plant to confine the penetration, establishment and reproduction of the disease-causing pathogen (Graham and Webb 1991). And though the genetic make-up of the plant species controls tolerance and resistance to pathogens, nutrient deficiency and toxicity in the soil and soil environment can affect them to a large extent and hence correct nutrient management can help in disease control for achieving a higher yield (Agrios 2005; Marschner 1995; Krauss 1999; Huber and Graham 1999; Graham and Webb 1991).

When a pathogen infects a susceptible host plant, it impairs its physiology including the uptake, assimilation, translocation and utilization of a nutrient. Some pathogens are also capable of immobilizing the nutrients in the infected regions or in the rhizosphere and interfering with their translocation or utilization causing nutrient deficiency or toxicity. Plant pathogens may also compete with the plants for nutrients utilizing a significant amount of nutrients for their growth causing nutrient deficiency and further increasing plant susceptibility to diseases (Marschner 1995; Huber and Graham 1999; Timonin 1965). The level of nutrients available in the soil has been also known to affect disease development either by affecting the pathogen, by affecting plant physiology or by impacting both by influencing the microclimate and hence infection and sporulation of the disease-causing pathogen (Marschner 1995). Hence, it becomes crucial to manage nutrient availability in soil in order to impact nutrient availability, and help in plant disease management.

Nitrogen (N) is the most important nutrient for plant growth and extensive literature is available regarding the effect of N on diseases because its function in disease resistance has been frequently demonstrated, but in spite of the fact that N is one of the most important nutrients for plant growth and disease development, there are several factors which impact the effect of N disease development such as the type of pathogen – obligate vs. facultative parasite, the form of N nutrition available to the host and the crop growth stage of N application (Table 28.3). For example, there is an increase in the severity of disease caused by obligate parasites such as *Puccinia graminis* and *Erysiphe graminis* at high N supply, whereas disease severity by facultative parasites such as *Xanthomonas vesicatoria* and *Alternaria solani* is greatly suppressed at high N availability. Similarly, the severity of infection of *Pythium* spp., *Rhizoctonia solani* and *Fusarium oxysporum* has been found to decrease at high nitrate (NO_3^-) availability while high ammonium (NH_4^+) caused decreased disease infection in *Pyricularia*, *Sclerotium rolfsii* and *Gibberella zeae*.

Phosphorus (P) is considered as the second most important nutrient for successful crop growth and development and it has been observed to play a significant part in influencing disease build-up. P application at the right crop growth stage has been

Table 28.3 Effect of N level on disease severity of several diseases (Dordas 2008)

Pathogen or disease		Low N	High N	References
Obligate parasite	*Puccinia graminis*	Decrease	Increase	Howard et al. (1994)
	Erysiphe graminis	Decrease	Increase	Büschbell and Hoffmann (1992)
	Oidium lycopersicum	Decrease	Increase	Hoffland et al. (2000)
	Plasmodiophora brassicae	Decrease	Increase	Kiraly (1976)
	Tobacco mosaic virus	Decrease	Increase	Singh (1970)
	Pseudomonas syringae	Decrease	increase	Hoffland et al. (2000)
Facultative parasite	*Xanthomonas vesicatoria*	Increase	Decrease	Chase (1989)
	Alternaria solani	Increase	Decrease	Blachinski et al. (1996)
	Fusarium oxysporum	Increase	Decrease	Woltz and Engelhar (1973)

Table 28.4 Effect of K level on disease severity of several diseases (Dordas 2008)

Pathogen or disease	Low K	High K	References
Puccinia graminae	Increase	Decrease	Lam and Lewis (1982)
Xanthomonas oryzae	Increase	Decrease	Chase (1989)
Tobacco mosaic virus	Increase	Decrease	Ohashi and Matsuoka (1987)
Alternaria solani	Increase	Decrease	Blachinski et al. (1996)
Fusarium oxysporum	Increase	Decrease	Srihuttagum and Sivasithamparam (1991)
Pyrenophora tritici-repentis	Increase	Decrease	Sharma et al. (2005)
Erysiphe graminis	Increase	Decrease	Menzies et al. (1992)

found to reduce blast disease and bacterial leaf blight in rice, *Pythium* root rot in wheat, root rot and soil smut in maize, pod and stem blight in soybean, downy mildew and leaf curl virus disease in tobacco and brown stripe disease in sugarcane. However, in some studies it has been shown that P application resulted in increased disease severity of flag smut in wheat and increased disease infection by *Bremia* in lettuce and *Sclerotinia* in several garden plants.

The role of potassium (K) in disease suppression is by way of decreasing disease susceptibility of the host to various obligate and facultative parasites through promoting the synthesis of high molecular-weight compounds (proteins, starch and cellulose) and development of thicker outer walls in epidermal cells to help in preventing disease attack. K application was found helpful in reducing disease incidence of black rust in wheat, bacterial leaf blight, sheath blight and stem rot in rice, bacterial leaf blight in cotton, tikka leaf spot in groundnut, sugary disease in sorghum, *Cercospora* leaf spot in mungbean and seedling rot by *Rhizoctonia solani* (Table 28.4) (Dordas 2008).

Micronutrients do not have a direct role in disease resistance but can affect disease suppression indirectly. Deficiency of micronutrients not only impairs the defence mechanism of the host but it also makes the host more prone to diseases by causing several metabolites like reducing sugars and amino acids to leak outside of

Table 28.5 Role of micronutrient deficiency on soil-borne diseases

	Micronutrient deficiency	Disease	Causal organism
1	Boron (Bo)	Tomato Wilt	*Verticillium albo-atrum*
		Beans Root rot	*Fusarium solani*
2	Zinc (Zn)	Take all of wheat	*G. graminis* var. *tritici*
		Rhizoctonia Root rot	*Rhizoctonia solani*
3	Manganese (Mn)	Take all of wheat	*G. graminis* var. *tritici*
4	Copper (Cu)	Take all of wheat	*G. graminis* var. *tritici*
		Powdery mildew of wheat	*Blumeria graminis*
		Take all of wheat	*G. graminis* var. *tritici*
		Sunflower	*Alternaria*

the plant cell, making it a more suitable feeding substrate. Micronutrients play a vital part in controlling the permeability of cell membranes and maintaining the structural integrity, deficiency of which causes the membranes to become unstable and leaky. The deficiency of micronutrients in soil and plants can also reduce the production of fungus-inhibiting natural antifungal compounds, which, in turn, can increase the susceptibility of the host plants to diseases (Table 28.5) (Dordas 2008; Chandrashekara et al. 2012).

Nutrient supply can impact the development of plant disease under field conditions either directly through the nutritional status of the plant or indirectly by affecting the environment, which can influence disease development such as crop population and density, difference in light interception and humidity within the crop stand. Disease control through nutrient management can be accomplished either through modifying the nutrient availability or by modifying its uptake. It is very crucial to supply the plants with balanced nutrition and at the optimum time when the nutrient can be most effective not just for achieving higher yields but also for disease control. On the other hand, nutrient uptake can be altered to suit our needs of disease suppression and higher yields by changing root absorption, translocation and metabolic efficiency (Dordas 2008).

28.5.1.2 Soil Organic Matter

Soil organic matter (SOM) is referred to as the index of soil fertility and sustainability of agricultural systems. Soil organic matter improves the physical, chemical and biological properties of soil, protects the soil surface from erosion and acts as a reservoir of plant nutrients. The quantity as well as quality of soil organic matter can influence not just the soil nutrient status but also several other soil functions associated with soil health, including nutrient availability through microbial activity, infiltration and water retention. Hence, soil organic matter can affect disease incidence indirectly, if not directly, by means of promoting plant growth by increasing plant resistance and escaping disease by altering the pathogen's environment. Stone et al. (2004) has proposed several organic matter-mediated mechanisms for disease suppresiveness which are listed below:

1. Microbiostasis
2. Microbial colonization of propagules of the disease-causing organism
3. Destruction of propagules
4. Antibiosis and antagonism
5. Competition for energy and nutrient sources and for substrate colonization
6. Competition for root infection sites
7. Induced systemic resistance or systemic acquired resistance

28.5.2 Soil Microbial Biomass

The importance of microorganisms in organic agriculture is irrefutable not only because abundance of beneficial microorganisms in the soil renders it healthy but also because a number of soil-borne diseases result from a reduction of ample microbial populations. Whereas conventional agriculture banks on synthetic chemical fertilizers and pesticides for crop production, organic agriculture utilizes the natural capital of the soil and its microbial population for agricultural production and sustainability. These processes involve different soil microorganisms and reinstating the soil with those beneficial microorganisms that tend to repel, attack or antagonize disease-causing organisms make a soil disease suppressive. And plants which are grown in disease-suppressive soils are more likely to resist disease attack better than those which are grown in soil with lower microbial flora and fauna.

Pathogens flourish in soil environments with abundance of free nutrients. The food for microorganisms comes from nutrients; therefore, putting a limit to available nutrients can play a crucial role in disease suppression. Beneficial microorganisms make the soil environment unfavourable for the disease-causing pathogen by competing for food and energy paired with secretion of antibiotics. And for the very reason that they secrete antibiotics and antifungal metabolites microorganisms are presently also being used as a substitute for synthetic pesticides and fertilizers for many different crops.

28.5.3 Soil pH

Soil pH has an important role to play in disease management, both directly and indirectly. Soil pH directly affects disease incidence by influencing pathogen population and its environment and indirectly by influencing the availability of nutrients to the pathogen as well as the host. Pathogens require a favourable environment with an optimum pH to reproduce, complete their lifecycle, flourish and cause disease. If the soil pH is not suitable for the plant pathogen it hinders with its activity and hence help in disease suppression by helping the host plant escape disease. Indirectly, soil pH affects the release of nutrients from soil making nutrients unavailable for utilization by the pathogens. Beneficial microorganisms thrive in slightly low pH, but if the pH is too high it affects the soil bacteria that are involved in the breakdown of organic matter, resulting in the nutrient getting locked up in the undecomposed

organic matter making it unavailable for the pathogens. Hence, plant diseases can be managed by altering soil pH to suit the plant and beneficial microorganisms and to hinder the growth of disease-causing organisms.

28.5.4 Soil Structure and Texture

Soil structure and texture do not have a direct impact on disease suppression but may have an indirect effect on disease development as soil structure and texture influence the nutrient status, water holding capacity and gas exchange. Beneficial organisms thrive in soils with good aeration but soils with poor aeration resulting from a poor soil structure and water-logged conditions make an environment conducive for the development of *Pythium* spp. which causes cavity spot in carrot. Increased bulk density resulting from soil compaction has been shown to affect fresh weights of pea due to infection from root rot incidence (Chang 1994). Thus, the soil structure and texture can be managed to play an important role in disease control.

28.5.5 Soil Moisture and Temperature

As discussed earlier, a pathogen requires a favourable environment to flourish, and a favourable environment would mean abundant nutrients, optimum pH and an optimal amount of soil moisture content with a favouring temperature. Well-drained soil seldom have disease attacks, but soils with poor water drainage leads to water logging and lowered temperature, resulting in an environment conducive for the growth of several soil-borne pathogens such as *Pythium* spp. and *Phytophthora* spp., which are responsible for causing damping off and seed decay in many different crops. Raised soil moisture due to poor drainage leads to reduced oxygen availability, hindering the ability of the host plant to defend itself and contrastingly favouring soil-borne pathogens to get established and multiply. The severity of cavity spot disease of carrot by *Pythium violae* and *Pythium sulcatum* and root rot complex of pea by *Fusarium* spp. have been found to be directly proportional to the amount of soil moisture (Agrios 1997).

28.5.6 Cropping System, Cultural Practices and Residue Management

28.5.6.1 Soil Amendment with Organic Fertilizers

Since synthetic chemical fertilizers are not permitted in organic agriculture, nutrients applied to organic farms are generally through organic fertilizers which include compost, farmyard manure and green manure. Owing to the multiple benefits that can be derived from organic wastes in agricultural practices, they provide us with myriad opportunities including a remarking potential in disease management. But

these organic amendments take time to decompose, which results in gradual nutrient supply to crops and hence need to be planned well beforehand.

Compost

Compost has been used traditionally for the countless benefits it provides, which include enhancing soil organic matter, enriching physico-chemical properties of the soil and encouraging microbial population and microbial activity. While, some studies have shown a decline in disease incidence of *Sclerotinia minor* in lettuce and *Pythium arrhenomanes* in sugarcane, others have recorded a significant reduction in the inoculum of *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *asparagi*, and *Verticillium dahlia* with continuous application of composts (Lumsden et al. 1983; Dissanayake and Hoy 1999; Blok et al. 2000).

Compost has been proven to be effective in disease management and suppression because it helps in nurturing a diverse soil environment with diverse life forms buzzing with activity. Compost serves as a food source for beneficial microorganisms which antagonize and parasitize plant pathogens and produce antibiotics. Successful suppression of *Pythium* spp. and *Phytophthora* spp. causing root rots in crops has been achieved from high diversity and activity of beneficial microorganisms present in compost (Harrison and Frank 1999).

There exists a direct correlation between the level of decomposition in compost and its ability to suppress a disease-causing pathogen. Fresh undecomposed organic matter offers a conducive environment for highly competitive pathogens like *Pythium* spp. and *Rhizoctonia* spp. to grow and colonize because immature composts have good supply of readily available carbon compounds which gradually decrease as the compost matures. So the level of decomposition plays a central part in disease suppression owing to the direct relationship among the level of decomposition, microbial population, diversity and activity and the degree of disease suppression.

Farmyard Manure

As discussed in the previous section, fresh organic matter is deleterious to crops since it promotes growth of soil-borne pathogens such as *Streptomyces* spp. However, studies reveal that application of liquid swine manure has a positive role in suppressing pathogens such as *Streptomyces* spp. and *Verticillium dahliae* and reducing plant pathogenic nematode population (Conn and Lazarovits 1999, 2000). Besides, liquid swine manure was also found to promote *Trichoderma* populations further enabling the management of soil-borne pathogens.

28.5.6.2 Tillage

Tillage is a necessary cultural practice for preparing a field for cultivation. Tillage practices have been shown to play a great role in displacing pathogens directly as well as indirectly – directly by the placement of the residues in the soil and indirectly by promoting the soil microbial activity (Cook 1990). Soil microorganisms are concentrated mainly within the top 15 cm of the soil and more tillage practice would pose a greater risk of soil erosion and causing disturbances to the top layer of the soil which is home to beneficial soil biota. Conventional agriculture involves

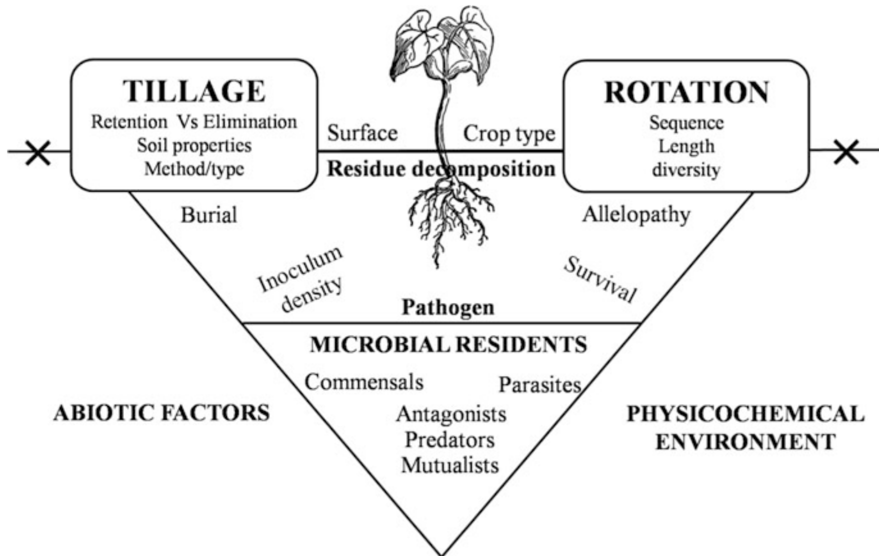

Fig. 28.4 Schematic diagram showing how agricultural practices recycle organic residues by the process of decomposition for utilization by the pathogen and beneficial microorganisms

intensive tillage practices, which result in considerable disturbance to the soil leading to low organic matter in the soil and therefore low microbial population and microbial activity in the soil (Lupwayi et al. 1999; Kennedy and Smith 1995). But the choice of tillage practice holds tremendous potential in disease suppression owing to its role in altering the ability of the pathogen to survive and its inoculum density.

Organic agriculture follows reduced tillage, minimum tillage or zero tillage systems which involve reduced mixing of the soil and thus retaining and maintaining healthy soil biota. These systems accumulate soil organic matter through carbon sequestration and stubble retention and encourage soil microfloral and microfaunal populations and activity (Fig. 28.4).

We have already discussed how soils with higher levels of organic matter have improved the soil structure, water infiltration, water retention and water drainage. Farming systems with reduced tillage practices have been shown to have lesser inoculum density of *Cochliobolus sativus* causing common root rot in wheat and barley compared to conventional tillage due to increased microbial activity, which created an antagonistic environment for the pathogen. Reduced tillage also led to increased populations of mycophagous amoebae as well as fungi which feed on *C. sativus* and creating a more hostile environment for its dormant spores. Reduced tillage systems also influence the availability of various nutrients in the soil and soil moisture impacting survival and infection of *Pythium* and *Phytophthora* spp. that cause root rots and damping-off (Tinline and Spurr 1991; Duczek 1983, 1986; Duczek and White 1986; Workneh et al. 1996).

28.5.6.3 Crop Rotation

Several diseases build-up in the soil only because the same type of crops are sown in the same field year after year. Crop rotation serves as the perfect strategy for managing those pathogens that survive in the soil or on the crop residue and for avoiding disease build-up by breaking this cycle. Since disease-causing organisms differ in the lengths of time for which they persist in the soil, crop rotation needs to be planned accordingly. Since pathogens usually attack plants belonging to the same family, it becomes helpful to group the susceptible host plant, related plants and alternate host pants together and keep them out during the rotation period (Fig. 28.4).

Crop rotation helps in disease suppression by either starving the pathogen or by killing it with toxic root exudates and for it to be most effective, crop rotation cycles should be 3–7 years between susceptible crops. A three-year crop rotation has been reported as the standard recommendation for *Fusarium oxysporum* causing stem rot, *Ceratocystis fimbriata* causing black rot, and *Monilochaetes infuscans* causing scurf in sweet potato. Three-year rotations also work best for several soil-borne pathogens such as *Fusarium* spp., *Verticillium* spp. and *Ralstonia* spp. that cause wilts in plants (Chandra and Baiswar 2007). However, crop rotation doesn't work effectively for those pathogens that survive for long periods in the soil without a host plant.

28.5.6.4 Organic Amendments and Organic Pesticides

Organic amendments prove to be an effective mechanism for disease suppression in organic agricultural systems because they enhance beneficial edaphic microorganisms along with antagonists which compete with the pathogens for available nutrients and suitable ecological niches. Organic amendments utilize natural products obtained from plants and animals for disease management also improving the soil structure, texture and water-holding capacity.

Neem seed and cake have been shown to effectively suppress many plant diseases, such as bacterial blight of rice and rhizome rot of ginger, and effectively control several disease pathogens such as *Rhizoctonia solani*, *Fusarium solani*, *Macrophomina phaseolina* and *Phytophthora capsici*. While cotton cake has been found to be effective in reducing disease incidence of seedling blight in eucalyptus, and several neem derivatives such as Nimbicidine, Neemark Neemoil have been reported to be effective against yellow mosaic virus in chickpea (Lazarovits et al. 2001; Bailey and Lazarovits 2003).

28.6 Conclusion

Crop protection in organic agriculture is not a simple matter. It heavily depends on a thorough knowledge of the crops grown and their likely pathogens. Successful organic crop protection strategies also rely on an understanding of the influence the abiotic factors such as soil, weather, climate and topography are likely to have on the crop performance as well as the disease-causing pathogens. Organic agriculture is rapidly expanding in its scope and area to include different food and fibre items for large-scale production, giving a strong competition to conventional farming systems.

Different innovative strategies to avoid, suppress and control persistent disease problems have already been established and several are being devised rapidly. We do need to optimize these strategies and look for further new avenues to tackle the disease problem in organic farms for which research needs to be done.

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Pest Risk Analysis and Plant Quarantine Regulations

29

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Abstract

International trade of agri-horticultural commodities and exchange of germplasm plays an important role in the long-distance dissemination of insect pests and pathogens, which may pose potential risk to the agriculture of the importing country. The National Plant Protection Organizations have the responsibility of protecting their countries from the unwanted entry of new insect pests and pathogens. The exclusion can be achieved by a combination of regulatory and technical approaches that can ensure biosecurity for a country/region. The International Plant Protection Convention (IPPC) of the Food and Agriculture Organization in the United Nations develops the International Standards for Phytosanitary Measures (ISPMs) which provide guidelines on pest prevention, detection and eradication. To date, 43 ISPMs have been developed and adopted. 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 quarantine inspection and disinfestation of agricultural commodities meant for commercial purpose. The imported germplasm materials including transgenics are subject to quarantine processing at the ICAR-National Bureau of Plant Genetic Resources, New Delhi. The strategies for biosecurity for insect pests and pathogens include pest risk analysis and stringent quarantine regulations for the imported material, domestic quarantine and use of certified disease-free seed and other planting materials within the country. Adopting a workable strategy such as post-entry quarantine (PEQ) growing in PEQ greenhouses/containment facility, electron microscopy, conventional, serological and molecular diagnostics, several pests including 45 viruses have so far been intercepted, which include 19 viruses not yet reported from India. Adopting the right strategy and appropriate technique for pest detection would go a long way in

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ensuring the biosecurity of Indian agriculture from transboundary introduction of plant viruses.

Keywords

Pest risk analysis · Quarantine · Pathogens · Viruses · Diagnostics · Germplasm · Commercial material · India

29.1 Introduction

Plant diseases substantially reduce crop production every year, resulting in serious economic losses throughout the world. Trade and exchange of germplasm at the international level play a key role in the long distance dissemination of a destructive pest 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 and other planting materials has the potential of introducing new pathogens and insect pests, which may pose potential risk to the agriculture of the importing country.

The devastating effects resulting from pathogens introduced, along with international movement of seed and other planting materials, are well documented. The Irish famine of 1845, which forced 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 materials without proper phytosanitary measures, indiscriminate exchange of germplasm and the distribution of seed and other planting materials 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*, *Peanut mottle virus,* etc. is attributed to the unrestricted exchange of seed lots.

Like in other countries, a number of exotic pathogens and insect pests got introduced in 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), potato tuber moth (*Pthorimaea operculella* in 1906), flag smut of wheat (*Urocystis tritici* in 1906), San Jose scale (*Quadraspidiotus perniciosus* in 1910), fluted scale (*Icerya purchasi* in 1912), codling moth (*Cydia pomonella* in 1919) and the weed, *Lantana camara* (in the

early part of nineteenth century). These introductions highlighted the fact that increased pace of international travel and trade had exposed countries to the danger of infiltration of exotic pathogens and insect pests 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 (NPPOs) assume responsibility for protecting their countries from the unwanted entry of new pests by applying appropriate measures. Such measures must either be based on international standards or based on risk assessment for the country. Therefore, plant quarantine measures should be based on pest risk analysis (PRA). 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. The PRA is calculating, speculating or extrapolating the “risk (known or perceived) involved” if an organism will enter, colonize and/or become established in an area where it is not known to occur. Most NPPOs determine the entry status of imported agricultural commodities based on perceived risk and economics involved in mitigation measures, in case the pest establishes. The PRA consists of risk assessment (scientific estimation of likelihood and magnitude of establishment of a given pest risk) and impact assessment (estimation of the consequences of the establishment of pest). It is a process of evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated and the strength of any phytosanitary measures to be taken against it. Risk analysis is an important element of biosecurity and forms the basis of prevention and initial control of problems arising from damaging new areas or introduction of new pests.

Theoretically for achieving zero risk, a country should not allow any imports of planting material/other commodities and be equally strict to not permit the movement of human beings and animals/birds from one part of the country to other. It is not easy to achieve this; therefore, if the overall risk is perceived to be high, the entry status of the imported material should be conservative, and conversely if the risk is perceived to be low the entry status should be liberal. Further to ensure that the best option available has been chosen out of all the available ones, PRA needs to be conducted for all. A PRA should be sufficiently documented so that when a dispute arises, the PRA will clearly state the source of information and the rationale used in reaching a management decision regarding phytosanitary measures taken or to be taken.

The risk analysis is carried out on the basis of information about the pest status in both importing and exporting countries. The justification for regulating pests requires that the pest should qualify as a quarantine pest or as Regulated Non-Quarantine Pest (RNQP), which is based on the PRA. The procedures to be followed for risk analysis of pests are given in the ISPM-2 (framework for pest risk analysis), ISPM-11 (pest risk analysis for quarantine pests) and ISPM-21 (pest risk analysis for regulated non-quarantine pests).

All the member countries of WTO are required to prepare and update lists of regulated pests to facilitate safe trade. In India, the Plant Quarantine (Regulation of Import into India) Order 2003 notified to the WTO Secretariat gives a list of >1200 regulated pests on ~700 host commodities. The Schedules V and VI pertain to the

specific requirements for regulated pests, but listing of RNQPs is yet to be done specifically. All the crops not listed under any of the present schedules can only be introduced in India only after carrying out detailed PRA. The requirements under these existing schedules become a part of the additional declarations sought from the importing country in the import permit. The same pest-free status and treatments given are followed by the exporting country and confirmed on the phytosanitary certificate.

29.2 Pest Risk Analysis

The PRA is evaluation of several factors through transparently collected data, quantitative or qualitative grading for the pest status, pathway, biology of the host, climatic conditions, economic impact and official control. Overall risk is then calculated and mitigation measures are discussed to reach to an objective of the minimum possible risk while carrying out trade under the prevailing situations. The preparation of PRA is an elaborate process requiring scientific input on various aspects of the pest and the associated risk involved in its inadvertent introduction. As per the IPPC, the term plant pest refers to all organisms harmful to plants or plant products including other plants, bacteria, fungi, insects and other animals, mites, molluscs, nematodes and viruses. Pests can be either regulated or not, and the IPPC recognizes and defines two categories of regulated pests of plants viz., quarantine pests and regulated non-quarantine pests. PRA assists with determining whether a pest fits either of these two categories.

A quarantine pest is defined by the IPPC as "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). In other words, it is any organism that is injurious or potentially injurious, directly or indirectly, to plants or plant products or by-products of plants and includes bacteria, fungi, insects, mites, molluscs, nematodes, other plants and viruses, not present in a specified area at risk or, if present, being controlled by an NPPO.

A regulated non-quarantine pest (RNQP) is defined by the IPPC as "a non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party" (FAO 2019). RNQPs are generally those that are established in the importing country, may be widely distributed and regulated on specified hosts to keep them below a level, at which they would cause an unacceptable economic impact.

The requirement for a PRA consists of three stages viz., initiation, risk assessment and risk management whose compilation should be fully documented in the event a review or whenever a dispute arises. The PRA should clearly document information sources and the rationale used in reaching a management decision regarding mitigation measures that have been taken or are to be taken. PRA is a process to answer the questions such as Is the organism a pest? What is the likelihood of introduction, establishment and spread? How much economic (including environmental and

social) damage (unacceptable impacts) does it cause? and What can be done to mitigate unacceptable impacts? Consequently, any phytosanitary measures implemented by a country to mitigate against a particular pest should be technically justified by the PRA.

29.2.1 Pest-Initiated vs. Pathway-Initiated PRAs

There are two widely adopted approaches to conducting a PRA, one focused on a pathway, the other focused on a particular pest associated with one or more pathways. A commodity PRA is one type of pathway PRA. In the case of a pathway PRA, it is necessary to conduct PRAs on those pests associated with the pathway that are identified as potential quarantine pests. In the case of a pest PRA, consideration needs to be given to all possible pathways or commodities with which the pest may be associated.

29.2.2 Stages in PRA

Pest risk analysis (PRA) consists of risk assessment (scientific estimation of likelihood and magnitude of establishment of a given pest risk) and impact assessment (estimation of the consequences of the establishment of pest). Therefore, to ensure that imported commodity presents a minimal pest or no pest risk to our agriculture and forestry, PRA must be conducted.

Initiating the process involves identification of pests or pathways for which the PRA is needed. The list of pests is generated by any combination of databases, literature sources or expert consultation. Once the list of pests has been established, it is preferable to prioritize it by using expert judgement before the next step. According to the results obtained, it may or may not be necessary to conduct a risk assessment on all pests on the list.

Pest risk assessment determines whether each pest identified as such, or associated with a pathway, is a quarantine pest, characterized in terms of likelihood of entry, establishment, spread and economic importance. In doing so, the PRA considers all aspects of each pest and in particular actual information about its geographical distribution, biology and economic importance. Expert judgement is then used to assess the establishment, spread and economic importance potential in the PRA area. Finally the potential for introduction into the PRA area is characterized.

Pest risk management involves developing, evaluating, comparing and selecting options for reducing the risk. A list of options for reducing risks to an acceptable level should be assembled. These options will primarily concern pathways and in particular the conditions for permitting entry of commodities. The pest risk management to protect the endangered areas should be proportional to the risk identified in the pest risk assessment.

A PRA should be sufficiently documented so that when a review or a dispute arises, the PRA will clearly state the sources of information and the rationales used in reaching a management decision regarding phytosanitary measures taken or to be taken. ISPMs specifically related to pest risk analysis.

29.2.3 International Standards for Phytosanitary Measures

The International Standards for Phytosanitary Measures (ISPMs) that are most relevant to pest risk analysis (PRA) rare given below:

29.2.3.1 ISPM No. 2 Framework for Pest Risk Analysis (Adopted in 2007 and Published in 2019)

This standard provides a framework that describes the pest risk analysis (PRA) process within the scope of the IPPC. It introduces the three stages of pest risk analysis – initiation, pest risk assessment and pest risk management. The standard focuses on the initiation stage. Generic issues of information gathering, documentation, risk communication, uncertainty and consistency are addressed.

29.2.3.2 ISPM No. 3 Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms (Adopted in 2005 and Published in 2017)

This standard provides guidelines for risk management related to the export, shipment, import and release of biological control agents and other beneficial organisms. The standard addresses biological control agents capable of self-replication (including parasitoids, predators, parasites, nematodes, phytophagous organisms and pathogens such as fungi, bacteria and viruses), as well as sterile insects and other beneficial organisms (such as mycorrhizae and pollinators), and includes those packaged or formulated as commercial products. Provisions are also included for import for research in quarantine stations of non-indigenous biological control agents and other beneficial organisms. The scope of this standard does not include living modified organisms, issues related to registration of biopesticides or microbial agents intended for vertebrate pest control.

29.2.3.3 ISPM No. 11, Pest Risk Analysis for Quarantine Pests (Adopted in 2013 and Published in 2019)

This standard provides details for the conduct of PRA to determine if pests are quarantine pests. It describes the integrated processes to be used for risk assessment as well as the selection of risk management options. Some explanatory comments on the scope of the IPPC with regard to environmental risks are given in Annex 1. Some explanatory comments on the scope of the IPPC regarding PRA for LMOs are given in Annex 2. Annex 3 deals with determining the potential for a living modified organism to be a pest.

29.2.3.4 ISPM No. 21 Pest Risk Analysis for Regulated Non-quarantine Pests (Adopted in 2004 and Published in 2019)

This standard provides guidelines for conducting pest risk analysis for regulated non-quarantine pests. It describes the integrated processes to be used for risk assessment and the selection of risk management options to achieve a pest tolerance level.

Although ISPMs are internationally agreed to and adopted, they are meant to be guidelines and their use is not mandatory within the framework of the IPPC. In addition, their interpretation and application on a national level varies from country to country. This variation is illustrated by the many different national systems and procedures that exist for carrying out PRA. Countries often take slightly different approaches to implement the standards while still staying true to their intent. A PRA conducted according to international standards can provide the basis for our national planners to allocate resources to problem areas posing the highest risk and thereby enable them to better protect our agriculture and environment from the entry and establishment of exotic pests.

29.2.3.5 Significance of PRA

Risk analysis may be defined as a science-based tool for decision-making; it is a broad term, which encompasses activities including risk assessment, pathway analysis, risk management and communication, border controls and emergency preparedness. International standards for these activities have been established and may be applied for prevention, management of alien pests at the international, regional or local levels.

International harmonization of phytosanitary measures can be successful only if contracting parties implement standards. Control systems are needed to verify whether this is done. If countries cannot resolve trade disputes bilaterally, a WTO dispute procedure can be initiated. The PRA becomes important as compliance by concerned countries with international standards is an important judgment criteria for decisions by WTO dispute panels.

29.3 Role of Diagnostics in Quarantine Against Transboundary Plant Pathogens

In the context of quality control, bulk samples need to be tested by drawing workable samples as per the 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 materials 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 trade and for exchange of germplasm. Early, sensitive, and accurate diagnosis is indispensable for certification of seed and other planting materials under exchange. The selection of a diagnostic

Table 29.1 Summary of various techniques for detecting pathogens of quarantine significance

Techniques	Fungi	Bacteria	Viruses	Viroids	Phytoplasma
Visual 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)	+	+	+	+	+

method for evaluating plant health depends on the host to be tested and the type of pathogens that may be carried in the seed and other planting materials. The technique should be reliable, reproducible within statistical limits, economical with regard to time, labour and equipment and should be rapid for quarantine requirements.

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 and are summarized below in Table 29.1.

29.4 Plant Quarantine Regulations

29.4.1 International Scenario

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 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, 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, establishment/spread of pests; or to prevent or limit other damages 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 provide guidelines on pest prevention, detection and eradication. To date, 43 ISPMs ((https://www.ippc.int/en/core-activities/standards-setting/ispms/) have been developed and adopted (Annexure-I).

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.

29.4.2 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 2016).

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 materials. 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 1st January, 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, clarifying specific queries raised by quarantine authorities of various countries, with revised lists of crops under the 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. The 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 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 six regional plant quarantine stations viz., Amritsar, Chennai, Kolkata, Mumbai, New Delhi and Bengaluru. There are 42 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, methyl bromide fumigation, etc. 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 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 green house complex. Keeping in view the biosafety requirements, National Containment Facility of level-4 (CL-4) has been established at ICAR-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 like *Fusarium nivale*, *Peronospora manshurica* and *Uromyces betae* and bacterium like *Xanthomonas campestris* pv. *campestris* (Bhalla et al. 2018a). 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 19 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), *Garlic virus-C* (GarV-C), Dioscorea latent virus (DLV), 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. 2004, 2005a, 2007, 2008, 2009a, 2009b, 2012a, b, d, 2013b, c, d, 2014a, b, c, 2015a, b, 2016a, b, 2018a, b; 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;

Singh et al. 2015). 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 in India was thus eliminated. All the plants infected by the viruses were uprooted and incinerated.

The infected samples were salvaged by using suitable techniques (Singh and Khetarpal 2005), 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.

29.4.3 National Scenario: Exports

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 agri-horticultural commodities. All the materials 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 and Farmers Welfare, Government of India, has notified 199 officers to grant Phytosanitary Certificate for export of plants and plant material (http://plantquarantineindia.nic.in/PQISPub/html/Export.htm).

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. ICAR-NBPGR has developed well-equipped laboratories and green house complex. ICAR-NBPGR undertakes detailed examination of germplasm meant for export for the 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 (Chalam and Mandal 2013; Jain and Chalam 2013).

29.4.4 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 seems to have been introduced in 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, 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) and West Bengal (WB) and banana plants, 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 the 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; the absence of concerted action and enforcement of internal quarantine regulations by the state governments; lack of interstate border quarantine check-posts 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; 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 need to review the status of existing domestic quarantine for establishment of interstate quarantine check-posts 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 in different parts of India.

There is a dire need to revisit the *existing domestic quarantine* scenario for strengthening interstate quarantine check-posts and eventually for monitoring the 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 removed 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 ring spot 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 the state level parallel to Australia and USA.

29.4.5 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 is 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 micro-organisms. It provides for modernising the legal framework to regulate safe movement of plants and animals within the country and in international trade, and harmonise 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 (i) regulating the import and export of plants, animals and related products; (ii) preventing the introduction of quarantine pests from outside India; and (iii) 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).

29.5 Challenges in Diagnosis of Pathogens in Quarantine

The issues related to the quarantine methodology were analyzed/reviewed by Khetarpal (2004) and Chalam and Khetarpal (2008). 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 the 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, ICAR-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, strains, etc. should be made available for its use as a ready reckoner by the scientists, extension workers and quarantine personnel. ICAR-NBPGR has compiled pests of quarantine significance for cereals (Dev et al. 2005; Chalam et al. 2005b), grain legumes (Chalam et al. 2012c), oilseeds (Gupta et al. 2013; Chalam et al. 2013a) and tropical and sub-tropical fruit crops (Bhalla et al. 2018b; Chalam et al. 2018b) for India. The Crop Protection Compendium of CAB International, UK, is an useful asset to scan for global pest data (http://www.cabi.org/cpc/).

As we face challenges to crops from intentional or unintentional introduction of pests, speed and accuracy of detection become paramount. Intense efforts are underway to improve detection techniques. The size of consignment received is very critical in quarantine from the 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 a 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 did not denote a false positive or a false negative sample. Removal of exotic viruses from the 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).

29.6 Perspectives

The plant pathogens have great potential to spread locally and globally due to the liberalized trade and exchange of research material and germplasm if stringent quarantine measures are not followed as per SPS/WTO norms. There is also a need to strengthen the domestic quarantine system to prevent the spread of viruses with limited distribution within the country. The way forward to ensure biosecurity is thus highlighted below:

- Strengthen Plant Quarantine Stations dealing with bulk samples in terms of manpower, infrastructure (well-equipped laboratories, treatment facilities and greenhouses) and expertise with special emphasis on advanced techniques for

detection of pathogens especially viruses/strains in bulk samples through regular trainings.

- Initiate pre-import inspection and strengthen the post-entry quarantine (PEQ) growing and inspection of the imported material. The inspection authorities should be given adequate support in terms of manpower and funds for undertaking the work. Also, impart training to Inspection Authorities on plant quarantine issues with special emphasis on post-entry quarantine requirements and methodology as per the notified SOPs on the same.
- Regular survey and surveillance programme needs to be undertaken to get a realistic picture of the status of pests in the country, and for authentic mapping of endemic pests present in localized pockets and this in turn will help in identification of pest-free areas and to include these pests under domestic quarantine. There is a need for development of eradication strategies for recently introduced pests and also for pests with limited distribution. This would give a boost to our exports when the importing country is assured by a certification agency that the produce is from pest-free areas.
- Detection and diagnosis of pests are crucial for application of mitigation strategies, trade and for exchange of germplasm. There is a need to accredit diagnostic laboratories at the central and state level for quick and accurate identification of pests and also for National Certification Programme for Seed Health in line with National Certification System for Tissue Culture-raised Plants, Department of Biotechnology, Govt. of India and need to review seed certification standards proposed in Seeds Act 1966. A National Repository of Diagnostics for Diseases including antisera Bank, database of primers, seeds of indicator hosts, reference collections, user-friendly diagnostics such as lateral flow strips/ dip sticks which can detect multiple viruses, multiplex RT-PCR protocols, LAMP and HDA protocols for detection of viruses in the field and at ports of entry, microarrays, DNA barcoding and ultimately, a cost-effective national biosecurity chip for diagnosis of all current threats to crop plants would be the backbone for strengthening the programme on biosecurity for pests. There is an urgent need to develop a *National Plant Pests Diagnostic and Certification Network* linking the research laboratories with seed/vegetative planting material testing laboratories and quarantine stations, which would be the backbone for strengthening the programme on biosecurity from plant pests including viruses (Chalam et al. 2017).
- Review the national regulatory framework and develop a mechanism for distribution or sale of pest-free seeds/ plants/ planting material within the country, be it seed distribution for multi-location testing under All India Coordinated Research Projects, inland supply of germplasm by ICAR-NBPGR or seed distribution by the National/State Seed Corporations/private organizations. Also, need to develop a national mechanism to monitor the movement of vegetatively propagated material and tissue culture-raised plants across the states. Also, strengthen the state certification mechanism to ensure the supply of pest-free nursery material.

- Database on all pests, including information on host range, geographical distribution, strains, etc. should be made available for its use as a ready reckoner by the scientists, extension workers and quarantine personnel.
- Establish proper authenticity for reports of new pests and deposition of reference cultures in the national repositories may be made mandatory. The exports may suffer due to wrong identification of pests and reporting the same as new reports.
- Use simulation models for developing an *early warning system* to predict outbreaks of diseases and insect pests. Remote sensing may also be used for the same.

Adopting 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, pest-free trade and exchange of germplasm. Besides preventing the introduction of exotic pathogens and insect pests, the role of the diagnostics especially advanced biotechnological interventions in certification of the planting material of the agri-horticultural crops against indigenous pests needs a great impetus in boosting our production and trade.

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30 Remote Sensing Technology and Its Applications in Plant Pathology

Ghada A. Khdery

Abstract

Early disease detection and plant health monitoring is a critical tool for reducing the spread of diseases. Thanks to its great importance in detecting infection before it occurs on plants, remote sensing is one of the modern science developments in the monitoring of plant pathogens. Remote sensing techniques will be a very useful tool for greatly tailoring the diagnostic results. Such innovative technologies are unparalleled instruments for making agriculture healthier and more sustainable and for reducing the unnecessary use of pesticides in crop safety. This chapter discusses the importance of remote sensing in the control of plant disease and its diagnostic methods, and some examples where remote sensing was used to monitor plant diseases. Finally, this chapter discusses the basic principles of hyper-spectrum measurements and the various types of hyperspectral sensors for plant defense and plant disease detection in various ranges.

Keywords

Plant pathology · Remote sensing · Hyperspectral technique · Spectral signature

30.1 Introduction

Innovative remote sensing technologies can supply new insights into host–pathogen systems and have the chance to replace general destructive investigation methods (Mutka and Bart 2014; Mahlein 2016). Among the various types of sensors, (chlorophyll-fluorescence- thermography, RGB, hyperspectral and multispectral)

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hyperspectral sensors have significant capabilities and various advantages for discriminating plant diseases and host–pathogen interaction. Chlorophyll fluorescence and thermography are able to reveal plant stress without determination of causes of an agent. With hyperspectral technology, it is possible to identify the responsible disease/pathogen (Bravo et al. 2004; Mahlein et al. 2010). Remote sensing can also help in plant conservation from potential attacks of fungi, pests or bacteria. Remotely sensed data and agricultural knowledge can give early warning and prevent disease or a pest from affecting the crops, by taking suitable action at an early stage. Detection of diseases at early stage is a lot easier less costly than currently used impractical human scouting techniques.

Remote sensing is the most important tool for previous diagnosis of plant diseases. It can provide a quick reaction compared to a manual scouting procedure typically to determine the presence of the lesion (Moran et al. 1997). The theoretical basis for remote sensing applications in the evaluation of crop diseases is that crop diseases cause some physiological changes and severe damage to plant tissues. As a result, infection from insect pests interferes with photosynthesis and plant structure and thus affects the amount of light energy and modifies the reflection feature of the plant (Hatfield and Pinter Jr. 1993). These changes are characterized as a significant change in the plant spectral pattern. Therefore, as a first step, there is a great need to determine the characteristics of spectral reflection in the case of infected and healthy plants. Second, these characteristics will be used for pre-visual diagnosis of a possible future infection. Many studies have been done about the ability of remote sensing technology in diagnosis and detection of plant diseases such as (Mahlein et al. 2010, 2012; Hillnhutter et al. 2011; Abdel et al. 2017). First of all we should understand what is remote sensing? (Table 30.1)

30.2 Remote Sensing Definition

There are a number of definitions for remote sensing, including the following:

Remote Sensing (RS) is the science of identification, observation and measurement of an object without direct contact with it. It is the science of deriving information from spectral characteristics acquired at a distance about the earth and water. Through aid of the eye, humans accomplish this mission. Car driving, newspaper reading and watching in front of you are all remote sensing practices. Many sensing devices record an object's information by measuring electromagnetic energy transmission from reflecting surfaces of an object, a camera is a remote sensor because it measures the reflected light without touching the photographed object. Besides, a camera is sensitive to certain light rays with its filters and photographic emulsions and radar instruments (Aggarwal 2004).

Table 30.1 Overview of plant pathosystems and plant diseases assessed by hyperspectral imaging

Host–pathogen system	Scale	Detection	Early detection	Quantification	References
Apple—*Venturia inaequalis*	Leaf	√	n.i.	√	Delalieux et al. (2009)
Barley—*Blumeria graminis* f.sp. *hordei*	Tissue	√	√	n.i.	Kuska et al. (2015)
Barley—*Blumeria graminis* f.sp. *hordei*	Leaf	√	√	√	Thomas et al. (2017)
Barley—*Blumeria graminis* f.sp. *hordei, Puccinia hordei, Pyrenophora teres*	Leaf	√	√	n.i.	Wahabzada et al. (2015)
Celery—*Sclerotinia sclerotiorum*	Canopy	√	n.i.	n.i.	Huang and Apan (2006)
Cucumber—CMV, CGMMV, *Sphaerotheca fuliginea*	Leaf	√	n.i.	√	Berdugo et al. (2014)
Sugar beet—*Cercospora beticola*	Leaf	√	n.i.	√	Bergstrasser et al. (2015)
Sugar beet—*Cercospora beticola*	Tissue	√	n.i.	√	Leucker et al. (2016)
Sugar beet *Cercospora beticola, Erysiphe betae, Uromyces betae*	Leaf	√	√	n.i.	Rumpf et al. (2010)
Sugar beet—*Cercospora beticola, Erysiphe betae, Uromyces betae*	Leaf	√	√	√	Mahlein et al. (2010, 2012)
Sugar beet—*Heterodera schachtii*[a], *Rhizoctonia solani*	Canopy	√	n.i.	√	Hillnhutter et al. (2011, 2012)
Oilseed rape—*Alternaria* spp.	Leaf	√	n.i.	√	Baranowski et al. (2015)
Wheat—*Blumeria graminis* f. sp. *tritici*	Canopy	√	n.i.	√	Cao et al. (2013)
Wheat—*Fusarium* spp.	Ear	√	√	√	Bauriegel et al. (2011)
Wheat—*Puccinia striiformis*	Canopy	√	n.i.	√	Bravo et al. (2003)
Wheat—*Puccinia striiformis*	Canopy	√	n.i.	n.i.	Bravo et al. (2003)
Wheat—*Puccinia striiformis*	Canopy	√	n.i.	√	Huang et al. (2007)
Wheat—*Puccinia striiformis*	Canopy	√	√	n.i.	Moshou et al. (2005)
Wheat—*Puccinia striiformis, Puccinia graminis, Puccinia triticin*	Leaf	√	n.i.	n.i.	Devadas et al. (2009)

Source: Thomas et al. (2017)
n.i. indicates a non-investigated aspect
[a]indicates nematodes

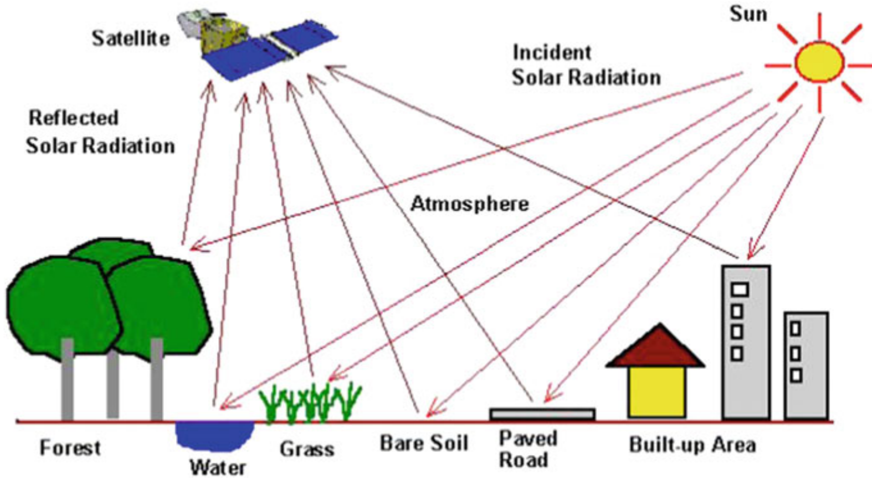

Fig. 30.1 Steps of remote sensing process. (Source https://crisp.nus.edu.sg/~research/tutorial/optical.htm)

30.2.1 Principles of Remote Sensing

Discrimination and identification of surface features or objects requires detection and recording of radiant energy produced or reflected by materials or objects on the surface (Fig. 30.1). Different objects depend on different amounts of energy in different bands of the electromagnetic spectrum, incident to it. It depends on the property of the substance (chemical, structural and physical), angle of incidence, intensity, roughness of the surface and wavelength of radiant energy. The electromagnetic radiation spectrum ranges from small, high to long wavelengths of energy. As an executor, the human eye only tests a fairly small segment of the spectrum from 0.4 to 0.7 nm in the visible field. The area between 0.4 and 5/Mm can be interpreted as the wavelengths reflected. Reflection is that phenomenon in which an impinging radiation beam of a specific wavelength is reflected back from the target without any alteration. This can be contrasted with emittance, which is the emission of radiant energy at a given wavelength due to an object's temperature. Emittance provides information which can be used for remote sensing applications to agricultural problems (Aggarwal 2004).

30.2.2 Types of Remote Sensing (Fig. 30.2)

Active Remote Sensing: When remote sensing is performed with a man-made source of radiation that is used to illuminate the body and reveal the shape of the reflected signal, for example, radar and lidar remote sensing (Fig. 30.2).

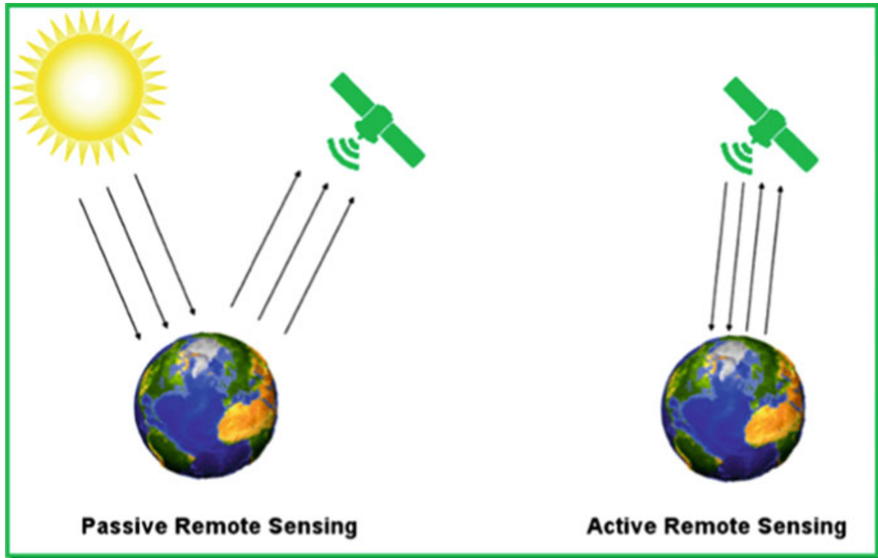

Fig. 30.2 Types of remote sensing. (Source: https://grindgis.com/remote-sensing/active-and-passive-remote-sensing)

Passive Remote Sensing: When remote sensing is performed with the help of electromagnetic radiations (signals) reflected by a natural body (sun and earth). For example, visible, NIR and microwave remote sensing (Fig. 30.2).

30.3 Spectral Signature

Spectral reflectance, as a function of wavelength, is the ratio of reflected energy to incident energy. Each material on Earth's surface has varying spectral properties. Different surface materials exhibit different properties of spectral reflection. Spectral reflection on a photograph of an object is responsible for the colour or tone. Trees represent the length of the green waves, so trees appear green. The spectral reflectance values of objects averaged over different, well-defined intervals of wavelengths are the spectral signature of the objects or features they can be distinguished by. The spectral reflectance is wavelength dependent; it has different values for a given terrain characteristic at different wavelengths, and its reflectance properties are its content of moisture, organic matter content, texture, structure and iron oxide. We can build a spectral signature for that object by measuring the energy that is reflected by targets on the earth's surface over a variety of different wavelengths. And we may be able to distinguish between them by comparing the response pattern of different features, which we may not be able to do if we only compare them at a single wavelength. For instance, water and vegetation reflect in

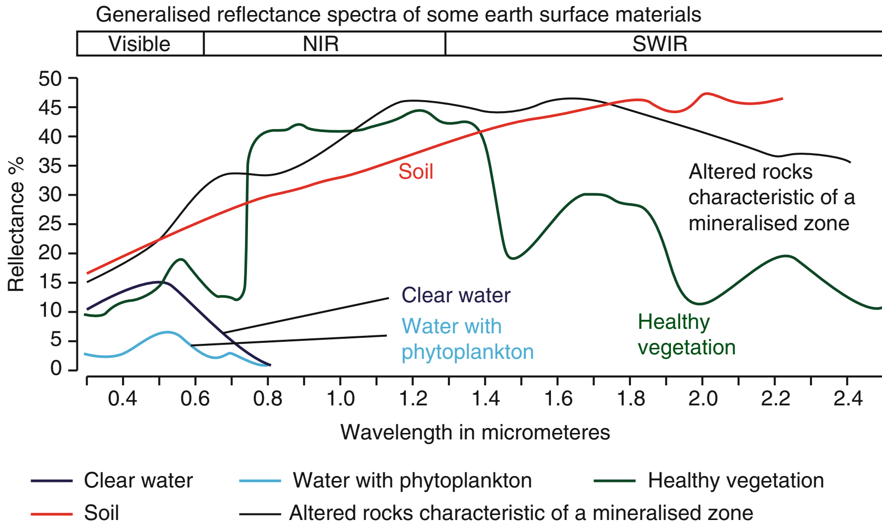

Fig. 30.3 Spectral signatures of water, vegetation and soil. (Source http://www.rsacl.co.uk/images/base2.jpg)

the visible wavelength somewhat similarly but not in the infrared (Aggarwal 2004) (Fig. 30.3).

30.3.1 Spectral Signature for Healthy and Infected Plant

Leaves have a low reflectance in the visible (0.4–0.7/m), a high reflectance in the near-infrared (0.7–1.2 Mm) and a low reflectance in the middle and far infrared (1.2 *Mm)* wavebands. This variability in the reflectance of the leaves has allowed the separation of the leaves from the soil, which tends to show little difference in reflectance across these wavelengths. As the leaf matures, reflectance tends to increase in individual leaves; however, the changes depend on wavelength. These variations derive from changes in the quality of intracellular water and chlorophyll. Increased reflectance is also caused by lesions and decreased chlorophyll content produced by a disease. Water stress increases the reflectance from an individual leaf by reducing the inner water content. Information collected from individual leaves provides a basic set of information about the process of changes occurring within a plant; however, it must be generalized to a canopy or field level to be of practical application. Leaves of green plants absorb most of the blue and red light to use in the photosynthesis process, and most of the green light is reflected from the leaves of plants. In Nir IR from 0.7 and 1.0 μm, there is also strong reflectance in the spongy mesophyll cells, which are located in the internal part of a leaf in (SWIR) short wave infrared. Water content is the major factor that affects spectral reflectance pattern. The occurrence of higher water content in plant leaves leads to a decrease of reflectance in short wave infrared zone (Gogoi et al. 2018).

Spectral characteristics of vegetation was discussed by (Sahoo et al. 2015) which are determined by their biochemical and biophysical nature and attributes such as senesced biomass, leaf area index, moisture content and pigment and structures. In the visible region (VIS: 0.4–0.7 nm), pigments which absorb main light are chlorophyll *a* and *b*, xanthophylls, carotenoids and polyphenols. Chlorophyll *a* exhibits the greatest absorption in the 0.41–0.43 and 0.60–0.69 nm regions, whereas Chlorophyll *b* display maximum absorption in the 0.45–0.47 nm range. These strong absorption bands induce a reflectance peak in the green zone at about 0.55 nm. In the near infrared zone (NIR: 0.7–1.3 nm), reflectance and transmittance reach their greatest values and absorption is very small. This is caused by interior diffusion at the cell–air–water interfaces within the leaves. In shortwave-infrared (SWIR) (1.3–2.5 nm), leaf properties are affected by other foliar constituents and water. The major bands of absorption take place at 1.45, 1.94 and 2.7 nm and other processes at 0.96, 1.12, 1.54, 1.67 and 2.2 nm (Sahoo et al. 2015). Changes in reflectance arise from changes in plant tissue's biophysical and biochemical properties (Khdery et al. 2019; Gamal et al. 2020a, b). When a plant is under stress, the chlorophyll production may decrease resulting in less absorption of palisade cells in the blue and red bands. So along with the green band, red and blue bands are also reflected. Therefore, in stressed vegetation yellow or brown colour is developed. As a result, dark patches are found in the image (Gogoi et al. 2018).

Basically, the visible region from 400 to 700 nm is related to the pigment composition (Blackburn and Steele 1999; Gitelson et al. 2001) while (NIR region) from 700 to 1100 nm is related to the water content, leaf traits and structure and influence (Blackburn and Steele 1999; Gitelson et al. 2001).

30.4 Applications of Remote Sensing in Plant Diseases

Remote sensing can help in identifying, diagnosing and controlling plant diseases, as well as the stress caused by a lack of water or nutrients. Remote sensing also helps protect plants from any possible attack of bacteria, fungi or pests. Dimension Remote sensing data can be combined with agricultural knowledge to provide early warning to prevent plant from crop diseases, by taking appropriate action at an early stage. The effects of such attack usually cause chlorophyll to break down, and by remote sensing we can detect the reduced chlorophyll concentration in the plants. In addition to chlorophyll loss, diseases and pests can cause destruction of whole leaves. Besides the loss of chlorophyll, pests and diseases may cause the destruction of entire leaves. This leads to a reduction in the total area of the leaf and, ultimately, to a reduction in the photosynthesis ability of the plant. By defining the leaf area index (LAI) for plant species, it is possible to identify an early pest attack and educate farmers to take appropriate measures. The study by Apan et al. (2004) demonstrated that Hyperion satellite hyperspectral imagery could be used to detect orange rust (*Puccinia kuehnii*) disease in sugarcane.

Many researchers studying application of remote sensing technologies in detection of plant diseases (Abdel et al. 2017; He et al. 2019; Deleon et al. 2017; Piou and Prévost 2013; Jiang et al. 2008; Hillnhutter et al. 2011; Mahlein et al. 2010, 2012).

Remote Sensing Techniques Based on Different Sensors:

Based on sensors, the following two groups of remote sensing techniques are used in monitoring plant diseases (Gogoi et al. 2018).

1. **Imaging Approaches**
   (a) RGB Camera
   (b) Multispectral imaging
   (c) Hyperspectral imaging
   (d) Thermal imaging
   (e) Fluorescence imaging

Multiple studies using hyperspectral imaging stated that high spatial resolution is crucial to avoid mixed spectral signals (Mahlein et al. 2012; Bravo et al. 2003; West et al. 2010). These investigations focused on fungal-plant disease detection in the field (Bravo et al. 2003; West et al. 2010) and in the laboratory (Mahlein et al. 2012). In these studies, it was possible to detect and differentiate plant diseases, and in some cases in early stages before they were visible to the human eye (Tables 30.2 and 30.3; (Rumpf et al. 2010)).

2. **Non-Imaging Approaches**
   (a) VIS and IR spectroscopy
   (b) Fluorescence spectroscopy (Table 30.4)

Table 30.2 Examples of studies on plant disease detection using imaging techniques

Plant	Disease	Statistical methods	Optimum spectral range	References
Wheat	Scab (*Fusarium* head blight) yellow rust, nutrient deficiency	Step discrimination and discriminant analysis self-organizing map-neural network, quadratic discriminant analysis, regression analysis	568,715 nm (550, 605, 623, 660, 697 and 733 nm)	Delwiche and Kim (2000) and Moshou et al. (2006)
Tomato	Late blight disease	Minimum noise fraction trans- formation and spectral angle mapping-based classification	700–750 nm, 750–930 nm, 950–1030 nm and 1040–1130 nm	Huang et al. (2007)
Grapefruit	Citrus canker	Principal component analysis	553, 677, 718 and 858 nm	Huang et al. (2007)
Sweet orange	Blue mold, browning rot	Difference in reflectance	540 and 680 nm	Qin et al. (2008)

Source: Sankaran et al. (2010)

Table 30.3 The specific wavelengths to identify the different infections

Samples	Wavelengths (nm)
Healthy young leaves	(548–557 nm) / (701–1387 nm)
Healthy old leaves	(1574–1597 nm) / (1749–1775 nm)
Infected young leaves (cotton leaf warm)	(542–559 nm) / (1580–1592 nm) / (1751–1763 nm)
Infected old leaves (cotton leaf warm)	(350–698 nm) / (1944–2500 nm)
Infected young leaves (aphid)	(1563–1567 nm) / (1785–1833 nm)
Infected old leaves (aphid)	(1569–1580 nm) / (1764–1781 nm)
Infected young leaves (whiteflies)	(1575–1579 nm) / (1764–1769 nm)
Infected old leaves (whiteflies)	(1782–1814 nm)

Source: Yones et al. (2019b)

30.5 Case Studies on Plant Pathology from Egyptian Environment

30.5.1 Case Study 1

Aboelghar and Abdel Wahab (2013) described a novel method for fungal characterization. The authors determined the spectral signatures of different *B. cinerea* isolates as well as various fungal genera. A unique spectral pattern was investigated at both the genus and isolate level. The short wave infrared II (2055–2315 nm) provided the best discrimination between the fungal samples observed. Moreover, the spectral analysis was performed on non-transformed data and investigated significant differences among the fungal genera as well as *B. cinerea* isolates, while the results investigated high similarity among replicates of the same isolate of *B. cinerea*. The results of each spectral test were obtained reproducibly without an expensive cost consumable during sample preparation and measurements. This innovative approach would allow identifying, discriminating and classifying fungi rapidly and inexpensively at the genus, species and isolating level (Figs. 30.4 and 30.5).

30.5.2 Case Study 2

Abd El Wahab et al. (2017) tested the capability of spectral measurement for detecting the asymptomatic Botrytis infection. The two diagnostic applications, spectroradiometer and qPCR, were evaluated to compare their reliability to distinguish Botrytis infected fruits from healthy ones. Both systems discriminated between the healthy and infected strawberry fruits and demonstrated their accordance in measurement results. Generally, the qPCR cycle and the spectral reflectance values of healthy fruits were higher than those of infected ones along with the whole sample collection. The different systems discriminated between healthy and infected strawberry fruits and showed agreement in calculating results. The qPCR period and

Table 30.4 Examples of studies on plant disease detection by different optical sensors. Source: Mahlein (1)

Sensor	Crop	Disease/pathogen	References
RGB	Cotton	Bacterial angular (*Xanthomonas campestris*), Ascochyta blight (*Ascochyta gossypii*)	Camargo and Smith (2009)
	Sugar beet	Cercospora leaf spot (*Cercospora beticola*), sugarbeet rust (*Uromyces betae*)	Neumann et al. (2014)
	Grapefruit	Citrus canker (*X. axonopodis*)	Bock et al. (2008)
	Tobacco	Anthracnose (*Colletotrichum destructivum*)	Wijekoon et al. (2008)
Spectral sensors	Barley	Net blotch (*Pyrenophora teres*), brown rust (*Puccinia hordei*),	Kuska et al. (2015)
	Wheat	Head blight (*Fusarium graminearum*), yellow rust (*Puccinia striiformis* f. sp. *tritici*)	Kuska et al. (2015) and Moshou et al. (2004)
	Sugar beet	Cercospora leaf spot (*C. beticola*), sugarbeet rust (*U. betae*)	Mahlein et al. (2010 and Hillnhutter et al. 2012)
	Tomato	Late blight (*Phytophthora infestans*)	Wang et al. (2008)
	Tulip	*Tulip breaking virus* (TBV)	Polder et al. (2014)
	Sugarcane	Orange rust (*Puccinia kuehnii*)	Apan et al. (2004)
Thermal	Sugar beet	Cercospora leaf spot (*C. beticola*)	Chaerle et al. (2004)
	Cucumber	Downy mildew (*Pseudoperonospora cubensis*), powdery mildew (*Podosphaera xanthii*)	Berdugo et al. (2014) and Oerke et al. (2006)
	Apple	Apple scab (*V. inequalis*)	Oerke et al. (2011)
Fluorescence imaging	Wheat	Leaf rust (*Puccinia triticina*), powdery mildew (*Blumeria graminis* f.sp. *tritici*)	Burling et al. (2011)
	Sugar beet	Cercospora leaf spot (*C. beticola*)	Chaerle et al. (2007 and Konanz et al. 2014)
	Bean	Common bacterial blight (*Xanthomonas fuscans* sub sp. *fuscans*)	Rousseau et al. (2013)

stable fruit spectral reflectance values were generally higher than those of the contaminated ones along with the entire collection of the samples. Spectral analysis demonstrated a higher reflectance in healthy fruits than that of infected ones throughout the visible near infrared (VNIR) spectral range, while the short wave infrared (SWIR) spectral zone showed different degrees of gray mold infection. Also, the results demonstrated that VNIR is the best spectral zone that would discriminate between infected and healthy fruits, while SWIR-2 is the best spectral zone to distinguish between population patterns of Botrytis within the infected fruits (Fig. 30.6).

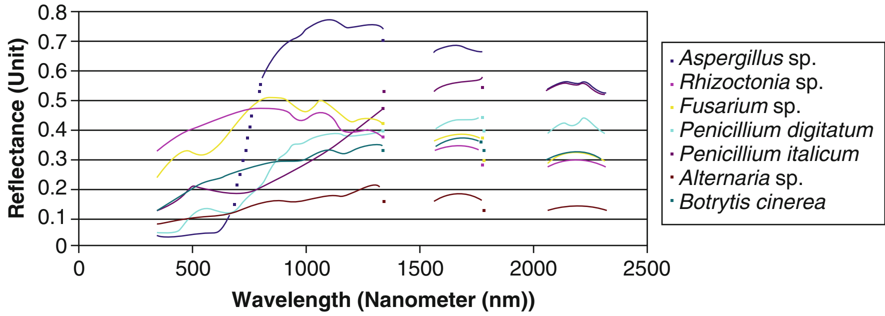

Fig. 30.4 Spectral reflectance pattern of different fungi using SWIR II at 2055–2315 nm. (Source: Aboelghar and Abdel Wahab 2013)

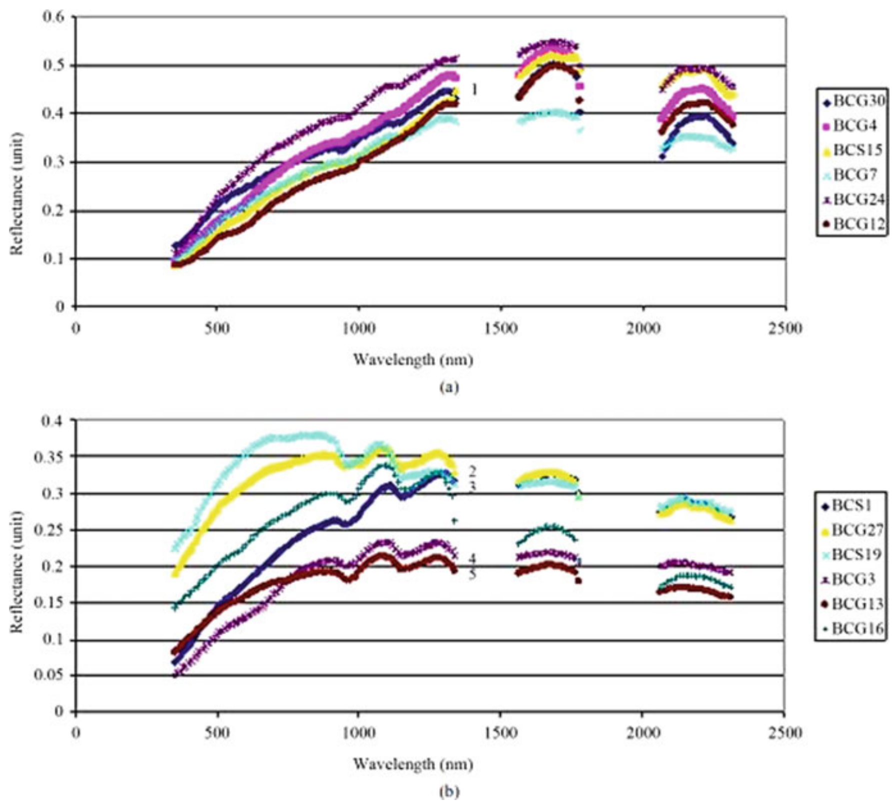

Fig. 30.5 Spectral reflectance pattern of different isolates of *B. cinerea*. (Source: Aboelghar and Abdel Wahab 2013)

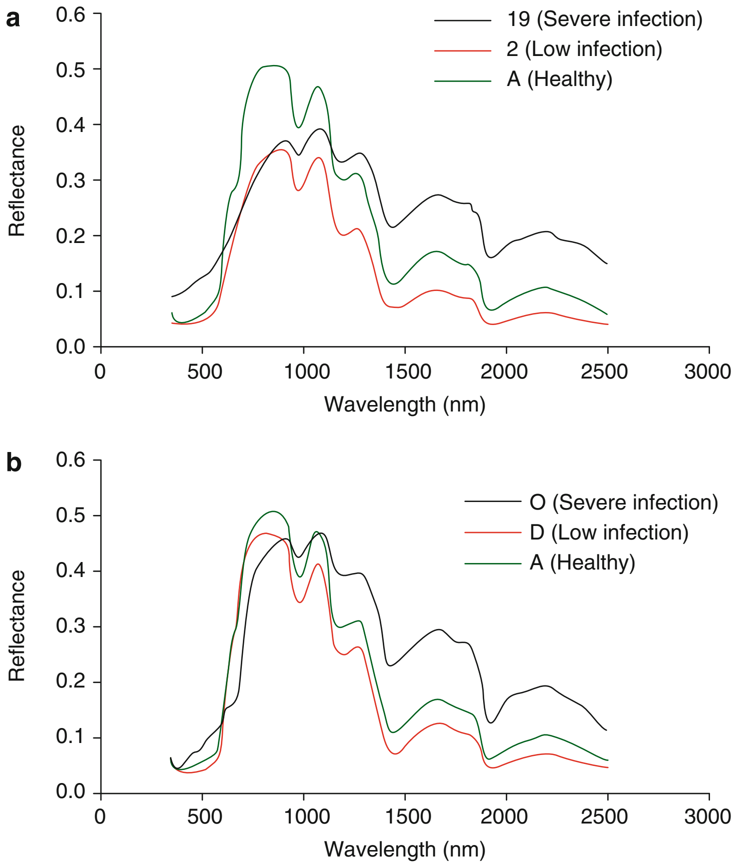

Fig. 30.6 Spectral reflectance pattern of healthy, low and severely infected strawberry fruits in two varieties (**a**) Festival and (**b**) Sweet Charlie. (Source: Abd El Wahab et al 2017)

30.5.3 Case Study 3

Yones et al. (2019a) differentiated various infections (aphid, white fly, cotton leaf worm, etc.) on leaves of sugar beet (old and young) using Field ASD spectroradiometer in Egypt. For all young and old leaves the spectral outline of reflection was identified. Results showed that the near infrared (NIR) and blue regions were the best zones to identify the three infections spectrally on young leaves. The results indicate that for the three infections on young leaves, near infrared (NIR) and blue regions are the best areas for spectrum define. The results suggest the possible use of remote sensing technologies for the identification of pests, allowing for successive control and site-specific pest management sequencing. Comparison of the reflectance of the plant infection shows the maximum reflectance (1000 nm) in the infrared zone, comparatively low reflectance (1650 nm) and minimum reflectance in the spectral zone (2200 nm). Discrimination results showed the best wavelength to spectrally identify infected and healthy plant table results (Figs. 30.7 and 30.8).

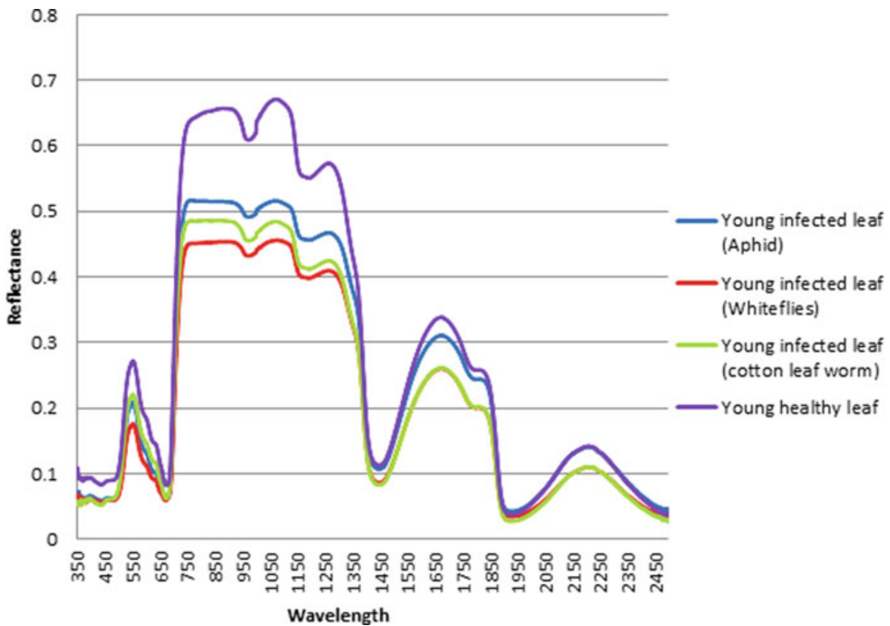

Fig. 30.7 The spectral reflectance pattern for young leaves of sugar beet plants with different kinds of infestation (cotton leaf worm, aphid and whiteflies) with healthy plant. (Source: Yones et al. 2019b)

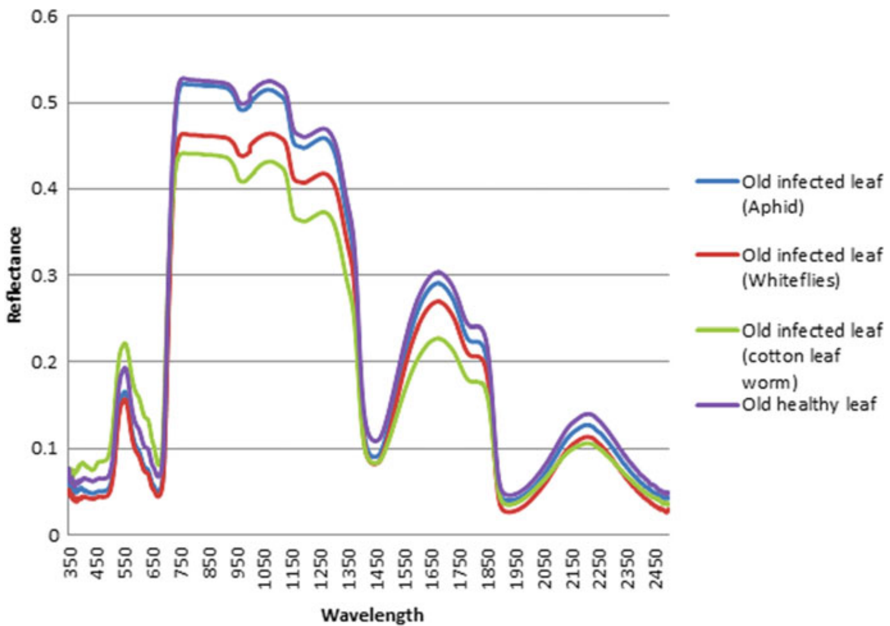

Fig. 30.8 The spectral reflectance pattern for old leaves of sugar beet plants with different kinds of infestation (cotton leaf worm, aphid and whiteflies) with healthy plant. (Source: Yones et al. 2019b)

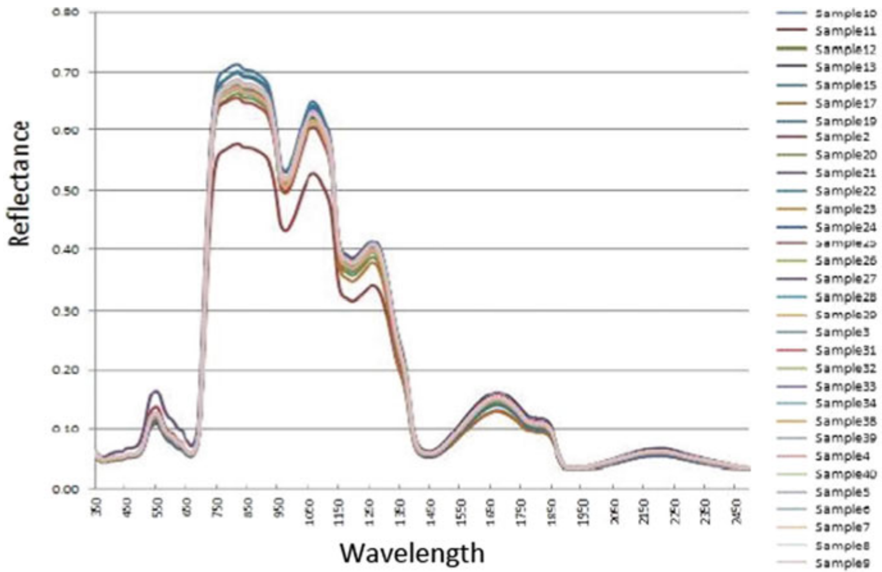

Fig. 30.9 The spectral reflectance pattern for cotton bolls with different levels of infestation with healthy bolls. (Source: Yones et al. 2019b)

30.5.4 Case Study 4

Yones et al. (2019b) developed a new approach to use the hyperspectral technique to detect infested cotton plant with PBW with no losses to boll. A study was aimed to identify the reflectance spectra of cotton plants with known PBW infestation and to identify optical wavelengths that are sensitive to PBW damage. The spectral measurements were performed using an ASD spectroradiometer in the spectral range of 350–2500 nm. The study indicates that invasion of PBW can be detected using hyperspectral data and its level identified, which could be used to monitor trade and predictions. Comparison of the reflection of healthy and contaminated cotton bollards reveals that the average spectral reflectance (1000 nm) in the infrared spectral region was relatively low reflectance (1650 nm) and minimum spectral zone reflectance (2200 nm) (Fig. 30.9).

30.5.5 Case Study 5

Yones et al. (2019c) used spectral data to differentiate between healthy- and pest-infested three arid-land plants: citrus lemon trees, sweet almond and olives. The reflection outline of the three healthy and infected plants was identified. The optimum waveband and wavelength/s were determined to distinguish between infected and healthy plants. The results showed that healthy plants gave higher reflection values in the visible spectra compared to plants with olives; however,

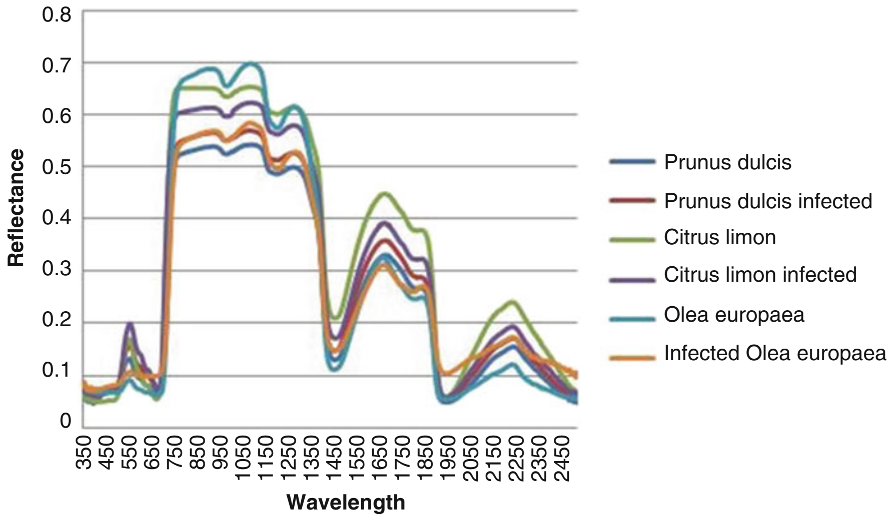

Fig. 30.10 Spectral reflectance pattern for three cultivated plants (healthy and infected). (Source: Yones et al. 2019c)

healthy plants showed a higher reflection than infected plants throughout the spectrum with other plants. Reflection measurements of the *Prunus dulcis* form showed a decrease in chlorophyll at 550 nm) as mites attack almond trees, feed on leaves, and remove chlorophyll. The spectral properties of C. lemon showed water stress, and this figure is apparent at 950, 1150 and 1450 nm. Spectral scales for *O. europaea*, the shape also indicates water stress, this is evident at 950, 1150 and 1450 nm (Fig. 30.10 and Table 30.5).

30.6 Recommendations

The strong relationship between the results of the remote sensing analysis and the pathology of the plant provides evidence of the value of hyperspectral reflectance data for performing rapid evaluations of the plant health condition effectively and without infection.

30.7 Conclusion

The chapter demonstrates the strong role remote sensing plays within the agricultural sector. Remote sensing technology is a powerful tool used in monitoring plant pathology. It can provide accurate and reliable information to guide decision-making in crop protection and hence has great potential for use in control of plant diseases.

Table 30.5 The optimal waveband to differentiate between healthy and infected plants

Species	Optimal wavelength zones (nm)
Citrus limon	355-363-371-379-387-395-403-411-419-427-435-443-451-459-467-475-483-491-499-507-515-523-531-539-547-555-563-571-579-587-595-603-611-619-627-635-643-651-659-667-675-683-691-699-707-715-723-731-739-747-755-763-771-779-787-795-803
Citrus limon infected	668-676-684-692-700-708-716-724-732-740-748-756-764-772-780-788-796-804
Infected *Olea europaea*	350-358-366-390-398-422-430-438-462-470-478-494-518-526-574-582-590-598-606-614-622-630-638-646-654-662-670-678-686-694-702-710-718-726-734-742-750-758-766-774-782-790-798-806
Olea europaea	357-365-381-389-397-405-413-421-429-437-445-453
Prunusdulcis	529-537-545-553-561-569-577-585-593
Prunusdulcis infected	530-538-546-554-562-570-578-586-594-602
Zizyphus vulgaris	351-359-367-375-383-391-399-407-415-423-431-439-447-455-463-471-479-487-495-503-511-519-527-535-543-551-559-567-575-583-591-599-607-615-623-631-639-647-655-663-671-679-687-695-703-711-719-727-735-743-751-759-767-775-783-791-799
Zizyphus vulgaris (infected)	616-624-632-640-648-656-664-672-680-688-696-704-712-720-728-736-744-752-760-768-776-784-792-800-808

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Decision-Making Tools for Integrated Disease Management

31

K. P. Singh, T. Aravind, Amit Kumar Srivastava, and C. S. Karibasappa

Abstract

The decision-making process is the core of any successful integrated disease management programme. The complexity of decision-making process in IDM is much higher as compared to conventional agriculture as it involves multiple factors related to the host, pathogen and environment to be considered. Hence, for taking the most efficient and economic decisions, a farmer or a scientist needs the help of decision-making tools. This need has led to the development of four such decision-making tools viz., warning services, expert systems, decision support systems and onsite devices. They differ in their objective, scope, architecture and complexity of data that they can handle. But the prime objective of these is to help the farming and the scientific community to take the best possible decision regarding plant disease management. At present, their adoption is limited and does not justify the cost and effort required for their development. However, more efficient and user-friendly tools are being developed after rectifying the drawbacks of the previous ones. Their efficient utilization will help in successful plant disease management and lead to the concept of sustainable agriculture.

31.1 Introduction

To satisfy the ever-growing food demand, agricultural production must increase by 70% by 2050 globally. However, pests and crop diseases put global food supplies at high risk (Carvajal-Yepes et al. 2019). Worldwide, yield losses caused by pests and

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diseases are estimated to average 21.5% in wheat, 30.0% in rice, 22.6% in maize, 17.2% in potato and 21.4% in soybean (Savary et al. 2019), which accounts for at least half of the global human calorie intake (FAOSTAT 2018). Therefore, quantifying the impacts of plant pests and associated diseases on crop production and making timely interventions represents one of the most important research questions for simulation models (Donatelli et al. 2017) for plant disease forecasting. Plant disease forecasting is a management system used to predict the occurrence or change in severity of plant diseases. At the field scale, these systems are used by growers to make economic decisions about disease treatments for control. Often the systems ask the grower a series of questions about the susceptibility of the host crop and incorporate current and forecast weather conditions to make a recommendation. Typically, a recommendation is made about whether disease treatment is necessary or not. Forecasting systems are based on assumptions about the pathogen's interactions with the host and environment, the disease triangle (Fig. 31.1). The objective is to accurately predict when the three factors – host, environment and pathogen – all interact in such a fashion that disease can occur and cause economic losses.

Moreover, a systems analysis approach is required to understand how pest and disease problems arise and how they may be tackled because of the complex relationships between crops and their pests and diseases and because of the dynamic nature of pest populations. This has become more complex as climate change is throwing new challenges due to change in temperatures and the rainfall amount and pattern. The shift to a non-stationary climate indicates that current datasets are no longer applicable to predict the behaviour of the production system. There is a plethora of evidence now available that pathogens which for decades have had no effect on crops are now becoming key determinants and affecting the crop yield (Gramaje et al. 2016; Parker and Warmund 2011).

The FAO has defined sustainable agricultural development as "the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued

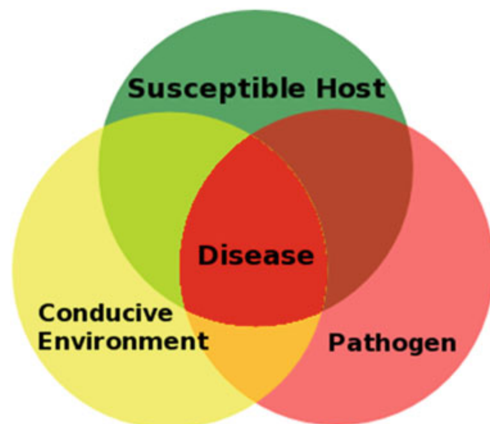

Fig. 31.1 The plant disease triangle represents the factors necessary for disease to occur. (Source: https://en.wikipedia.org/wiki/Plant_disease_forecasting)

satisfaction of human needs for present and future generations. Such development conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable and socially acceptable". The concept of integrated pest management has emerged from this concept of sustainability. It has shifted our focus from conventional chemical-based management to an integrated approach for ensuring environmental safety and ecological sustainability. Integrated disease management (IDM) can be viewed as harmonious integration of different disease management strategies for the effective and economical management of plant diseases below the economic threshold level based on the sound understanding of the whole crop ecosystem (Agrios 2005). It requires a thorough understanding of the etiology and epidemiology of the disease, cost-benefit analysis and deeper knowledge of the plant protection measures. IDM relies upon the integration of different plant protection practices as compared to the sole dependence on synthetic pesticides in conventional agriculture. The decision-making process is the core of any IPM/IDM module (European commission 2009). Decisions are usually made based on a combination of empirical data, analysis of the situation in hand and personal expertise on the subject. The complexity in the decision-making process depends on the complexity of factors governing the situation (McCown 2002). The simple task requires only a little knowledge and a bit of experience in handling such a situation. However, more complex decisions with greater consequences may require experience, judgment and quantitative analysis. In this regard, the decision making from an agricultural perspective is often complicated as it involves complex interactions of many factors. It requires the information and application knowledge from various interacting fields of science starting from the knowledge on edaphic factors to the much complex economics and marketing strategies (Reddy and Rao 1995). This type of complex decision-making ability is usually lacking in the farming community, especially in those belonging to the under-developed and developing countries.

The decisions taken in an agricultural farm can be broadly divided into three viz., strategic, tactical and operational (Rossi et al. 2012). Strategic decisions are taken based on the consideration of the long-term plans (many years) of the whole farm. It is taken by the owner/director of a farm and gives guidelines for the farm management. The tactical decisions refer to the decisions having an impact for a few days to weeks or a crop season and are taken by the manager in charge of the farm. The operational decisions are taken by the employees and mainly deal with the implementation of the strategic or tactical decisions taken earlier (Rabbinge et al. 1993). The chain of decision-making process starts with the identification and analysis of the problem followed by the evaluation of possible solutions. The best possible solution is selected and converted into action and the results are validated. It is a continuous process and requires regular monitoring of onsite field problems and analyzing the impact of the remedial measures adopted (March 1994).

Diverse control measures are currently available for the management of plant diseases. These include various cultural, mechanical, physical, biological and chemical methods (Odile et al. 2010). Of these, the use of chemical control is most popular among the farming community. Since the advent of the concept of sustainable

agriculture, there is a common consensus among the scientific and farming communities that the sole application of any of these management techniques will be ineffective and unsustainable in the long run. Hence, integrated pest and disease management is becoming more popular. Compared to disease management practices in conventional agriculture, IDM involves more complex decision making (Rossi et al. 2012).

For an individual farmer or a farm manager, it is difficult to make complex decisions as it requires a thorough understanding of the multiple factors governing the impact of decisions taken. Hence, they require tools that can help them in selecting the best possible and effective decisions that can be adopted to yield maximum economic benefits. Predictive systems are of much importance in this regard for making decisions for integrated plant disease management. "Predictive system" is used as a general term for formalized algorithms that assess disease risk factors that inform the need for crop protection (Gent et al. 2013). The most successful predictive systems take into account the whole of a crop ecosystem, using simulation models, previously developed databases and decision-making rules to analyze a situation and to provide the most appropriate action to be taken (De Wolf and Isard 2007). The decisions are mostly taken based on the etiology and epidemiology of the disease, prevailing weather parameters, crop growth stage, previous history of the disease occurrence, etc. The different predictive systems used in plant pathology include the warning services, expert systems, decision support systems and on-site devices.

31.2 Warning Services

Warning services are based on general IPM/IDM guidelines or any of the disease-forecasting models (Rossi et al. 2000). They do not take into consideration the factors affecting the disease spread in an individual farm and are given on a regional scale. They are usually carried out by the government agencies, NGOs or even the private extension agents for free or on paid basis. The information is passed on to the beneficiaries through mass media like radio, newspaper, television, etc. or personal contacts through SMS/emails (Beta 2011).

31.3 Expert Systems

The complex agricultural decision requires the expertise and knowledge of all aspects of agriculture. Such personal expertise is widely lacking in our farming and scientific community. This necessitated the help of computer programmes that are programmed to help the farmers in the quality decision regarding the diverse agricultural processes, which lead to the evolution of the concept of Expert System (ES). ES, sometimes called as Knowledge Base System, is defined as "a computer programme designed to model the problem-solving ability of a human expert" (Durkin 1994). It is intended to cater to the needs of making quality decisions as

well for technology dissemination. In agriculture, ES finds its applications in the selection of crop varieties best suited for the locality, detection and diagnosis of pest and diseases and their effective management, nutrient and water management, etc. (Abu-Naser et al. 2008). It can be viewed as an excellent tool for relieving the mounting pressure on limited expertise available in developing countries. It is capable of gathering and analysing the vast data from many experts from multiple fields of agriculture and allied fields (Chen et al. 2012). Moreover, it will help in speeding up the process of pest identification and suggesting management strategies for the same (Mahaman et al. 2003)

Shafinah et al. (2013) have described 13 diverse methodologies used to develop ES. These include a rule-based system, knowledge-based system, neural network system, fuzzy expert system, case-based reasoning, object-oriented methodology, etc. In case of pest and disease detection and management, the rule-based ES is most commonly employed. It employs IF (condition) and THEN (action) to solve the problems. There are two types of rule-based ES viz., forward chaining (or data-driven) and backward chaining (or goal-driven) expert systems. In the data-driven ES, the process starts from the analysis of the initial facts and the conclusions are drawn using the rules. In case of goal-driven ES, it begins with the highest level rules. It searches and determines the rules that are allowed to be further sustained (Shafinah et al. 2013).

The basic components of an ES include the knowledge base, inference protocol, user interface and a knowledge acquisition mechanism (Feigenbaum 1992; Pham and Pham 1988). The knowledge base encompasses the knowledge regarding the problem like the facts, information and rules for judgment. The stores' knowledge is further analyzed and manipulated using the inference mechanism which is also called as the control structure or reasoning mechanism. The user interface acts as a communication bridge between the user and the ES. Knowledge acquisition domain is used for further updating and upgrading the knowledge base and the ES. An additional component, i.e. a memory component, used to store the temporary data was suggested by Mahaman et al. (2003).

Some of the successful examples of ES in crop protection include:

(i) VEGES (Yialouris et al. 1997) – for the diagnosis of nutritional disorders, pests and diseases of six greenhouse vegetables
(ii) AGRES (Ganesan 2006) – for the diagnosis of pest and diseases of major crops in Kerala
(iii) RUBEXS -04 (Balasubramani and Lekshmi 2001) – for disease diagnosis in rubber plants

31.4 Decision Support Systems

Decision support systems (DSS) are interactive computer-based systems that aid in making quality decisions. Turban (1995) has defined DSS as "an interactive, flexible and adaptable computer-based information system, specially developed for

supporting the solution of a non-structured management problem for improved decision making. Apart from agriculture, DSS finds its application in a broad range of areas like natural resource management, business management, health sector and environmental studies (Eom and Kim 2006). In agriculture, DSS are frequently developed and used in agronomy, soil science and crop protection studies (Kumbhar and Singh 2013).

There are four important components of DSS viz., database management subsystem, knowledge management subsystem, user interface and user (Mir and Quadri 2009). The first step in the creation of a DSS is the collection of the data on the problem. For a DSS about plant disease, we need to collect data on the previous occurrence of the disease, epidemiological factors governing the disease, survival and spread of the pathogen, management measures adopted and the efficiency and economy of the measures in managing the disease. The database creation is the most critical step for any successful DSS. The database management subsystem manages and provides access to the existing database through the Database Management System (DBMS). DBMS creates and manages the database and regulates access to the database. The knowledge-based management sub-system mostly consists of a model or an inference protocol that solves the problem and provides a decision. The accuracy of the decision making depends on the database and the efficiency of the model used. Most DSS use "if-then" strategy to solve the problems. The user interface is the part of the DSS that interacts with the end-user. It acts as a bridge between the system and the end user. Mostly the user interface is a desktop or a smartphone using which the end-user can feed the data and get desired results. The user interface is also used for updating the database and the models already existing with the system. The user can be a farmer, extension agent or a scientist who seeks the right decision from the system (Fig. 31.2). According to Marakas (2003), the five basic components of the DSS include data management system, model management system, knowledge engine, user interface and the user. The model management system performs the modelling and necessary data analysis, whereas the knowledge engine is used for the problem recognition and finding out their solution.

In agriculture, DSS finds its application in entire aspects starting from the selection of seeds to sale. It can be used as a decision making aid for plant protection, integrated nutrient management agricultural extension, climate prediction, farm mechanization, land use planning, etc. (Hansen 2002; Suarez de Cepadaet al. 2005; Jorgensen et al. 2006; Mosseddaq et al. 2005; Sodtke 2005). There is no such thing called an ideal DSS. All the DSS developed has got its pros and cons. The success of DSS is mainly assessed by the accuracy of the decision making and the level of adoption by the end-users. Bhargava and Power (2001) have classified DSS into five groups viz., model-driven, communication-driven, data-driven, document-driven and the knowledge-driven DSS. The model-driven DSS relies on the access to and manipulation of statistical, financial, optimization or simulation model. The communication-driven DSS emphasizes on shared decision making based on communication and collaboration. The knowledge-driven DSS, most commonly used in plant disease diagnosis and management, is specialized for problem-solving based

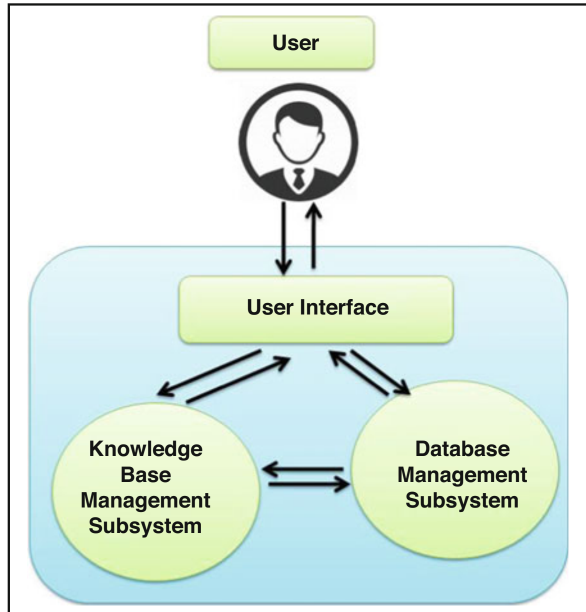

Fig. 31.2 Components of a decision support system

on the stored database, facts and rules. The different types, categories and fields of application of DSS in agriculture have been reviewed by Manos et al. (2004).

Many unique features of the plant protection measures make them an ideal area for development and use of the DSS. They include a wide range of strategies currently available for disease management, lack of needed expertise especially in remote villages, complex interactions with the other crop production practices and farming economics, etc. (Shtienberg 2013). Many a time, the scientific experts become unavailable for solving the issues under real field conditions. Moreover, the passage of human expertise from one expert to the successor is also fairly low. In this aspect, DSS can play a vital role as the expertise of an individual stored as a computer programme will be available at all times. It will help in bridging the gap from lab to land and inconveniences caused due to the absence of the expert in person. The apple scab predictive systems FAST and BLITECAST are the weather-based disease prediction systems that are the predecessors of modern-day DSSs (Jones et al. 1980; MacKenzie 1981; Madden et al. 1978). For successful adoption of these systems, they have to be highly user-friendly, based on a scientific basis, farm and user-specific and be able to provide simple, robust and most efficient solutions to the problems (Cabrera 2012).

31.5 Onsite Devices

Onsite devices help in decision making on an individual farm. They are programmed to collect data (e.g. weather data) from within the farm and give recommendations regarding disease management practices based on the factors of the particular farm where they are installed (Rossi et al. 2012). The best example of an onsite weather data collection device is the μMETOS. The device can be operated via mobile phone, personal computer or a notebook. The plugin devices used in precision agriculture can also be used for collecting the onsite data for pest and disease management. At present, attempts are being made to collect the weather data by linking with GIS and GPS systems for accurate decision making. Such an onsite device has been developed for the accurate prediction of apple scab in the Garhwal Himalayas (Singh and Kumar 2005).

31.6 Case Study 1: Late Blight of Potato

Late blight of is one of the most important diseases of potato causing widespread crop loss worldwide. It is caused by the Oommycete pathogen *Phytophthora infestans* (Mont.) de Bary (Kingdom *Chromista*; Phylum *Oomycota*, Class *Oomycetes*; Order *Pythiales*; Family *Pythiacea*) (Singh 2009). The disease is prevalent in all the major potato growing regions of the world. The occurrence and severity of the disease vary across years and from region to region depending upon the climatic conditions. The pathogen survives in the soil as oospores and in the infected tubers, which serve as the primary inoculum for the next season.

The disease is influenced by low temperature along with relative humidity, dew, rainfall and wind velocity. Taking into consideration the economic importance of the disease, the studies on the forecasting of the late blight epidemic had been undertaken from 1926 by Van Everdingen (Dutch Rules). According to him, the late blight can occur in severe form if the night temperature is below dew point for atleast 4 hrs, minimum temperature of 10 °C or slightly above, clouds on the next day and rainfall during the next 24 hours of atleast 0.1 mm (Van Everdingen 1926). Since then, a number of empirical and fundamental models have been developed to forecast the epidemic (Table 31.1). The importance of weather parameters in the development of late blight has been extensively reviewed well by Rotem et al. (1971) and Harrison (1992).

Other than these basic models, certain decision support systems were also developed for adopting the timely management strategies for the late blight of potato. The first of these kind (BLITECAST) was developed by Krause and his co-workers in 1975 at the Pennsylvania State University (Krause et al. 1975). Basically it combined the concept of severity values (given by Wallin in 1962) and blight favourable days (given by Hyre in 1947). The system gives the management recommendations based on the weather parameters send by the growers for their locality. This was further modified and incorporated into another programme WISDOM (crop management programme) by Mac Hardy (1979). The other

Table 31.1 Various forecasting models developed for late blight of potato (Singh et al. 2012)

Sl. no.	Fundamental models	Sl. no.	Emperical models
1.	BLIGHTCAST (Krause et al. 1975)	1.	Beaumont's rules (Beaumont 1947)
2.	Mac Hardy (1979)	2.	Cook's system (1949)
3.	Fry et al. (1983)	3.	Hyre's system (1954)
4.	Grunwald et al. (2000)	4.	Smith's system (1955)
5.	Runno and Koppel (2002)	5.	Wallin's system (1962)

Table 31.2 Mills' table and our university data to arrive at the incubation period based on temperature and leaf wetness

	Minimum wetting hours of leaves for infection (approx. hours)[a]		
Average daily temperature (°C)	As per Mills' table	As per Univ. data	Days required (after infection for symptom appearance)
25	11	9	
16 and 24	9	6	9
15	10	8	12
14	10	8	13
13	11	9	13
12	11–15	9	14
11	12	10	15
10	14	13	16
9	15	13	17

[a]The infection period is considered to start at the beginning of the rain

important decision support system for late blight of potato include PhytoPRE (Forrer et al. 1993), SIMPHYT 1 and 2 (Gutsche 1993), NEGFRY (Hansen et al. 1995), SIMBLIGHT (Benno kleinhenz et al. 2007), etc.

There are four major late blight forecasting models developed for the Indian conditions:

1. Ten day moving graph – Chaudhary and Pal (1959)
2. Seven day moving precipitation model – Bhattacharyya et al. (1983)
3. JHULSACAST – Singh et al. (2000)
4. INDO-BLIGHTCAST – Singh et al. (2016a)

31.6.1 INDO-BLGHTCAST

It was developed by Singh and his colleagues at the Central Potato Research Instiute, Shimla, Himachal Pradesh, India. It was developed by using the late blight occur dates and meteorological parameters at four different locations in the Indo gangetic

plains. It involves the calculation of night relative humidity (RH) for seven consecuitive days and P-days (Physiological days – moving cumulative effective temperature). Late blight is predicted to occur within 15 days if RH and P-days exceed 525 and 52.5, respectively, for seven consecutive days.

31.7 Case Study 2: Apple Scab Prediction Model for Garhwal Himalayas

Apple scab, caused by *Venturia inequalis* (Cke.) Aderh. is one of the most dreadful diseases of crops leading to serious crop loss both under field conditions and post-harvest stages. It was first time reported in India, in Kashmir, in 1935, followed by Himachal Pradesh in 1977. Ever since its introduction through planting materials, the disease has caused a series of the epidemic in the two states (Singh et al. 2015, 2016b). Over 60% of the area under sweet varieties of the total of 17,542 ha was engulfed by apple scab during 2000 (Singh and Kumar 2008, 2009). The major economic loss is caused in the form of scabbed apple fruits that are unfit for marketing and consumption. Besides this, the disease also cause a decrease in plant vigour, reduction in crop yield and lead to the gradual death of a tree. According to Singh (2019), the average economic loss due to reduced marketability because of scabbed fruit is 24%.

31.7.1 Symptomatology

Young plants are more susceptible to scab infections and the pathogen attack all the above-ground plant parts. The symptoms of scab infection are first observed on the under-surface of young leaves. Young lesions are olive green in colour which gradually changes to velvety brown and later on to metallic black. Mycelium radiate from the mature lesions. The leaf may develop a puckered appearance due to the infection. On the fruits, many lesions coalesce to give a distorted appearance and cracks may develop on such fruits (Fig. 31.3). Severe infection of the tree often leads to heavy fruit drop. Primary infection lead to the development of isolated scattered spots whereas the secondary infection cause severe leaf spotting and leaf distortion (Singh et al. 2001).

31.7.2 Etiology of the Pathogen

The pathogen belongs to the kingdom *Fungi*, phylum *Ascomycota,* subphylum *Pezizomycotina*, class *Dothidiomycetes*, order *Pleosporales* andfamily *Venturiaceae* (Dube 2013). The conidial stage, *Spiloceapomi,* develops in the leaves and fruits on the tree. In the host, the light-coloured mycelium remains sub-epidermal which later on turns brownish. The reddish-brown conidia are mostly flame-shaped and one- or

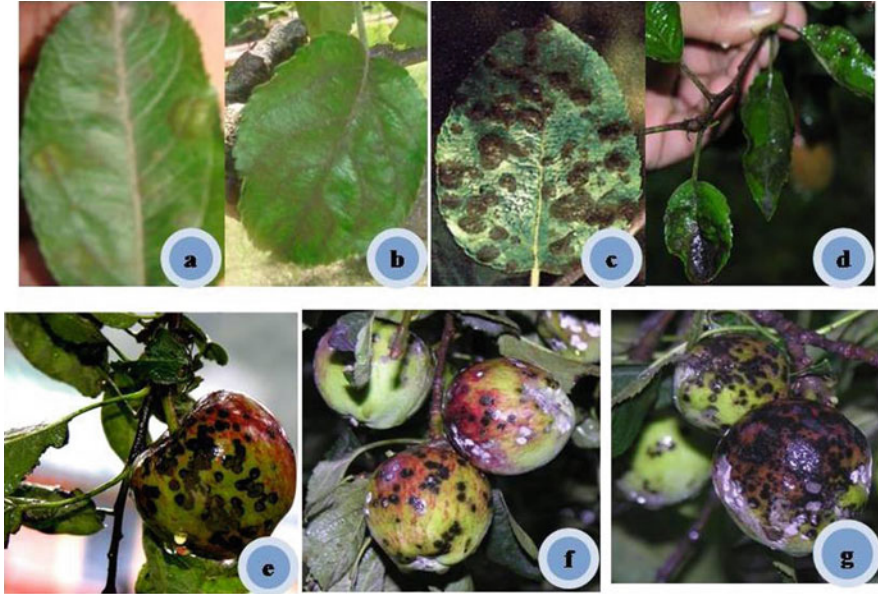

Fig. 31.3 (**a–d**) Scab on leaves as velvety brown to olive spots**;** (**e–g**) Mousy black secondary scab lesions and sign on the fruit surface of delicious apple

two-celled. The fungi produce pseudoothecia in the fallen leaves which produce bicelledascospores that cause the primary infection (Singh 2009).

31.7.3 Survival and Spread

The pathogen survives in the dead and fallen infected leaves as saprophytes. In the Garhwal Himalayas, the fungi start producing pseudothecia in November and mature pseudothecia release infectious ascospores during May–June (Singh 2019). The intermittent rains or heavy snowfall followed by dry climate are conducive for pesudothecia development and ascospore release. Mature ascospores are discharged into the air during periods of rain. This ascospore produces primary infections. The conidial stage develops in the infected leaves and cause secondary infection in the orchard. In apple scab development, the conidial stage has long been recognized as an important phase, particularly for the rapid build up and spread of scab from tree to tree within the orchard and from one orchard to another in the late spring and summer. The optimum temperature and relative humidity regime for conidial production and infection was 16–20 °C and 90%, respectively (Singh et al. 2010).

31.7.4 Disease Cycle

The pathogen has two distinct phases *viz.,* the saprophytic and the parasitic (Fig. 31.4). The saprophytic stage (primary cycle) occurs in the fallen leaves on the orchard floor whereas the parasitic phase (secondary cycle) is observed on the leaves and fruits on the tree. The pathogen completes its sexual cycle during the saprophytic stage whereas the parasitic phase is caused by the conidial stage of the pathogen. The primary cycle starts production of pseudothecia in the overwintering leaves in the late winter. Pseudothecia are spherical to sub-spherical and vary in size from 90 to 160 μm with a prominent ostiole (Singh and Kumar 1999). It starts producing the ascospores during the bud break stage of the tree which continues up to the petal fall stage. The maximum ascospore release and primary infection occur in May.

The secondary cycle begins with the induction of the asexual stage of the fungus at the primary infection foci. The pathogen produces copious amounts of conidiophores and conidia sub-epidermally. The conidiophores are around 90 μm long and 5–6 μm thick with anolivaceous brown appearance. Conidia are produced singly at the tip of conidiophores and then successively by proliferation through scars of the fallen conidia that result in characteristic and distinct annellations on the conidiophores. Conidia are 0–1 septate, and 12–30 × 6–10 μm, obpyriform to

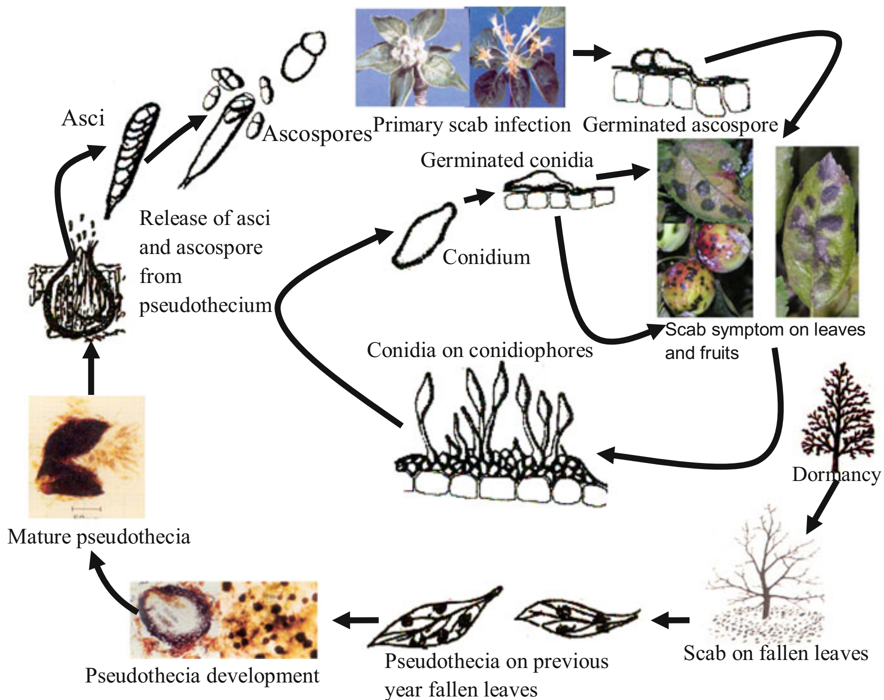

Fig. 31.4 Disease cycle of scab pathogen

obclavate and pale to mid-olivaceous brown. The discharge of conidia is mediated by rains and the spread to adjacent trees/orchard is mediated by the winds.

31.7.5 Modification of Mills Rules for Garhwal Himalayas

In 1942, Mills proposed a rule for predicting the infection of *V. inaequalis* using the leaf wetness period and temperature. According to him, if the product of leaf wetness duration (in hours) and the minimum temperature exceeds 140, the infection is likely to result. This does not hold good under all the apple-growing regions due to variations in the other host, environment and pathogen-related factors. For example, based on the published works of literature, MacHardy and Gadoury (1989) have reported that the infection by the ascospores of the scab pathogen requires approximately three hours less than that was reported by Mills. Similarly, Sys and Soenen (1970), Schwabe (1979), Olivier et al. (1983), Stensvand et al. (1997), Thakur and Khosla (1999) and Belete and Boyraz (2017) have also revalidated the Mills table for their localities. Using the apple scab predictor and μMETOS system, Singh (2005) have developed an apple scab warning system for the efficient use of fungicides for the management of the scab. μMETOS is an onsite weather data collecting system and the apple scab predictor simulated the data on temperature, relative humidity, rainfall and leaf wetness to a modified Mills table to give the intensity of infection period and incubation period for the appearance of scab symptoms. Once the infection is predicted and there is availability of mature ascospores, relevance of these infection periods as predicted by the apple scab predictor and other weather monitoring equipment can be seen under orchard conditions to judge whether this Mills' Infection Period Table is valid under Indian conditions or not (Singh 2019).

Series of trials were conducted in the Gangothri valley and the results revealed that weather conditions were very favourable for the infection to occur with maximum infection period reported in May as the weather conditions are favourable and the tree is at the most susceptible phonological stage, i.e. bloom to near petal fall (Fig. 31.5). The Revised Mills' Table revealed the minimum continued wetting period (in hours) required for primary infection of apple leaves to occur. We defined our minimum infection time as the minimum time required for successful infection of any quality of tissue on trees. This observation indicated that at 10 °C, according to Mills' table, the time needed for symptom appearance was 16 days. Our result revealed that during April and May, 5–8 mild infection periods occurring could initiate primary infection. Minimum 9–14 days were required for symptom expression under prevailing environmental conditions (Table 31.1 and Fig. 31.6).

31.7.6 Disease Prediction Model

An apple scab prediction model developed for the Uttarakhand Himalayas by Singh and Kumar (2005).The model provides the same information on ascospore maturity as a predictive degree-day model, but by tracking the future development of

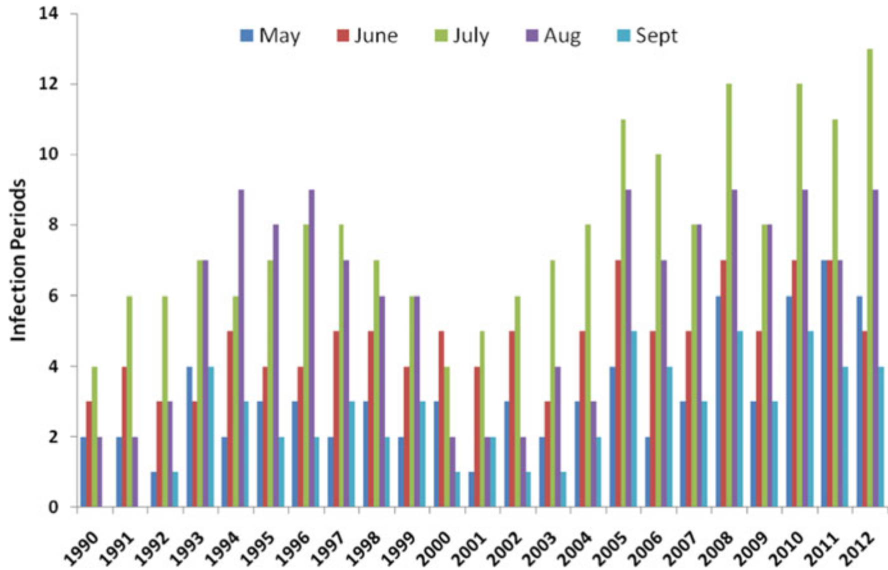

Fig. 31.5 Occurrence of apple scab infection periods in the Gangotri valley of Uttarakhand hills

Fig. 31.6 μMETOS system

ascospores, it allows a grower to plan and schedule fungicide applications and to integrate fungicide applications with insecticide and miticide applications with greater efficiency. A degree day is calculated by subtracting the base temperature from the average minimum and maximum temperature of each day. The total of each day is added to the total of the previous day. The degree day and the ascospore

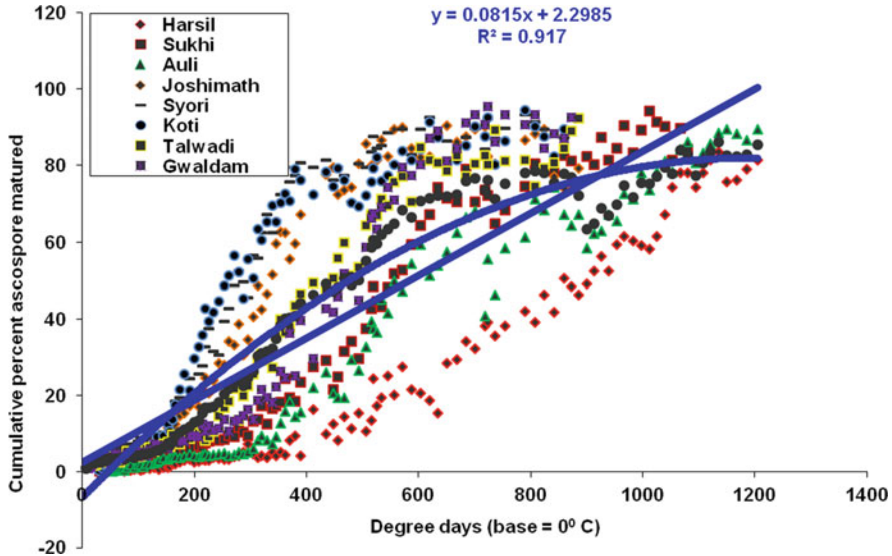

Fig. 31.7 Cumulative percentage of matured ascospore against degree days

discharge data of 15 years were subjected to regression analysis to obtain a meaningful correlation to forecast the ascospore maturation based on accumulated degree days (Singh 2019). The significant relationship was observed between the daily temperature and ascospore maturity. Probit transformation was further carried out to obtain the linear relationship between ascospore maturity and daily temperature. Based on these data, two linear lines were developed, one for the use when the cumulative degree days from 1 February to 15 May were <618 and another for use when the cumulative degree days for those dates were > 618. Our data showed that 50% and 95% ascospore maturation occurs after 338 and 859 cumulative degree days for the orchards situated at 1900–2200 m asl whereas it was >1167 for those situated above 2200 m asl (Figs. 31.7 and 31.8). The need to quantify the ascosporic inoculum has been met by a procedure that estimates potential ascospore dose (PAD), i.e. the expected production of ascospores per m^2 orchard floor.

$$\text{PAD} = \text{LD} \times \text{LLD} \times \text{PD} \times \text{AD} \times \text{n}$$

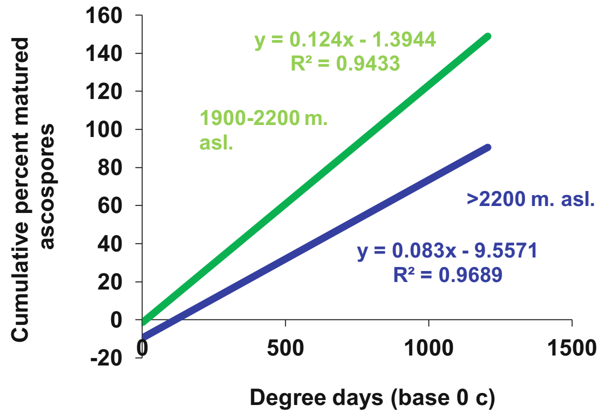

Fig. 31.8 Linear relationship between per cent ascospore maturation and degree days in different altitudes of Uttaranchal Himalayas

Where,

PAD	Potential ascospore dose
LD	Lesion density, number of lesions on leaves per square meter of orchard floor at leaf fall
LLD	Leaf litter density, the proportion of the orchard floor covered by leaf litter at bud break
PD	Pseudothecial density, number of mature pseudothecia/visible lesion multiplied by a lesion fertility factor
AD	Ascus density, number of asci/pseudothecium
n	Number of ascospores/ascus

31.7.7 Scab Prediction System

Based on the findings of the study, an onsite prediction system, which provides infection alert and disease forecast, was developed for the major apple-growing region in Garhwal Himalayas by Singh and Kumar (1999, 2005, 2008, 2009). The onsite weather data is automatically recorded by the µMETOS system installed at different locations in the region of the Garhwal Himalayas. Each of these systems also contains an inbuilt model that simulates the inoculum production and the infection process. Based on the onsite weather parameters, cultivar susceptibility, host phenological stages and the pathogen parameters specific to the orchard, the disease forecast will be generated (Singh and Kumar 2009). The forecast thus developed was passed on to the growers using All India Radio, local newspapers, SMS alerts, etc. which helped the orchardists to accordingly plan the plant protection measures against the apple scab.

31.7.7.1 Upscaling Disease Forecasting System at the Regional–National Scale: a Systems Analysis

A comprehensive and detailed analysis of the production systems at the national level, which is currently resource and data limited, is the need of time. However, emerging issues like impacts of climate change on crop diseases and their eventual effect on crop production could be addressed via model-based, spatially explicit scenario analysis of the national production system. One major output would be the identification of potential hotspots of vulnerability to climate change. Due to the fact that a national assessment of the food production systems may require a high level of generalization, which would not be able to account for the specificity of local or regional production systems, a two-phase approach is required. The approach is based on a finer resolution analysis of local/regional production systems using cropping system-integrated disease models combined with farm-scale models to represent locally relevant bio-physical and bio-economic drivers. Then the results of the first-phase analysis can be integrated into national food system models taking into account economic and physical equilibrium of demand and supply at the national scale. A possible pathway for such a two-phase analysis is presented in Fig. 31.9, showing possible feedback to the supply and demand module of the nation-wide model integration chain. In a biophysical analysis of disease occurrence at the level of landscape units, generalization from the production systems as analyzed above, and integration of the information about the percentage of areas used by a crop, soil typologies topography, climate and cropping intensity (population density could also be used as a proxy for cropping intensity but only after validation), would produce multiple sub-grid-scale simulation results per unit area. At the regional scale, this would not be useful only to the bio-economic analysis to explore different production systems, but would also create a key input to the development of a modelling layer to build a link between simulation and statistics. The production constraints due to prices can be successfully accounted for via the linking of bio-physical disease models and economic models at the national level. Structural constraints instead are context-specific, and must be considered in the regional analysis outlined above.

The whole concept of crop yield forecasting and envisaged modelling capacity would in turn combat detrimental effects of climate change as well and would be helpful in:

- Informing the farmers and consultants with real-time information about their crops, giving risk-assessment information and monitoring decision support relevant to farm management.
- Strengthening resilience and adaptive capacity to climate-related hazards on crops.
- Improving institutional capacity on climate change mitigation, adaptation, impact reduction and early warning.
- Integrating climate change measures into national policies, strategies and planning.

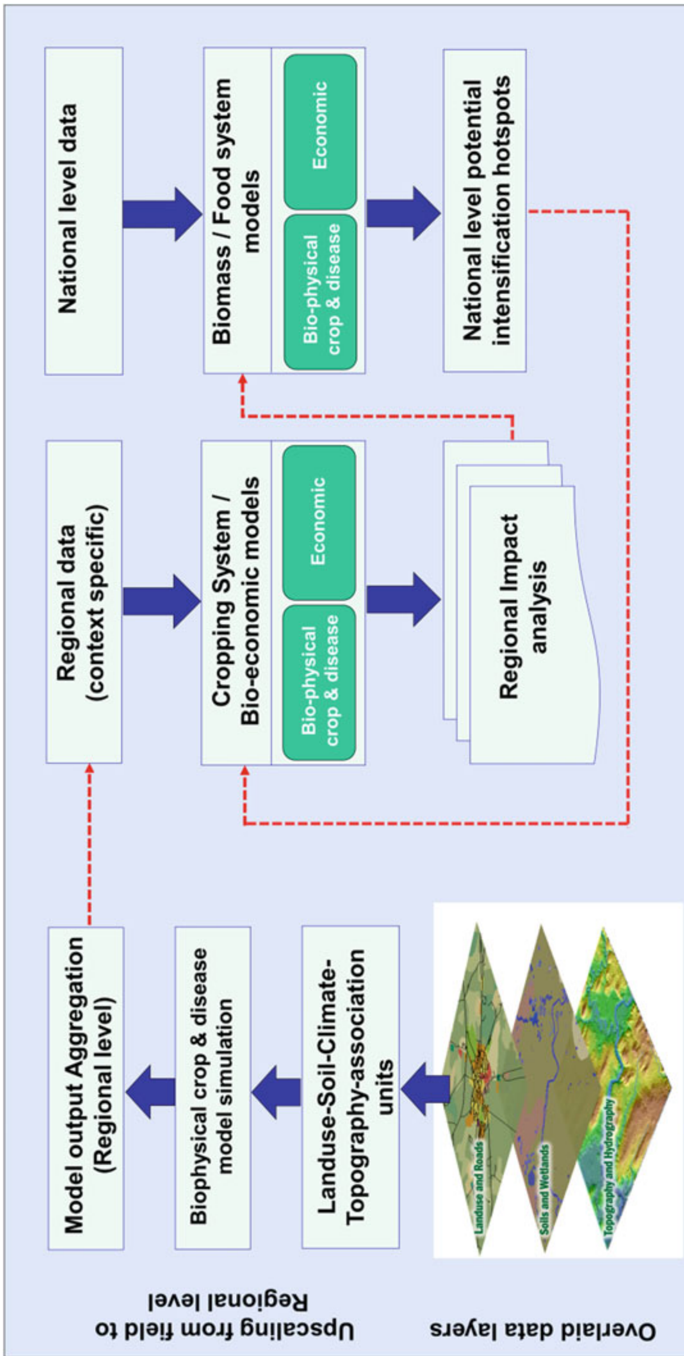

Fig. 31.9 Schematic presentation of a two-way approach of production systems analysis

Addressing such issues would, therefore, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, which strengthen capacity for adaptation to climate change.

31.8 Conclusion

Thus, from the available literature sources cited above, it can be concluded that the decision-making tools offer a viable option for taking appropriate disease management decision. Though the present adoption rate of these technologies is limited among both the scientific and farming community, they have got great potential to cater to the needs of both in the near future. For this, however, the flaws in these systems need to be rectified to increase their trust and acceptance among the end-users. The apple scab warning service based on the onsite devices in the Garhwal Himalayas offers an exciting example for the successful implementation of such technologies for the benefit of the farmers. The adoption of these tools will help to improve the decision making in IDM programmes, which will lead to better management of the plant diseases and take us one step closer to achieving the goal of sustainable agriculture.

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Bioinformatics in Plant Pathology

32

Aamir Khan, Sakshi Singh, and Vinay Kumar Singh

Abstract

Unprecedented success and availability of enormous next-generation sequencing data of host-pathogen in the public domain give us opportunities to understand the disease system biologically. The availability of genome data of host-pathogen in popular depository systems provides strong and proper help to retrieve, annotate, analyze and identify the functional elements for characterization at gene and genome levels for application development. The primary goal of bioinformatics is to enhance the understanding of biological processes using sequence pattern recognition, biological data mining, machine learning algorithms for biological datasets and visualization of biological data and molecules. Significant research efforts in the field include databases, software and tools development, genome analysis, anthropology, forensic genetics, sequence alignment, gene finding, genome assembly, drug design, drug discovery, protein structure alignment, protein structure prediction, gene expression analysis, microarray data analysis, protein–protein interactions and genome-wide association studies. Scientists, Paulien Hogeweg and Ben Hesper coined the term in 1970 to refer to the study of biological information processes in biotic systems. Margaret Oakley Dayhoff, the mother and father of bioinformatics compiled one of the first protein sequence databases. Elvin A. Kabat, the scientist

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who pioneered biological sequence analysis, developed the approach in 1970. Bioinformatics tools, techniques and databases can be used to identify potential genes, and target protein for host–pathogen interaction, drug designing and discovery and harvesting biological information from the plant genomes and their genes. Bioinformatics applications can be very beneficial in the improvement of crops and helpful for the development of designer crops.

Keywords

Bioinformatics · Plant pathology · Designer crops · Plant genomics · Next-generation sequencing

32.1 Introduction

Quality and quantity based designer crops and disease-free crops are in demand today. For that, crop improvement and protection is the first priority, in which computational biology approach for sequenced plant genomes plays a very important role and helps in crop improvement by maximizing the yield, quality-based fruits and grains production and disease resistant crops varieties (Chen and Chen 2008; King 2004; Mochida and Shinozaki 2010; Batley and Edwards 2016; Moody 2004). Development of sequence markers based on single nucleotide polymorphism and simple sequence repeat identification has now become feasible method for crop improvement. Lots of techniques, databases, tools and software have been developed to understand and analyze the biological system fully. Here standard bioinformatics techniques with specific tools and software are described.

32.2 Bioinformatics Techniques

32.2.1 Comparative Analysis

A comparative analysis is a field of biological sequence analysis in which the genomic sequence features of different organisms are compared. The genomic features may include the DNA sequence, regulatory region sequence genes and gene order. The major principle of comparative analysis is that to identify the common features between homologous sequences, it will often be encoded within the DNA that is evolutionarily conserved between them or differ region which are involved in diversity (Hardison 2003; Ong et al. 2016; Gebhardt et al. 2005; Sayers et al. 2019) (Fig. 32.1).

32.2.2 Sequence Analysis

Sequence analysis is the process of subjecting a DNA, RNA or protein homologous gene (orthologous and paralogous genes) sequence to understand its evolution,

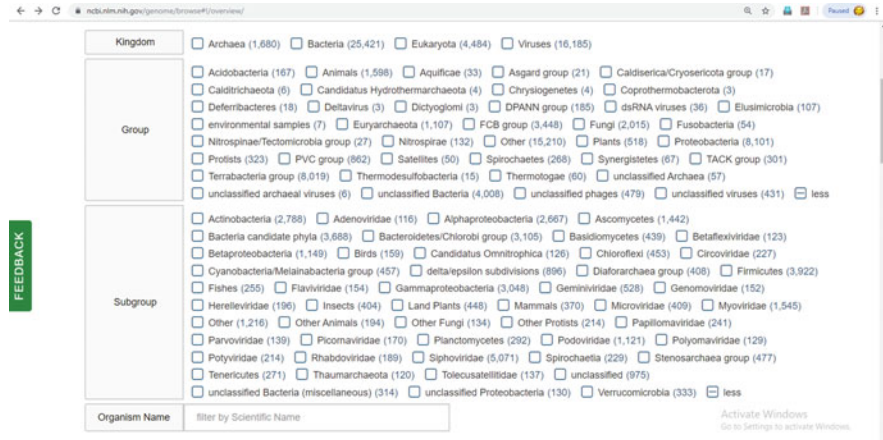

Fig. 32.1 Genome availability details in the NCBI database for retrieval and comparison of sequences

function, structure or features based on sequence alignment and searches against biological sequence databases like reference genes, proteins, UniProtKB/swiss-prot, protein data bank, etc. Sequence analysis includes the comparison of common region homologous sequences in order to find similarity and dissimilarity; identification of intrinsic features of the sequence such as active sites, post-translational modification sites, gene-structures, reading frames and distributions of introns and exons and regulatory elements; identification of sequence differences and variations such as point mutations, single nucleotide variants (SNV) and single nucleotide polymorphisms (SNPs) in order to get the genetic marker, revealing the evolution and genetic diversity of sequences and organisms and identification of molecular structure from sequence alone. A basic local alignment tool is the best tool for revealing the evolutionary and genetic diversity of sequences and organisms and identification of molecular structure from sequence (Aljanabi 2001; Bolger et al. 2018; Martinez 2013; Demuth and Hahn 2009; Lyons and Freeling 2008; Altschul et al. 1990; McClure et al. 1994; Pirovano and Heringa 2008; Bawono et al. 2017) (Fig. 32.2).

32.2.3 Gene Identification

Gene hunting, gene finding or gene prediction refers to the process of identifying the regions of genomic DNA that encode genes. Gene identification is one of the first and most important steps in understanding the gene and genome of organisms once they are sequenced and available to the public domain. Gene finding is one of the key steps in genome annotation, following genome sequence assembly and the filtering of non-coding (intronic) regions and coding (exonic) regions (Alioto 2012; Wang et al. 2004; Mochida and Shinozaki 2010) (Fig. 32.3).

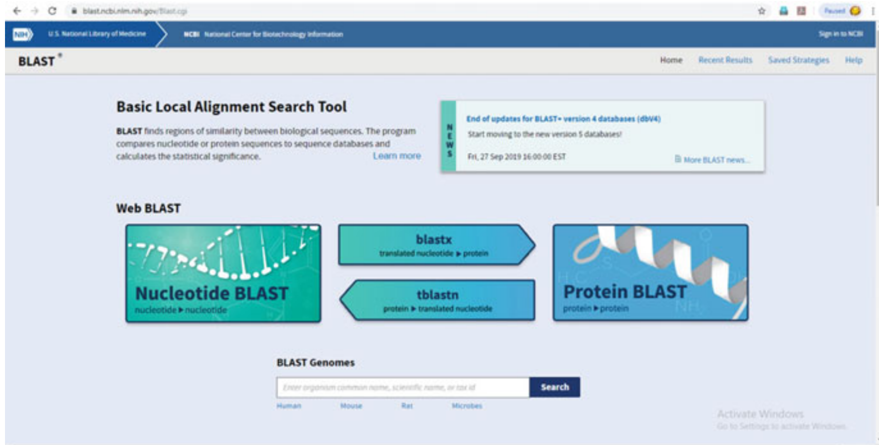

Fig. 32.2 Basic local alignment search tool web page for sequence similarity analysis

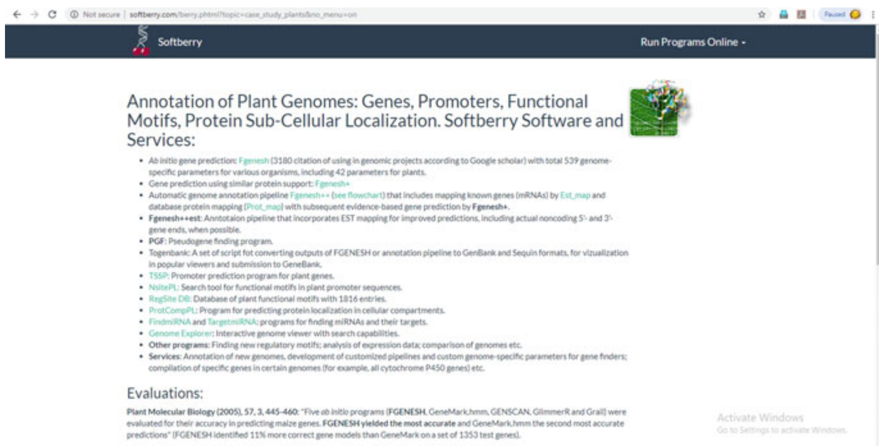

Fig. 32.3 Softberry server is a collection of software tools for genomic research focused on computational methods for high throughput biomedical data analysis

32.2.4 Phylogenetic Analysis

Phylogenetic analysis is the study of the evolutionary relationships among groups of homologous genes from organisms (e.g. species or populations). These phylogenetic relationships are discovered based on phylogenetic inference methods (distance-matrix methods: Neighbor-Joining (NJ), UPGMA (Unweighted Pair Group Method with Arithmetic mean) and WPGMA (Weighted Pair Group Method with Arithmetic mean), Fitch–Margoliash method, using outgroups, etc.; Maximum parsimony: Branch and bound, Sankoff-Morel-Cedergren algorithm, MALIGN and POY; Maximum likelihood; Bayesian inference) using sequence or morphological data. A phylogenetic tree is a branching tree diagram that represents the evolutionary

relationships among selected biological organisms or species. The phylogeny inferences based on similarities and differences in their genetic or physical characteristics. Phylogenetic analyses have become central to understanding genomes, diversity, evolution and ecology (Thompson et al. 1994, 2002).

32.2.5 Protein–Protein Interaction

Protein–protein interactions (PPIs) are the physical contacts between two or more protein molecules with high specificity based on biochemical events directed by hydrophobic effect and electrostatic forces. In STRING database known interactions based on curated databases or experimentally determined, predicted interactions based on gene neighbourhood or gene fusions or gene co-occurrence and other interactions based on textmining or co-expression or protein homology (De Las Rivas and Fontanillo 2010; Kozakov et al. 2017; Szklarczyk et al. 2019) (Figs. 32.4 and 32.5).

32.2.6 Microarray Data Analysis

NCBI developed the Gene Expression Omnibus (GEO) database in 2000 for high-throughput gene expression data. Microarray data analysis is used to infer information from the data generated from DNA, RNA and protein microarray experiments; these information allows researchers to investigate the expression level of a huge number of genes of the entire organism genome in a single experiment. Gene Expression Omnibus (GEO) is a public database using MIAME (Minimum Information About a Microarray Experiment) compliant data submissions. Sequence and

Fig. 32.4 STRING is a database for functional protein association networks

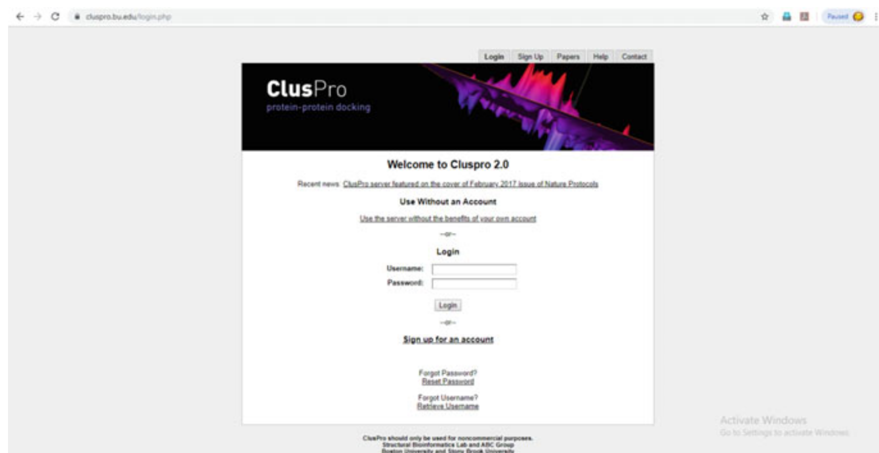

Fig. 32.5 ClusPro server is a web-based server for the direct docking of two interacting proteins

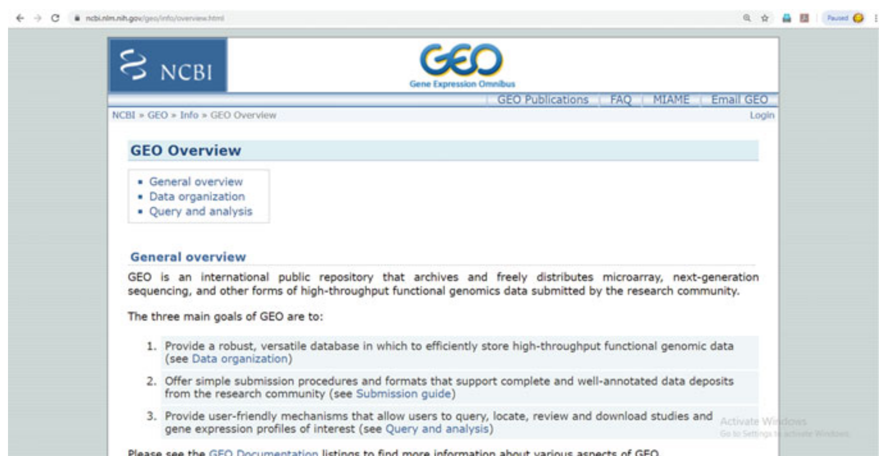

Fig. 32.6 Gene Expression Omnibus (GEO) is a database repository of high throughput gene expression data and microarrays

array-based data are accepted by the repository. Techniques and tools are available to help researchers query and download experimental datasets and gene expression profiles. GEO has collected repository and it consists freely available microarray data, next-generation sequencing data, and other high-throughput functional genomics data submitted by the scientific community (Clough and Barrett 2016). Due to the complexity of data which are generated by experiments are analyzed by bioinformaticians and bio scientists with specialized softwares. GEO has developed many tools for data query, analysis and visualization that can be analyzed directly on the GEO server (Fig. 32.6).

32.2.7 Structure Prediction and Refinement

Protein structure prediction is the construction of the three-dimensional (3D) structure of a protein from its amino acid sequence. In three-dimensional structure, the 3D prediction contains folds and secondary and tertiary structures from its primary sequence. It is highly important in drug designing and in the designing of 3D novel enzymes (Krieger et al. 2003; Xiang 2006; França 2015; Cavasotto and Phatak 2009; Xu et al. 2000).

32.2.8 Molecular Docking Calculation

Molecular docking is the interaction of two or more molecules to provide a stable complex structure. Based on the binding properties of the ligand and target, it generates a three-dimensional structure complex. Molecular docking is an approach to predict the orientation of one molecule to second molecule in the bound structure, which forms a stable complex. Knowledge of the active site orientation in turn may be useful in predicting the binding strength or binding affinity between receptor-ligand molecules using scoring functions. Molecular docking is a prominent method for structure-based drug design, due to the prediction of the binding-conformation of molecular ligands to the target receptor binding site. Characterization of the active binding behaviour plays an important role in rational design of novel pesticides, herbicides, insecticides and fungicides (Ferreira et al. 2015; Guedes et al. 2014; Morris and Lim-Wilby 2008; Meng et al. 2011; de Ruyck et al. 2016; Pagadala et al. 2017; Zhao and Caflisch 2015; Kroemer 2007; Sousa et al. 2006; Jones and Willett 1995; Lybrand 1995; Goodsell et al. 1996; Gschwend et al. 1996; Trosset and Cavé 2019).

32.3 Bioinformatics Databases

Biological Data Model

Biological data model is a library of biological life sciences information and biological databases; it has a collection of computational analysis tools, literature and high-throughput experimental data. Biological database contains information from research areas including genomics, phylogenetics, proteomics, metabolomics microarray gene expression and phenomics. Information contained in biological databases includes gene structure and function, macromolecular structure, cellular and chromosomal localization and SNP and mutations in sequences and structures (Wheeler et al. 2005; Galperin and Fernández-Suárez 2012). NCBI is a data model that contains popular search engine Entrez. Entrez is NCBI's retrieval system and primary text search that integrates the PubMed and PMC database of biomedical literature with so many molecular databases including genome, gene, DNA, genetic variation, gene expression, protein sequence and structure.

32.3.1 NCBI

NCBI stands for the National Center for Biotechnology Information and is strongly associated with the National Library of Medicine (NLM) and National Institutes of Health (NIH), Bethesda, Maryland. The NCBI was founded in 1988 by Senator Claude Pepper. NCBI resources contain chemicals and bioassays data, data and software, DNA and RNA sequence data, domains and structures, genes and expression data, genetics and medicine, genomes and maps, homology data, literature, protein sequence and structure, sequence analysis, taxonomy, training and tutorials data and variation data (NCBI Resource Coordinators 2016; Wheeler et al. 2005) (Figs. 32.7, 32.8, and 32.9).

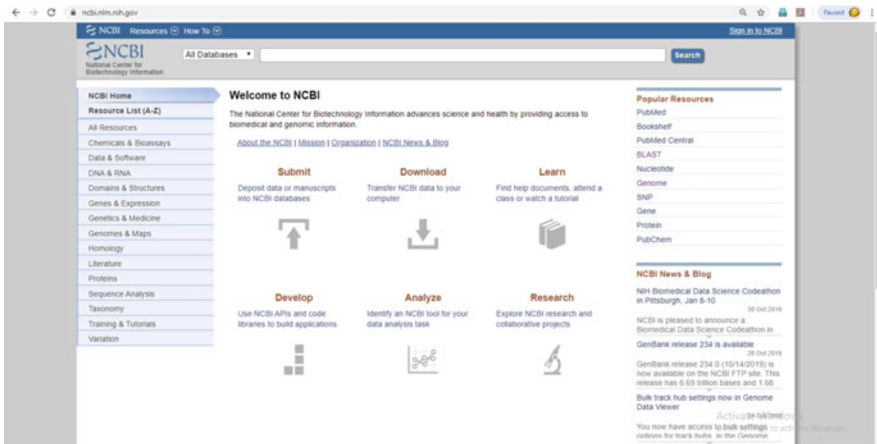

Fig. 32.7 National Center for Biotechnology Information web page

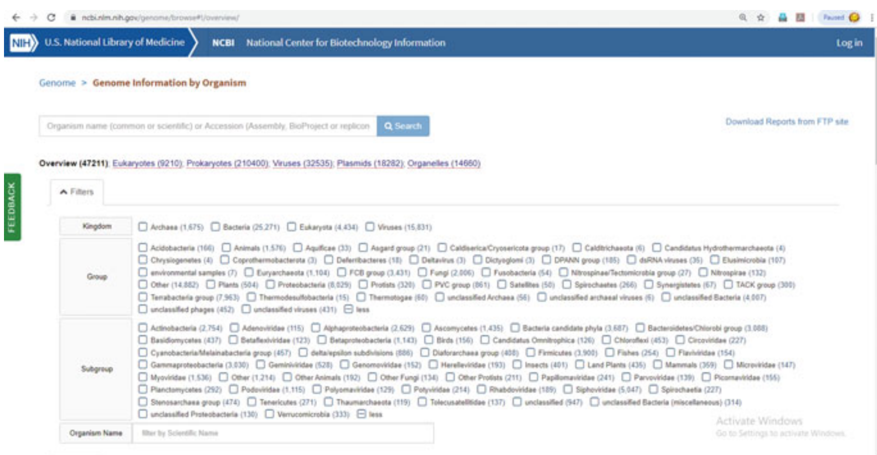

Fig. 32.8 NCBI genome details page1 (The genome information can search by different kingdoms, groups, subgroups, organism name present in the NCBI database)

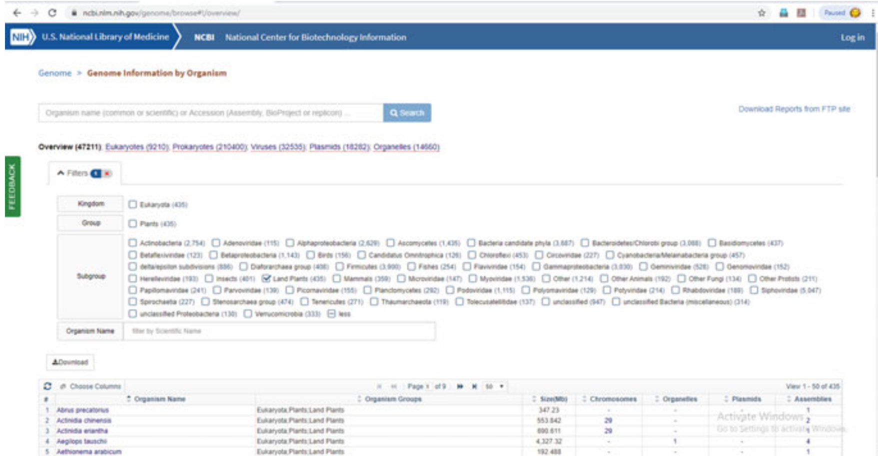

Fig. 32.9 NCBI genome details page2 (The genome information of eukaryota kingdom, plants group with their subgroups)

32.3.2 DDBJ

DDBJ (DNA Data Bank of Japan), founded in 1986, is a biological databank that mainly contains DNA sequence information. DDBJ is located at National Institute of Genetics (NIG), Shizuoka prefecture, Japan. It is also a member of INSDC (International Nucleotide Sequence Database Collaboration). The INSDC consists of a joint effort to collect and share DNA and RNA sequence data with GenBank (USA) and the European Nucleotide Archive (UK). DDBJ Sequence Read Archive (DRA), NCBI Sequence Read Archive (SRA) and EBI Sequence Read Archive (ERA) share new data and updated data on nucleotide sequences, and each of the three databases (DDBJ, NCBI and EMBL) are synchronized on a daily basis through continuous interaction between the staff at each of the collaborating organizations (Kodama et al. 2012) (Fig. 32.10).

32.3.3 EMBL

European Molecular Biology Laboratory (EMBL) is a research institution supported by 25 member states. EMBL was founded in 1974 and is a molecular biology research organization funded by public money from its member states conducted by approximately 85 independent groups. The web-based submission systems include WebIn at EMBL-EBI, Sakura ("cherry blossoms") at DDBJand BankIt at the NCBI (Madeira et al. 2019) (Fig. 32.11).

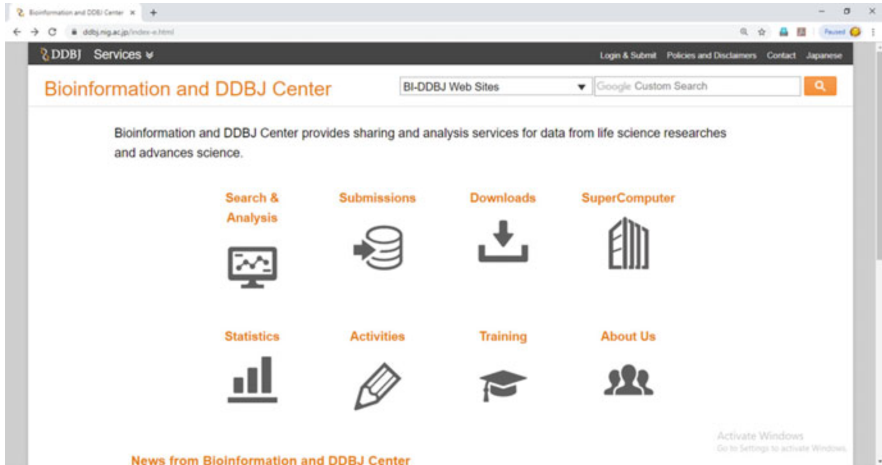

Fig. 32.10 DNA Data Bank of Japan web homepage

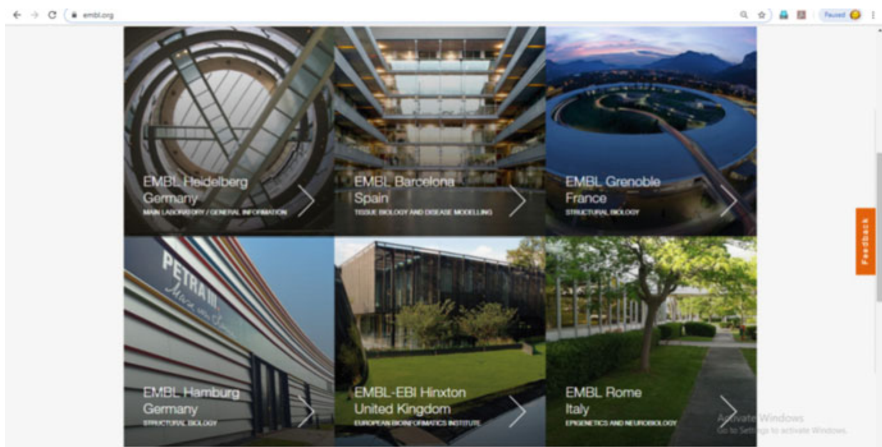

Fig. 32.11 European Molecular Biology Laboratory web page

32.3.4 Ensembl Plants

Ensembl Plants is an integrative database containing genome-scale information of plants. Ensembl Plants database includes genome sequence, gene models, polymorphic loci and functional annotation and various tools for analysis of sequence data. It contains various additional information, such as variation data, individual genotype data, linkage, population structure and phenotype data (Bolser et al. 2016, 2017) (Fig. 32.12).

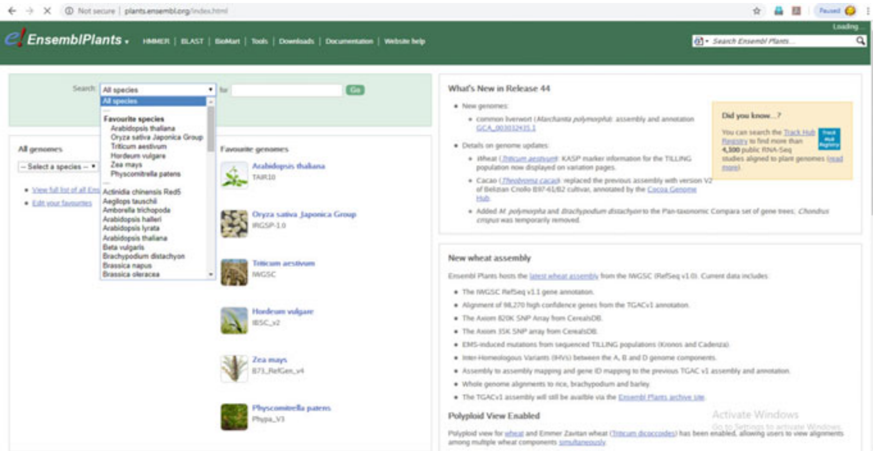

Fig. 32.12 Ensembl Plants front page for genome-scale information of plant species

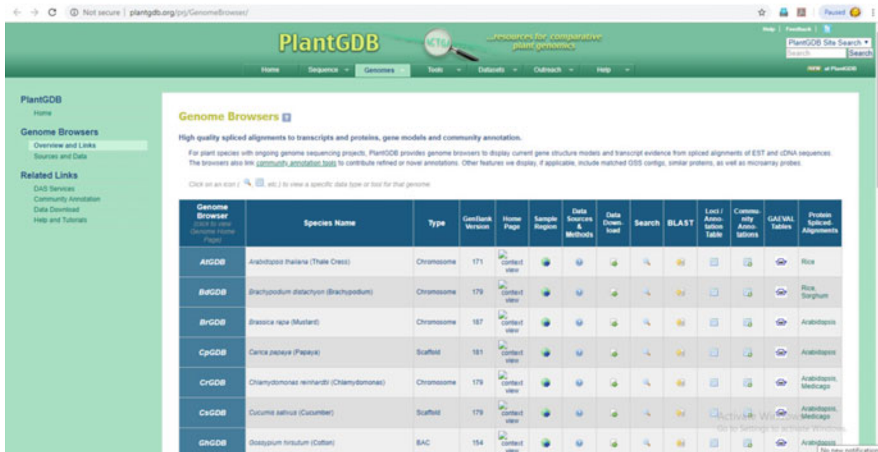

Fig. 32.13 PlantGDB database for the comparative plant genomics information

32.3.5 PlantGDB

PlantGDB is a resource for comparative genomics and a database of molecular sequence data for plant genomes. PlantGDB contains assembled unique transcripts (PUT), genome survey sequence assemblies (GSS), genome browsers and workflow Management (Dong et al. 2004; Duvick et al. 2008) (Fig. 32.13).

32.3.6 Phytozome

Phytozome is a comparative hub for plant genomes and gene family's data and analysis. Phytozome provides a view of genome organization, gene family, gene structure and the evolutionary history of gene at the level of sequence. It also provides access to the sequences and functional annotations of plant genomes and genes (Goodstein et al. 2012) (Fig. 32.14).

32.3.7 UNIPROT

UniProt database is a freely accessible database for protein sequence and functional annotation information, many entries being derived from different genome sequencing projects. UniProt contains a large amount of biological function of protein information derived from the literature mining. The main aim of UniProt is to provide a freely accessible resource, comprehensive and high-quality information of protein sequence and functional annotation information to scientific community (UniProt Consortium 2018) (Fig. 32.15).

32.3.8 PDB

PDB (Protein Data Bank) is a databank for the three-dimensional (3D) structural data of a large number of biological molecules, such as nucleic acids and proteins. The structural data is typically obtained by X-ray crystallography, NMR spectroscopy and cryo-electron microscopy. They are submitted by structural biologists from all around the world and are freely accessible on the net via website URLs. PDBmain

Fig. 32.14 Homepage of Phytozome database

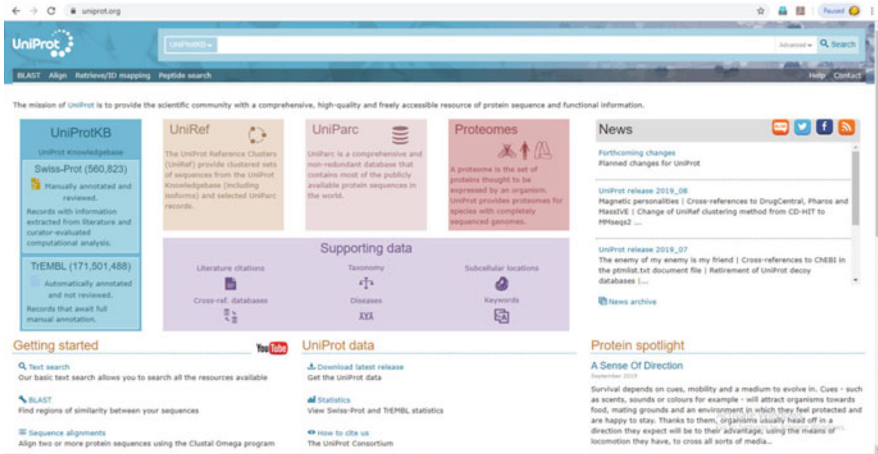

Fig. 32.15 UniProt database

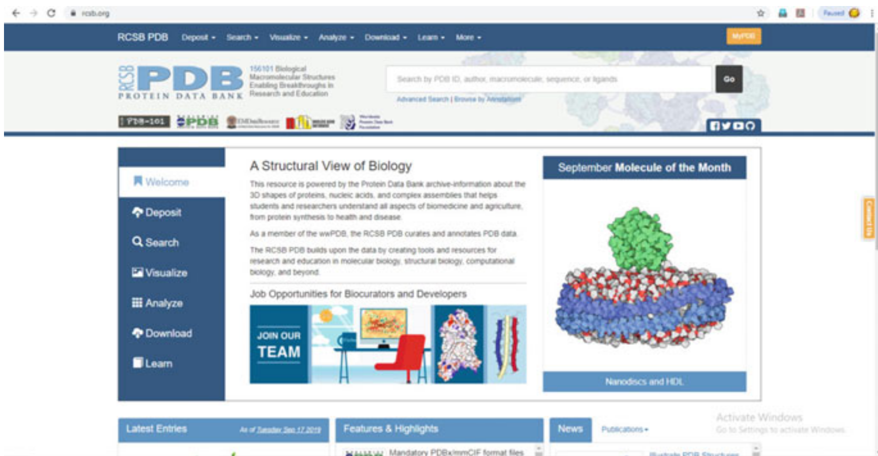

Fig. 32.16 Protein Data Bank homepage

member organizations are PDBe, PDBj, RCSB and BMRB. The PDB is overseen by an international organization called the Worldwide Protein Data Bank, wwPDB (Berman et al. 2000; Berman 2008; Laskowski et al. 1997) (Fig. 32.16).

32.3.9 MMDB

The Molecular Modeling Database (MMDB) is a three-dimensional biomolecular structure database of experimentally determined macromolecules and hosted by the National Center for Biotechnology Information (Chen et al. 2003) (Fig. 32.17).

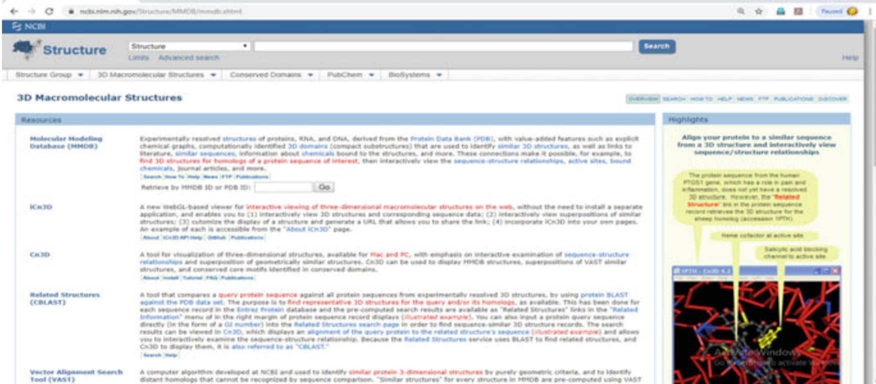

Fig. 32.17 Molecular modeling database of NCBI

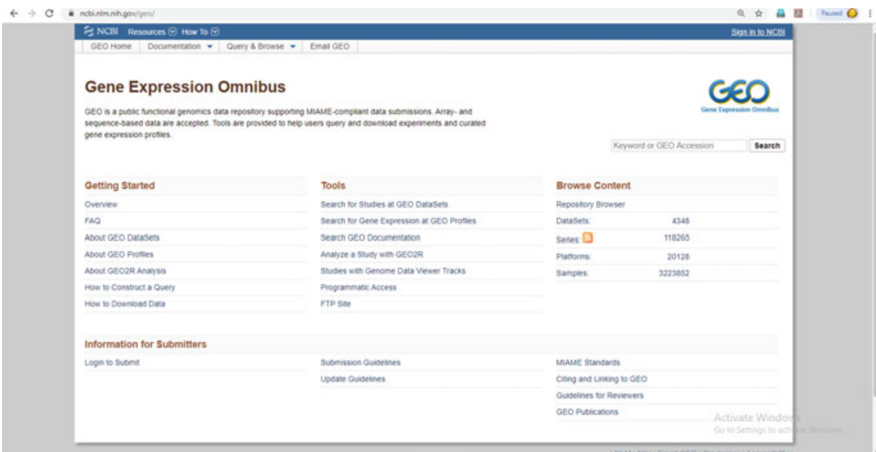

Fig. 32.18 Gene Expression Omnibus database of deposited high-throughput gene expression profiling data

32.3.10 GEO

GEO (Gene Expression Omnibus) is a gene expression database that archives and freely distributes microarray datasets, next-generation sequencing analysis details and other high-throughput functional genomics datasets deposited by the research community. The main goals of GEO are to provide versatile and robust database in which researchers can efficiently store high-throughput functional genomic data, offer simple submission procedures and formats to the research community that supports complete and well-annotated data deposits and provide user-friendly mechanisms to researchers that allow users to review, query, locate and download studies and gene expression profiles of interest for query and analysis (Clough and Barrett 2016) (Fig. 32.18).

32.4 Bioinformatics Tools and Software

32.4.1 BiGGEsTS

BiclusterinG Gene Expression Time Series (BiGGEsTS) is a free tool and graphical application based on bi-clustering algorithms mainly developed for analysis of gene expression time series data (Gonçalves et al. 2009) (Fig. 32.19).

32.4.2 HCE

HCE (Hierarchical Clustering Explorer) consists of hierarchical clustering algorithm to enable researchers to determine the grouping of data with informative dendrogram and colour mosaic visual feedback and dynamic query controls (Seo et al. 2006) (Fig. 32.20).

32.4.3 ClustVis

ClustVis is a web tool which allows researchers to upload their data and create Heat maps and PCA (Principal Component Analysis) plots. Data can be uploaded as a file or by pasting data to the text box (Metsalu and Vilo 2015) (Fig. 32.21).

Fig. 32.19 BiclusterinG Gene Expression Time Series

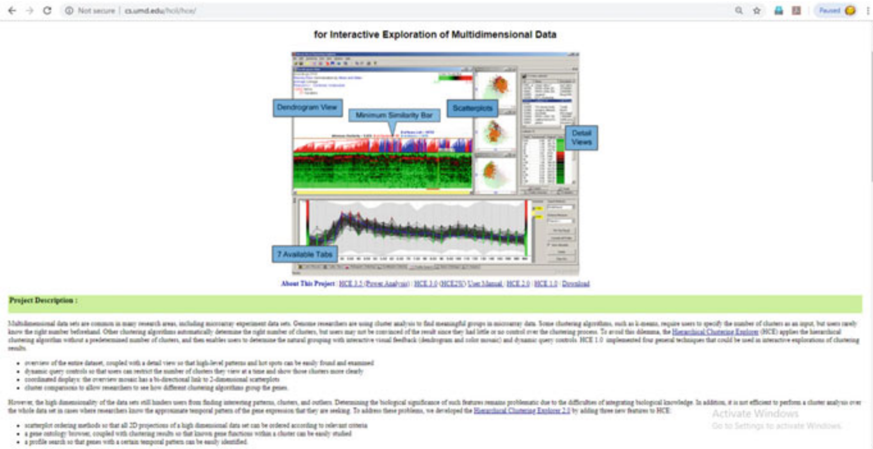

Fig. 32.20 Hierarchical Clustering Explorer

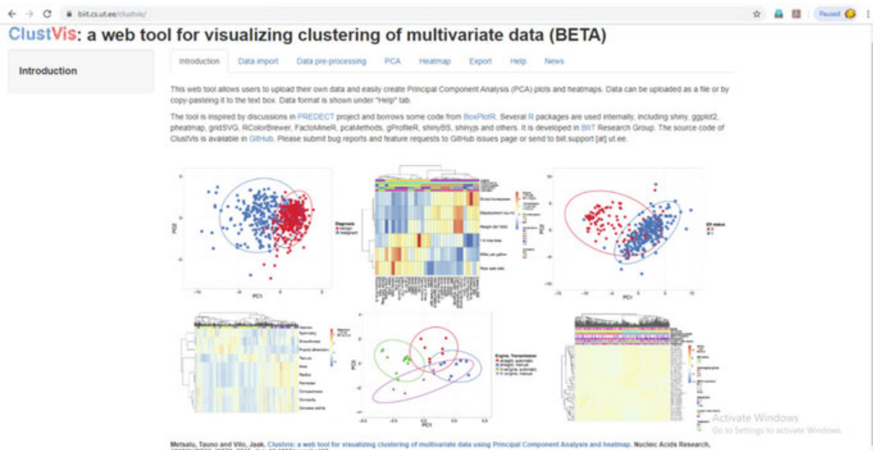

Fig. 32.21 ClustVis web tool

32.4.4 BLAST

BLAST (Basic Local Alignment Search Tool) finds regions of similarity and dissimilarity between sequences. The BLAST programme compares nucleotide or protein sequences to sequence databases and calculates identity with statistical significance (Altschul et al. 1990; Mount 2007) (Fig. 32.22).

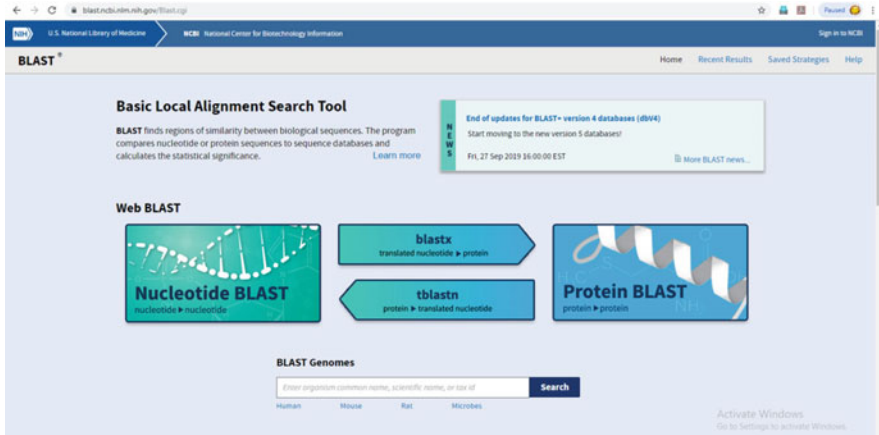

Fig. 32.22 Basic Local Alignment Search Tool

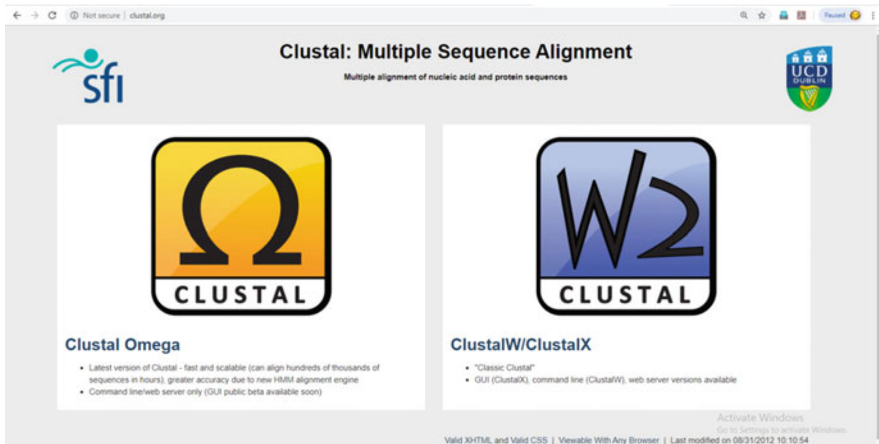

Fig. 32.23 Clustal series homepage

32.4.5 Clustal

Clustal omega, *Clustalw* and Clustalx (Clustal series) are widely used programmes for multiple sequence alignment (Higgins et al. 1996; Chenna et al. 2003; Sievers and Higgins 2014) (Fig. 32.23).

32.4.6 Bioedit

BioEdit is a free sequence alignment editor for editing and manipulation of sequence alignment data (Tippmann 2004) (Fig. 32.24).

Fig. 32.24 BioEdit is a biological sequence alignment editor tool

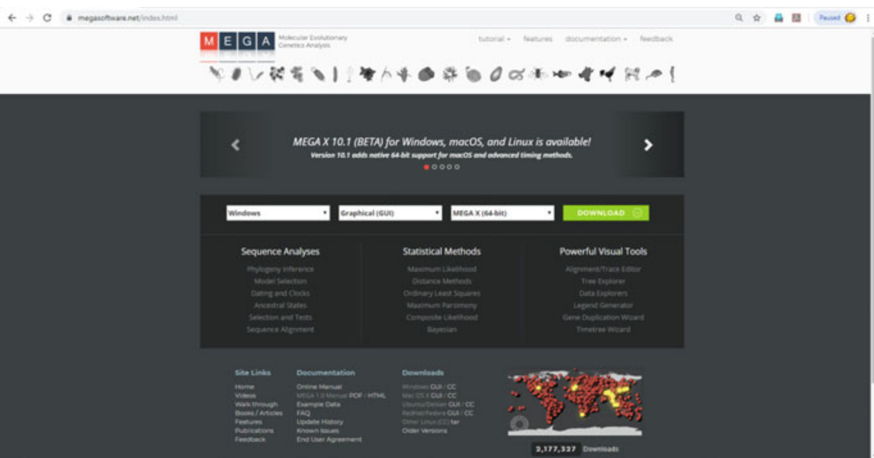

Fig. 32.25 Molecular evolutionary genetic analysis

32.4.7 MEGA

MEGA is a tool for manual and automatic sequence alignment, phylogenetic tree preparation, estimating rates of molecular evolution, web-based database mining and testing evolutionary hypotheses (Kumar et al. 2018) (Fig. 32.25).

32.4.8 Figtree

Figtree is a graphical viewer of phylogenetic tree visualization and for producing publication-ready figures of phylogenetic trees (Rambaut 2012) (Fig. 32.26).

32.4.9 Circos

Circos server is basically for identification and analysis of similarities and dissimilarity/differences generated from gene and genome comparisons (Krzywinski et al. 2009) (Fig. 32.27).

32.4.10 Prosite

PROSITE server is protein database that consists of protein families, functional domains and functional signature sites and amino acid profiles and patterns in sequence (Sigrist et al. 2002) (Fig. 32.28).

32.4.11 CDD

Conserved Domain Database (CDD) is a protein database that consists of well-annotated multiple sequence alignments as position-specific score matrices (PSSMs) for identification of conserved domains via RPS-BLAST. CDD includes NCBI-curated functional domains based on 3D-structure information to define domain boundaries and provide functional insights into sequence/structure/function

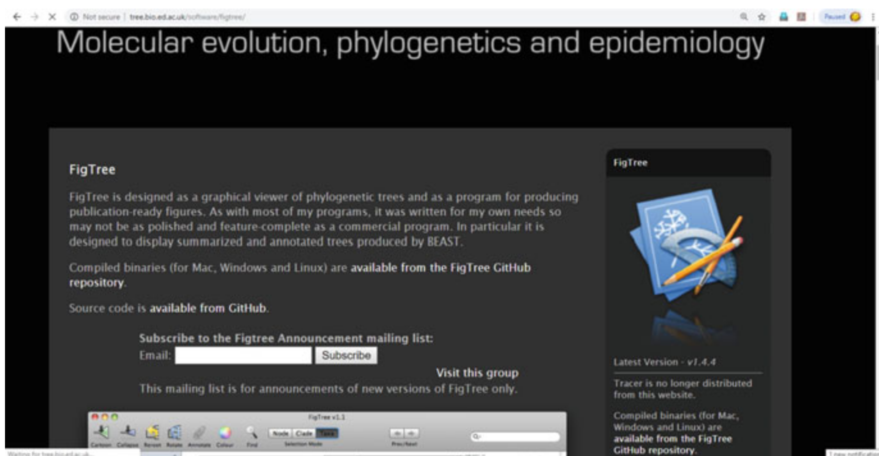

Fig. 32.26 FigTree server

Fig. 32.27 Circos server

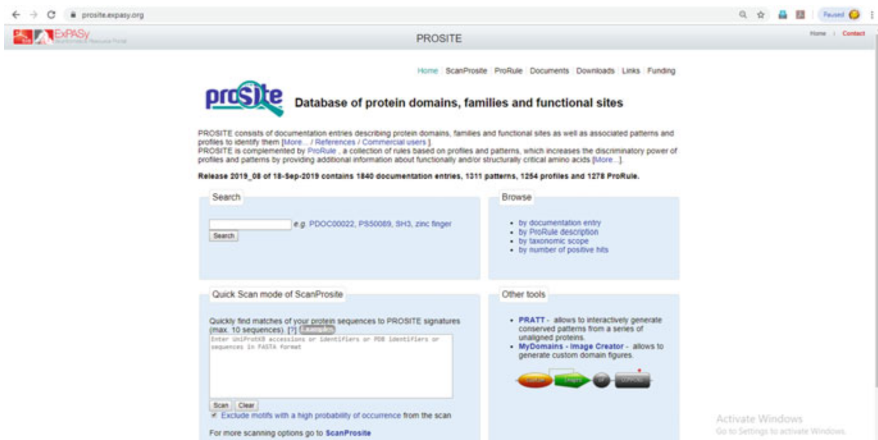

Fig. 32.28 PROSITE server

relationships, using Pfam, SMART, COG, PRK and TIGRFAMs databases (Marchler-Bauer et al. 2017) (Fig. 32.29).

32.4.12 Interproscan

InterProScan is a server to annotate protein families and domains automatically. InterPro provides functional signature analysis of proteins by classifying them into families, domains and important sites (Mitchell et al. 2019) (Fig. 32.30).

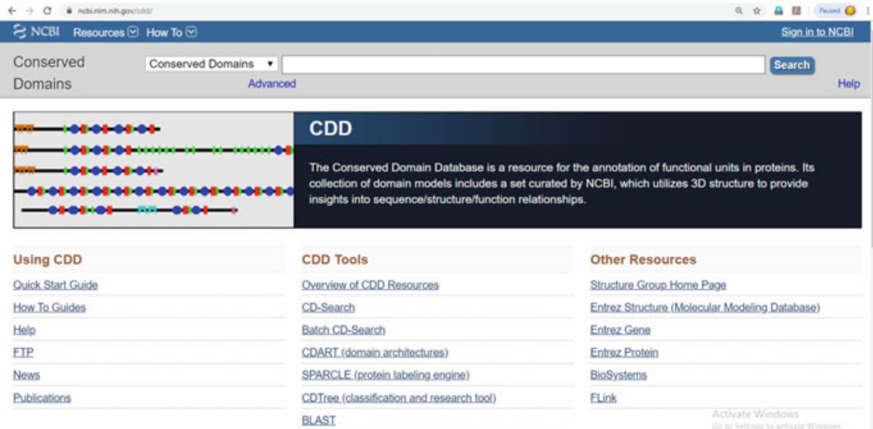

Fig. 32.29 Conserved Domain Database

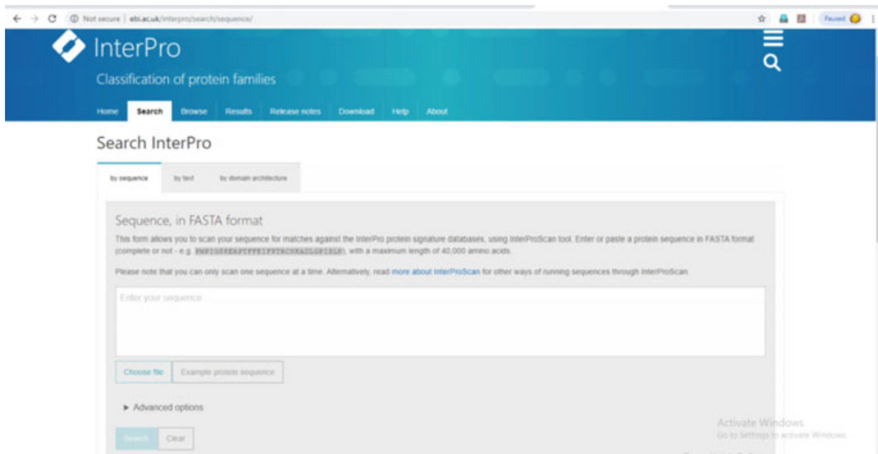

Fig. 32.30 InterProScan server

32.4.13 EasyModeller

EasyModeller is a graphical user interface programme used for homology modeling for predicting models of protein tertiary structures (Kuntal et al. 2010) (Fig. 32.31).

32.4.14 RAMPAGE/PROCHECK

PROCHECK server checks the stereochemical quality of a protein structure model; it produces Ramachandran plot to analyze the overall and residue-by-residue geometry (Laskowski et al. 2017; Lovell et al. 2003) (Figs. 32.32 and 32.33).

Fig. 32.31 EasyModeller

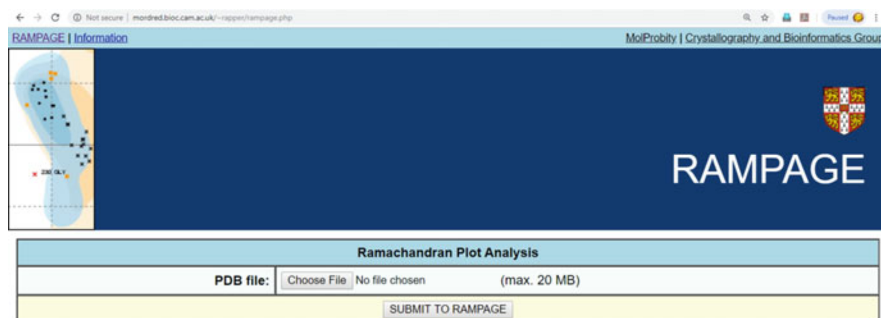

Fig. 32.32 RAMPAGE server

32.4.15 VERIFY3D

VERIFY3D server is used for determination of an atomic model (3D) with its amino acid sequence, by assigning a structural class based on alpha, beta, loop, polar, non-polar, etc. location and comparing the results to template structures (Eisenberg et al. 1997) (Fig. 32.34).

32.4.16 YASARA

YASARA (Yet Another Scientific Artificial Reality Application) is a computer programme for molecular vizualization, modeling and docking (Krieger and Vriend 2014) (Fig. 32.35).

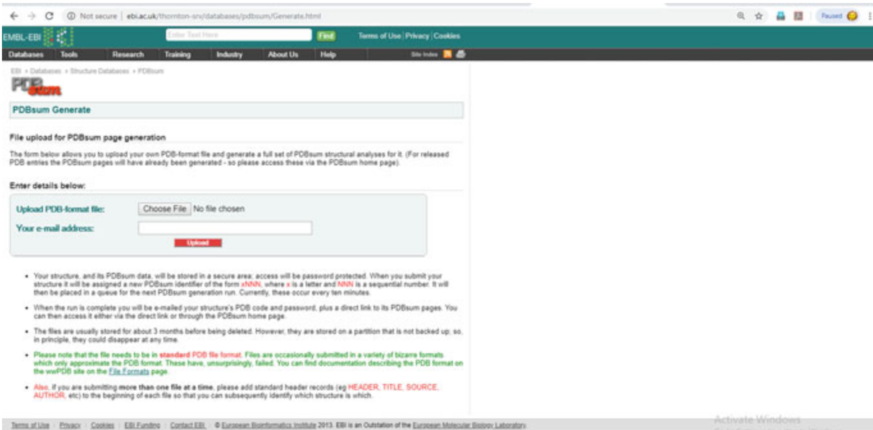

Fig. 32.33 PDBSum

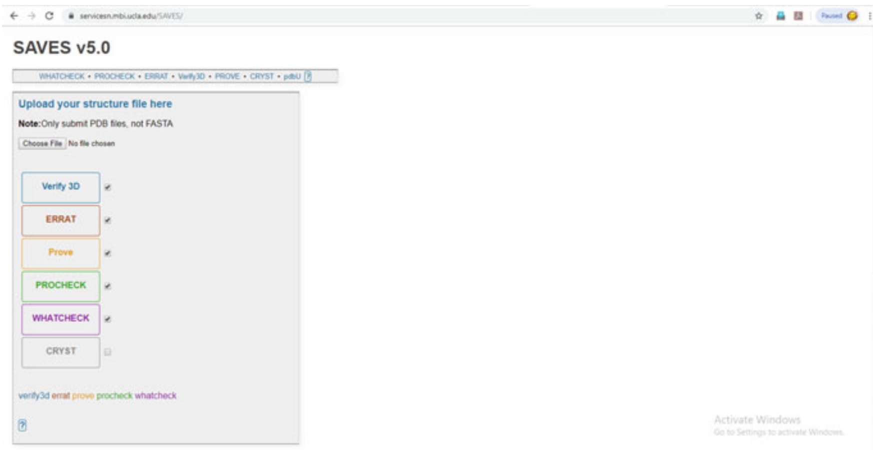

Fig. 32.34 SAVES server

32.4.17 BIOVIA Discovery Studio 2019

BIOVIA Discovery Studio contains BIOVIA Pipeline Pilot used for simulations, macromolecule design and analysis, antibody modeling, structure-based design, pharmacophore and ligand-based design, QSAR, ADMET and predictive toxicology, X-ray and visualization (Fig. 32.36).

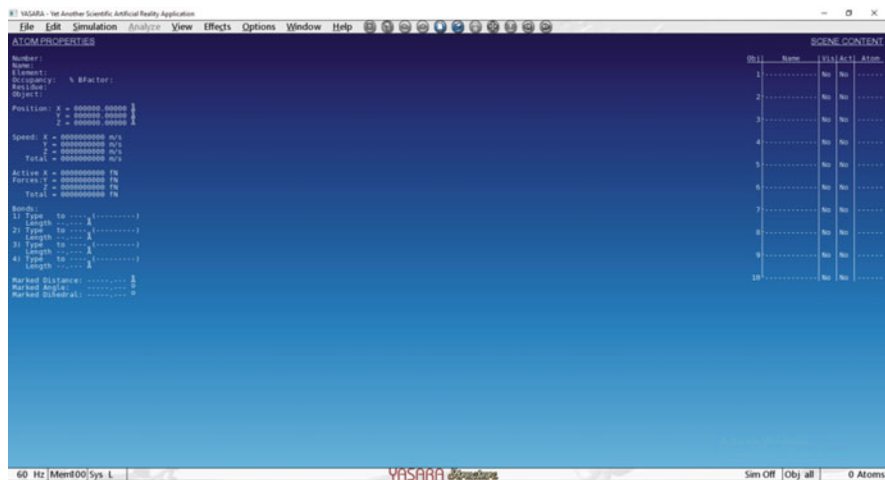

Fig. 32.35 Yet Another Scientific Artificial Reality Application

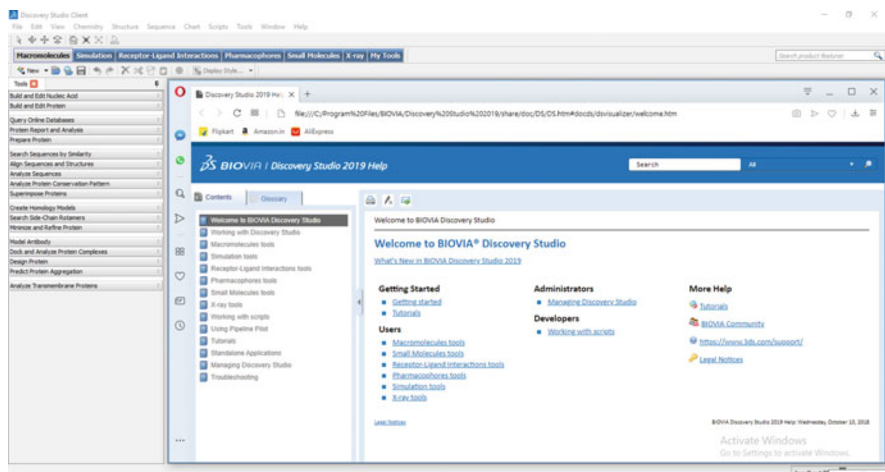

Fig. 32.36 BIOVIA Discovery Studio

32.4.18 Patchdock

The PatchDock server performs protein–protein docking and generates protein-small molecule complexes (Schneidman-Duhovny et al. 2005) (Fig. 32.37).

Fig. 32.37 PatchDock server

Fig. 32.38 Hex server

32.4.19 Hex

Hex tool/server is a graphics programme for docking calculation and visualizing docking modes of pairs of protein and DNA molecules. Hex is also useful for calculation of protein-ligand docking; it can superpose molecules (Macindoe et al. 2010) (Fig. 32.38).

32.5 Plant and Pathogen Genomics

Five main types of pathogenic organisms that cause plant diseases are viruses, bacteria, fungi, protozoa and worms/nematodes, which can lead from damage to death. The genome availability of plants and pathogens gives us opportunities to understand the bio systems and disease mechanisms (Tables 32.1, 32.2, and 32.3).

Table 32.1 List of important plant diseases with their causing organism, in which most of pathogen genomes are available in the NCBI database

Disease	Causing organism (pathogen)
Bacterial leaf blight	*Pseudomonas syringae subsp. syringae*
Aster yellows	*Phytoplasma*
Bacterial wilt	*Erwinia tracheiphila*
Bacterial blight	*Xanthomonas campestris, Xanthomonas axonopodis, Pseudomonas syringae*
Crown gall	*Agrobacterium tumefaciens*
Bacterial soft rot	*Erwinia, Pectobacterium and Pseudomonas*
Scab	*Venturia inaequalis, Streptomyces scabies*
Anthracnose	*Colletotrichum*
Black knot	*Dibotryon morbosum* or *Apiosporina morbosa*
Blight	*Cryphonectria parasitica*, *Cochliobolus heterostrophus*, *Colletotrichum capsici*
Chestnut blight	*Cryphonectria parasitica*
Late blight	*Phytophthora infestans*
Canker	*Sirococcusclavigignenti-juglandacearum*, *Seiridiumcardinale*, *Gibberellabaccata*, *Diplodiaquercina*, *Leptosphaeria coniothyrium, Cryptosporella umbrina*, *Colletotrichum coccodes*
Clubroot	*Plasmodiophora brassicae*
Damping-off	*Pythium*
Dutch elm disease	*Claviceps purpurea*
Fusarium wilt Panama disease	*Fusarium oxysporum*
Leaf blister	*Taphrina caerulescens*
Downy mildew	*Pseudoperonospora cubensis*
Powdery mildew	*Podosphaera xanthii*, *Erysiphe cichoracearum*
Oak wilt	*Ceratocystis fagacearum*
Rot	*Oomycota*
Basal rot	*Botrytis, Fusarium, and Penicillium*
Graymold rot	*Botrytis cinerea*

(continued)

Table 32.1 (continued)

Disease	Causing organism (pathogen)
Rust	*Phragmidium spp.*
Blister rust	*Cronartium ribicola*
Cedar-apple rust	*Gymnosporangium juniperi-virginianae*
Coffee rust	*Hemileia vastatrix*
Scab	*Venturia inaequalis*
Smut	*Sporisorium scitamineum*
Bunt	*Tilletia tritici*
Corn smut	*Ustilago maydis*
Sooty mold	*Cladosporium and Alternaria*
Verticillium wilt	*Verticillium*
Curly top	(*Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragrovirus, Grablovirus, Mastrevirus, Topocuvirus, Turncurtovirus*)
Mosaic	*Tobacco mosaic virus*
Psorosis	*Citrus psorosisophiovirus*
Spotted wilt	*Tomato spotted wilt virus*
Root-knot nematodes	*Meloidogyne*
Witchweed	*Pratylenchus*

Table 32.2 List of important plant pathogen genome details

Organism name	Organism groups	Size(Mb)	Assemblies
Abutilon Brazil virus	Viruses; Geminiviridae	0.005271	1
Abutilon golden mosaic virus	Viruses; Geminiviridae	0.002629	1
Abutilon mosaic Bolivia virus	Viruses; Geminiviridae	0.005399	1
Abutilon mosaic Brazil virus	Viruses; Geminiviridae	0.005282	1
Abutilon mosaic virus	Viruses; Geminiviridae	0.005217	1
African cassava mosaic Burkina Faso virus	Viruses; Geminiviridae	0.00277	1
African cassava mosaic virus	Viruses; Geminiviridae	0.005503	1
Ageratum enation virus	Viruses; Geminiviridae	0.00276	3
Ageratum leaf curl Cameroon virus	Viruses; Geminiviridae	0.002792	1
Ageratum leaf curl virus – [G52]	Viruses; Geminiviridae	0.002735	1
Ageratum yellow vein China virus – OX1	Viruses; Geminiviridae	0.002739	1
Ageratum yellow vein Hualian virus	Viruses; Geminiviridae	0.002756	2
Ageratum yellow vein Sri Lanka virus	Viruses; Geminiviridae	0.002748	1
Ageratum yellow vein virus	Viruses; Geminiviridae	0.002768	12
Agrobacterium tumefaciens	Bacteria; Proteobacteria; Alphaproteobacteria	7.2733	69
Alfalfa leaf curl virus	Viruses; Geminiviridae	0.002745	1
Allamanda leaf curl virus	Viruses; Geminiviridae	0.002755	1
Allamanda leaf mottle distortion virus	Viruses; Geminiviridae	0.005462	1
Alternanthera yellow vein virus	Viruses; Geminiviridae	0.002745	3
Alternaria alternata	Eukaryota; Fungi; Ascomycetes	32.9908	6
Alternaria arborescens	Eukaryota; Fungi; Ascomycetes	33.9434	5
Alternaria atra	Eukaryota; Fungi; Ascomycetes	35.1121	1
Alternaria brassicae	Eukaryota; Fungi; Ascomycetes	34.1411	1
Alternaria brassicicola	Eukaryota; Fungi; Ascomycetes	31.0365	2
Alternaria consortialis	Eukaryota; Fungi; Ascomycetes	34.2409	1
Alternaria gaisen	Eukaryota; Fungi; Ascomycetes	34.3469	1
Alternaria gansuensis	Eukaryota; Fungi; Ascomycetes	75.0519	1
Alternaria solani	Eukaryota; Fungi; Ascomycetes	32.7791	2
Alternaria sp. MG1	Eukaryota; Fungi; Ascomycetes	34.6956	1
Alternaria tenuissima	Eukaryota; Fungi; Ascomycetes	35.7042	7

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Andrographis yellow vein leaf curl virus	Viruses; Geminiviridae	0.002754	1
Aster yellow witches'-broom phytoplasma	Bacteria; Terrabacteriagroup; Tenericutes	0.72397	1
Asystasia mosaic Madagascar virus	Viruses; Geminiviridae	0.005404	1
Axonopus compressus streak virus	Viruses; Geminiviridae	0.002858	1
Bean calico mosaic virus	Viruses; Geminiviridae	0.005175	1
Bean chlorosis virus	Viruses; Geminiviridae	0.005279	1
Bean dwarf mosaic virus	Viruses; Geminiviridae	0.005191	1
Bean golden mosaic virus	Viruses; Geminiviridae	0.005197	2
Bean golden yellow mosaic virus	Viruses; Geminiviridae	0.005255	2
Bean leaf crumple virus	Viruses; Geminiviridae	0.002598	1
Bean leaf curl Madagascar virus	Viruses; Geminiviridae	0.002754	1
Bean white chlorosis mosaic virus	Viruses; Geminiviridae	0.005163	1
Bean yellow dwarf virus	Viruses; Geminiviridae	0.002561	1
Bean yellow dwarf virus	Viruses; Geminiviridae	0.002561	1
Bean yellow mosaic Mexico virus	Viruses; Geminiviridae	0.002641	1
Beet curly top Iran virus	Viruses; Geminiviridae	0.002859	4
Beet curly top virus	Viruses; Geminiviridae	0.002994	8
Bhendi yellow vein Bhubhaneswar virus	Viruses; Geminiviridae	0.002757	1
Bhendi yellow vein Delhi virus [2004: New Delhi]	Viruses; Geminiviridae	0.002751	1
Bhendi yellow vein Haryana virus	Viruses; Geminiviridae	0.00274	1
Bhendi yellow vein India virus [India: Dharwad OYDWR2:2006]	Viruses; Geminiviridae	0.002739	1
Bhendi yellow vein mosaic virus	Viruses; Geminiviridae	0.002747	6
Bipolaris maydis	Eukaryota; Fungi; Ascomycetes	32.9292	2
Bitter gourd yellow vein virus	Viruses; Geminiviridae	0.005453	1
Blainvillea yellow spot virus	Viruses; Geminiviridae	0.0053	1
Blechum interveinal chlorosis virus	Viruses; Geminiviridae	0.005285	1
Boerhavia yellow spot virus	Viruses; Geminiviridae	0.002621	1
Botrytis cinerea	Eukaryota; Fungi; Ascomycetes	42.6301	4
'Brassica napus' phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.743598	1
Bretziellafagacearum	Eukaryota; Fungi; Ascomycetes	26.782	1
Bromuscatharticus striate mosaic virus	Viruses; Geminiviridae	0.002797	1
Cabbage leaf curl Jamaica virus	Viruses; Geminiviridae	0.005069	1
Cabbage leaf curl virus	Viruses; Geminiviridae	0.005096	1
Candidatus Phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.687137	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Candidatus Phytoplasma aurantifolia	Bacteria; Terrabacteria group; Tenericutes	0.474669	1
Candidatus Phytoplasma australiense	Bacteria; Terrabacteria group; Tenericutes	0.959779	2
Candidatus Phytoplasma mali	Bacteria; Terrabacteria group; Tenericutes	0.601943	1
Candidatus Phytoplasma oryzae	Bacteria; Terrabacteria group; Tenericutes	0.533195	2
Candidatus Phytoplasma phoenicium	Bacteria; Terrabacteria group; Tenericutes	0.541091	2
Candidatus Phytoplasma pini	Bacteria; Terrabacteria group; Tenericutes	0.474136	1
Candidatus Phytoplasma pruni	Bacteria; Terrabacteria group; Tenericutes	0.598511	1
Candidatus Phytoplasma solani	Bacteria; Terrabacteria group; Tenericutes	0.821322	3
Candidatus Phytoplasma ziziphi	Bacteria; Terrabacteria group; Tenericutes	0.750803	1
Capraria yellow spot Yucatan virus	Viruses; Geminiviridae	0.005208	1
Cassava mosaic Madagascar virus	Viruses; Geminiviridae	0.00551	1
'Catharanthus roseus' aster yellows phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.603949	1
Catharanthus yellow mosaic virus	Viruses; Geminiviridae	0.002752	1
Centrosema yellow spot virus	Viruses; Geminiviridae	0.002675	1
Chayote yellow mosaic virus	Viruses; Geminiviridae	0.002787	1
Chenopodium leaf curl virus	Viruses; Geminiviridae	0.002626	1
Chickpea chlorosis Australia virus	Viruses; Geminiviridae	0.002572	1
Chickpea chlorosis virus	Viruses; Geminiviridae	0.002603	3
Chickpea chlorosis virus-A	Viruses; Geminiviridae	0.002582	1
Chickpea chlorotic dwarf virus	Viruses; Geminiviridae	0.002587	5
Chickpea redleaf virus	Viruses; Geminiviridae	0.002605	1
Chickpea yellow dwarf virus	Viruses; Geminiviridae	0.002547	1
Chickpea yellows virus	Viruses; Geminiviridae	0.002557	1
Chilli leaf curl Ahmedabad virus-India [India/Ahmedabad/2014]	Viruses; Geminiviridae	0.002744	1
Chilli leaf curl India virus	Viruses; Geminiviridae	0.002755	1
Chilli leaf curl Kanpur virus	Viruses; Geminiviridae	0.002754	1
Chilli leaf curl Vellanad virus	Viruses; Geminiviridae	0.002788	1
Chilli leaf curl virus	Viruses; Geminiviridae	0.002858	7
Chino del tomate Amazonas virus	Viruses; Geminiviridae	0.002615	1
Chino del tomate virus	Viruses; Geminiviridae	0.005213	3
Chloris striate mosaic virus	Viruses; Geminiviridae	0.00275	1
'Chrysanthemum coronarium' phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.739592	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Chrysanthemum yellows phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.659699	1
Citrus psorosis virus	Viruses; Aspiviridae	0.011278	1
Cladosporium cladosporioides	Eukaryota; Fungi; Ascomycetes	33.2257	1
Cladosporium phlei	Eukaryota; Fungi; Ascomycetes	32.816	1
Cladosporium sp. SL-16	Eukaryota; Fungi; Ascomycetes	35.8569	1
Cladosporium sphaerospermum	Eukaryota; Fungi; Ascomycetes	26.8942	1
Claviceps purpurea	Eukaryota; Fungi; Ascomycetes	32.0914	3
Cleome golden mosaic virus	Viruses; Geminiviridae	0.002566	1
Cleome leaf crumple virus	Viruses; Geminiviridae	0.005386	1
Clerodendron golden mosaic virus	Viruses; Geminiviridae	0.005524	1
Clerodendron yellow mosaic virus	Viruses; Geminiviridae	0.00276	1
Clerodendrum golden mosaic China virus	Viruses; Geminiviridae	0.005515	2
Clerodendrum golden mosaic Jiangsu virus	Viruses; Geminiviridae	0.002753	1
Cnidoscolus mosaic leaf deformation virus	Viruses; Geminiviridae	0.005254	1
Coccinia mosaic Tamil Nadu virus	Viruses; Geminiviridae	0.00544	1
Colletotrichum acutatum	Eukaryota; Fungi; Ascomycetes	52.1291	2
Colletotrichum chlorophyti	Eukaryota; Fungi; Ascomycetes	52.387	1
Colletotrichum coccodes	Eukaryota; Fungi; Ascomycetes	50.122	2
Colletotrichum falcatum	Eukaryota; Fungi; Ascomycetes	48.1864	1
Colletotrichum fioriniae	Eukaryota; Fungi; Ascomycetes	50.1509	3
Colletotrichum fructicola	Eukaryota; Fungi; Ascomycetes	55.9157	3
Colletotrichum gloeosporioides	Eukaryota; Fungi; Ascomycetes	61.9165	6
Colletotrichum godetiae	Eukaryota; Fungi; Ascomycetes	35.0343	1
Colletotrichum graminicola	Eukaryota; Fungi; Ascomycetes	51.6443	2
Colletotrichum higginsianum	Eukaryota; Fungi; Ascomycetes	50.7161	3
Colletotrichum incanum	Eukaryota; Fungi; Ascomycetes	53.2546	2

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Colletotrichum lentis	Eukaryota; Fungi; Ascomycetes	56.1001	1
Colletotrichum lindemuthianum	Eukaryota; Fungi; Ascomycetes	99.1667	2
Colletotrichum musae	Eukaryota; Fungi; Ascomycetes	49.1188	1
Colletotrichum nymphaeae	Eukaryota; Fungi; Ascomycetes	49.9563	1
Colletotrichum orbiculare	Eukaryota; Fungi; Ascomycetes	89.7483	1
Colletotrichum orchidophilum	Eukaryota; Fungi; Ascomycetes	48.5565	1
Colletotrichum salicis	Eukaryota; Fungi; Ascomycetes	48.3734	1
Colletotrichum sansevieriae	Eukaryota; Fungi; Ascomycetes	51.2013	1
Colletotrichum shisoi	Eukaryota; Fungi; Ascomycetes	69.6677	1
Colletotrichum siamense	Eukaryota; Fungi; Ascomycetes	55.9616	1
Colletotrichum sidae	Eukaryota; Fungi; Ascomycetes	86.8278	1
Colletotrichum simmondsii	Eukaryota; Fungi; Ascomycetes	50.4742	1
Colletotrichum sp. JS-367	Eukaryota; Fungi; Ascomycetes	87.1965	1
Colletotrichum spinosum	Eukaryota; Fungi; Ascomycetes	82.7349	1
Colletotrichum sublineola	Eukaryota; Fungi; Ascomycetes	64.8486	2
Colletotrichum tanaceti	Eukaryota; Fungi; Ascomycetes	57.9125	1
Colletotrichum tofieldiae	Eukaryota; Fungi; Ascomycetes	52.7196	5
Colletotrichum trifolii	Eukaryota; Fungi; Ascomycetes	109.66	1
Colletotrichum truncatum	Eukaryota; Fungi; Ascomycetes	57.9128	2
Common bean mottle virus	Viruses; Geminiviridae	0.005235	1
Common bean severe mosaic virus	Viruses; Geminiviridae	0.00519	2
Corchorus golden mosaic virus	Viruses; Geminiviridae	0.005352	2
Corchorus yellow spot virus	Viruses; Geminiviridae	0.005195	1
Corchorus yellow vein mosaic virus	Viruses; Geminiviridae	0.002743	1
Corchorus yellow vein virus – [HoaBinh]	Viruses; Geminiviridae	0.005415	1
Cotton chlorotic spot virus	Viruses; Geminiviridae	0.00532	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Cotton leaf crumple virus	Viruses; Geminiviridae	0.00518	2
Cotton leaf curl Alabad virus	Viruses; Geminiviridae	0.002744	4
Cotton leaf curl Allahabad virus [India: Karnal:OY77:2005]	Viruses; Geminiviridae	0.002744	1
Cotton leaf curl Bangalore virus	Viruses; Geminiviridae	0.002751	1
Cotton leaf curl Gezira virus	Viruses; Geminiviridae	0.00278	9
Cotton leaf curl Kokhran virus	Viruses; Geminiviridae	0.002759	3
Cotton leaf curl Multan virus	Viruses; Geminiviridae	0.002754	5
Cotton leaf curl Shahdadpur virus	Viruses; Geminiviridae	0.002748	1
Cotton leaf curl virus	Viruses; Geminiviridae	0.002753	1
Cotton yellow mosaic virus	Viruses; Geminiviridae	0.005482	2
Cowpea golden mosaic virus	Viruses; Geminiviridae	0.002728	1
Crassocephalum yellow vein virus – Jinghong	Viruses; Geminiviridae	0.002745	1
Cronartiumribicola	Eukaryota; Fungi; Basidiomycetes	94.3329	1
Croton yellow vein mosaic virus	Viruses; Geminiviridae	0.002757	2
Croton yellow vein virus	Viruses; Geminiviridae	0.002744	1
Cucurbit leaf crumple virus	Viruses; Geminiviridae	0.005232	1
'Cynodondactylon' phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.483935	1
Dalechampia chlorotic mosaic virus	Viruses; Geminiviridae	0.005214	1
Datura leaf curl virus	Viruses; Geminiviridae	0.002782	1
Datura leaf distortion virus	Viruses; Geminiviridae	0.005163	1
Deinbollia mosaic virus	Viruses; Geminiviridae	0.005461	1
Desmodium leaf distortion virus	Viruses; Geminiviridae	0.005083	1
Desmodium mottle virus	Viruses; Geminiviridae	0.00548	1
Dicliptera yellow mottle virus	Viruses; Geminiviridae	0.005204	2
Digitariaciliaris striate mosaic virus	Viruses; Geminiviridae	0.002816	2
Digitariadidactyla striate mosaic virus	Viruses; Geminiviridae	0.002762	1
Digitaria streak virus	Viruses; Geminiviridae	0.002701	1
Digitaria streak virus	Viruses; Geminiviridae	0.002701	1
Dolichos yellow mosaic virus	Viruses; Geminiviridae	0.005494	2
Dragonfly-associated mastrevirus	Viruses; Geminiviridae	0.00265	2
Duranta leaf curl virus	Viruses; Geminiviridae	0.002759	1
East African cassava mosaic Cameroon virus	Viruses; Geminiviridae	0.005543	1
East African cassava mosaic Kenya virus	Viruses; Geminiviridae	0.005573	1
East African cassava mosaic Malawi virus	Viruses; Geminiviridae	0.005558	2
East African cassava mosaic virus	Viruses; Geminiviridae	0.005576	4
East African cassava mosaic Zanzibar virus	Viruses; Geminiviridae	0.005548	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
'Echinacea purpurea' witches'-broom phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.545427	1
Eclipta yellow vein virus	Viruses; Geminiviridae	0.002748	2
Emilia sonchifolia yellow vein Thailand virus	Viruses; Geminiviridae	0.002746	1
Emilia yellow vein virus-[Fz1]	Viruses; Geminiviridae	0.002725	1
Eragrostiscurvula streak virus	Viruses; Geminiviridae	0.002754	2
Eragrostis minor streak virus	Viruses; Geminiviridae	0.002689	1
Eragrostis streak virus	Viruses; Geminiviridae	0.002746	1
Erectites yellow mosaic virus	Viruses; Geminiviridae	0.002751	1
Erwinia tracheiphila	Bacteria; Proteobacteria; Gammaproteobacteria	4.71727	3
Eupatorium yellow vein mosaic virus	Viruses; Geminiviridae	0.002778	1
Eupatorium yellow vein virus	Viruses; Geminiviridae	0.002767	5
Euphorbia caput-medusae latent virus	Viruses; Geminiviridae	0.002683	2
Euphorbia leaf curl Guangxi virus	Viruses; Geminiviridae	0.002747	1
Euphorbia leaf curl virus	Viruses; Geminiviridae	0.002746	1
Euphorbia mosaic Peru virus	Viruses; Geminiviridae	0.0026	1
Euphorbia mosaic virus	Viruses; Geminiviridae	0.005215	1
Euphorbia yellow leaf curl virus	Viruses; Geminiviridae	0.002731	1
Euphorbia yellow mosaic virus	Viruses; Geminiviridae	0.005187	2
Exomismicrophylla associated virus	Viruses; Geminiviridae	0.002974	1
French bean leaf curl virus	Viruses; Geminiviridae	0.002741	1
French bean severe leaf curl virus	Viruses; Geminiviridae	0.002771	1
Fusarium oxysporum	Eukaryota; Fungi; Ascomycetes	61.3869	129
Golovinomyces cichoracearum	Eukaryota; Fungi; Ascomycetes	65.8869	3
Gossypium darwinii symptomless virus	Viruses; Geminiviridae	0.00274	1
Gossypium punctatum mild leaf curl virus	Viruses; Geminiviridae	0.005462	1
Grapevine red blotch virus	Viruses; Geminiviridae	0.003206	2
Hedyotisuncinella yellow mosaic virus	Viruses; Geminiviridae	0.002749	1
Hemidesmus yellow mosaic virus	Viruses; Geminiviridae	0.002825	1
Hemileiavastatrix	Eukaryota; Fungi; Basidiomycetes	543.605	2
Hollyhock leaf crumple virus	Viruses; Geminiviridae	0.002755	1
Hollyhock leaf curl virus	Viruses; Geminiviridae	0.002748	1
Hollyhock yellow vein mosaic Islamabad virus	Viruses; Geminiviridae	0.002741	2
Hollyhock yellow vein mosaic virus	Viruses; Geminiviridae	0.00275	2
Honeysuckle yellow vein Kagoshima virus	Viruses; Geminiviridae	0.002762	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Honeysuckle yellow vein mosaic virus	Viruses; Geminiviridae	0.002759	2
Honeysuckle yellow vein virus	Viruses; Geminiviridae	0.002784	15
Horsegram yellow mosaic virus	Viruses; Geminiviridae	0.005405	1
Horseradish curly top virus	Viruses; Geminiviridae	0.00308	1
Indian cassava mosaic virus	Viruses; Geminiviridae	0.00546	4
Ipomoea yellow vein virus	Viruses; Geminiviridae	0.002791	1
Italian clover phyllody phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.597245	1
Jacquemontia mosaic Yucatan virus	Viruses; Geminiviridae	0.005193	1
Jacquemontia yellow mosaic virus	Viruses; Geminiviridae	0.005189	1
Jacquemontia yellow vein virus	Viruses; Geminiviridae	0.002585	1
Jatropha leaf crumple virus	Viruses; Geminiviridae	0.002735	1
Jatropha leaf curl Gujarat virus	Viruses; Geminiviridae	0.002758	1
Jatropha leaf curl virus	Viruses; Geminiviridae	0.002844	2
Jatropha leaf yellow mosaic Katarniaghat virus	Viruses; Geminiviridae	0.002744	1
Jatropha mosaic India virus	Viruses; Geminiviridae	0.00274	1
Jatropha mosaic Nigeria virus	Viruses; Geminiviridae	0.002781	1
Jatropha mosaic virus	Viruses; Geminiviridae	0.005198	1
Jatropha yellow mosaic virus	Viruses; Geminiviridae	0.002757	1
Kenaf leaf curl virus-[India: Bahraich:2007]	Viruses; Geminiviridae	0.00274	1
Kudzu mosaic virus	Viruses; Geminiviridae	0.005403	1
Leonurus mosaic virus	Viruses; Geminiviridae	0.002652	1
Linderniaanagallis yellow vein virus	Viruses; Geminiviridae	0.00274	1
Lisianthus enation leaf curl virus	Viruses; Geminiviridae	0.002759	1
Ludwigia yellow vein Vietnam virus	Viruses; Geminiviridae	0.002751	1
Ludwigia yellow vein virus	Viruses; Geminiviridae	0.002758	1
Luffa yellow mosaic virus	Viruses; Geminiviridae	0.005455	1
Lycianthes yellow mosaic virus	Viruses; Geminiviridae	0.005456	1
Macroptilium bright mosaic virus	Viruses; Geminiviridae	0.002636	1
Macroptilium common mosaic virus	Viruses; Geminiviridae	0.00523	1
Macroptilium golden mosaic virus	Viruses; Geminiviridae	0.005158	1
Macroptilium golden yellow mosaic virus	Viruses; Geminiviridae	0.005224	1
Macroptilium mosaic Puerto Rico virus	Viruses; Geminiviridae	0.005186	1
Macroptilium yellow mosaic Florida virus	Viruses; Geminiviridae	0.005247	1
Macroptilium yellow mosaic virus	Viruses; Geminiviridae	0.005223	2
Macroptilium yellow net virus	Viruses; Geminiviridae	0.005197	1
Macroptilium yellow spot virus	Viruses; Geminiviridae	0.00266	1
Macroptilium yellow vein virus	Viruses; Geminiviridae	0.002656	1
Maize bushy stunt phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.576118	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Maize streak Reunion virus	Viruses; Geminiviridae	0.002882	1
Maize streak Reunion virus	Viruses; Geminiviridae	0.002882	1
Maize streak virus	Viruses; Geminiviridae	0.002701	12
Maize striate mosaic virus	Viruses; Geminiviridae	0.002746	2
Malachra yellow mosaic virus	Viruses; Geminiviridae	0.002739	1
Malvastrum bright yellow mosaic virus	Viruses; Geminiviridae	0.005213	1
Malvastrum leaf curl Guangdong virus	Viruses; Geminiviridae	0.002767	1
Malvastrum leaf curl Philippines virus	Viruses; Geminiviridae	0.002742	1
Malvastrum leaf curl virus	Viruses; Geminiviridae	0.002745	1
Malvastrum yellow mosaic Helshire virus	Viruses; Geminiviridae	0.002609	1
Malvastrum yellow mosaic Jamaica virus	Viruses; Geminiviridae	0.005192	1
Malvastrum yellow mosaic virus	Viruses; Geminiviridae	0.002728	1
Malvastrum yellow vein Baoshan virus	Viruses; Geminiviridae	0.002745	2
Malvastrum yellow vein Cambodia virus	Viruses; Geminiviridae	0.002737	1
Malvastrum yellow vein Changa Manga virus	Viruses; Geminiviridae	0.002754	1
Malvastrum yellow vein Honghe virus	Viruses; Geminiviridae	0.00274	1
Malvastrum yellow vein virus	Viruses; Geminiviridae	0.002731	1
Malvastrum yellow vein Yunnan virus	Viruses; Geminiviridae	0.002747	1
Melochia mosaic virus	Viruses; Geminiviridae	0.005213	1
Melochia yellow mosaic virus	Viruses; Geminiviridae	0.005288	1
Meloidogyne arenaria	Eukaryota; Animals; Roundworms	284.032	3
Meloidogyne enterolobii	Eukaryota; Animals; Roundworms	162.967	1
Meloidogyne floridensis	Eukaryota; Animals; Roundworms	74.846	2
Meloidogyne graminicola	Eukaryota; Animals; Roundworms	38.185	1
Meloidogyne hapla	Eukaryota; Animals; Roundworms	53.013	1
Meloidogyne incognita	Eukaryota; Animals; Roundworms	183.532	3
Meloidogyne javanica	Eukaryota; Animals; Roundworms	150.345	2
Melon chlorotic leaf curl virus	Viruses; Geminiviridae	0.005325	3
Merremia mosaic Puerto Rico virus	Viruses; Geminiviridae	0.005225	1
Merremia mosaic virus	Viruses; Geminiviridae	0.005085	2
Mesta yellow vein mosaic Bahraich virus	Viruses; Geminiviridae	0.002737	1
Mesta yellow vein mosaic virus	Viruses; Geminiviridae	0.002752	2
Milkweed yellows phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.583806	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Mimosa yellow leaf curl virus	Viruses; Geminiviridae	0.002757	1
Mirabilis leaf curl virus	Viruses; Geminiviridae	0.002778	1
Miscanthus streak virus	Viruses; Geminiviridae	0.002672	1
Miscanthus streak virus	Viruses; Geminiviridae	0.002672	1
Mungbean yellow mosaic India virus	Viruses; Geminiviridae	0.005361	1
Mungbean yellow mosaic virus	Viruses; Geminiviridae	0.005398	1
New Jersey aster yellows phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.652092	1
Oat dwarf virus	Viruses; Geminiviridae	0.00274	1
Oat dwarf virus	Viruses; Geminiviridae	0.00274	1
Okra enation leaf curl virus	Viruses; Geminiviridae	0.002738	1
Okra enation leaf curl virus [India: Munthal EL37:2006]	Viruses; Geminiviridae	0.002724	1
Okra leaf curl Cameroon virus	Viruses; Geminiviridae	0.002764	1
Okra leaf curl India virus [India:Sonipat EL14A:2006]	Viruses; Geminiviridae	0.002723	1
Okra leaf curl Oman virus	Viruses; Geminiviridae	0.002788	1
Okra leaf curl virus	Viruses; Geminiviridae	0.002386	1
Okra mottle virus	Viruses; Geminiviridae	0.005313	1
Okra yellow crinkle virus	Viruses; Geminiviridae	0.002795	3
Okra yellow mosaic Mexico virus	Viruses; Geminiviridae	0.005194	1
Onion yellows phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.853092	1
Ophiognomoniaclavigignenti-juglandacearum	Eukaryota; Fungi; Ascomycetes	52.5149	3
Oxalis yellow vein virus	Viruses; Geminiviridae	0.002661	1
Panicum streak virus	Viruses; Geminiviridae	0.002736	9
Papaya leaf crumple virus-Panipat 8 [India:Panipat:Papaya:2008]	Viruses; Geminiviridae	0.002736	1
Papaya leaf curl China virus	Viruses; Geminiviridae	0.002751	5
Papaya leaf curl Guandong virus	Viruses; Geminiviridae	0.002764	2
Papaya leaf curl virus	Viruses; Geminiviridae	0.002769	12
Paspalum dilatatum striate mosaic virus	Viruses; Geminiviridae	0.002806	1
Paspalum striate mosaic virus	Viruses; Geminiviridae	0.002816	2
Passionfruit leaf distortion virus	Viruses; Geminiviridae	0.005172	1
Passionfruit severe leaf distortion virus	Viruses; Geminiviridae	0.005316	1
Pavonia mosaic virus	Viruses; Geminiviridae	0.005367	1
Pavonia yellow mosaic virus	Viruses; Geminiviridae	0.005378	1
Pea leaf distortion virus	Viruses; Geminiviridae	0.002738	1
Peanut witches'-broom phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.566694	1
Pectobacterium actinidiae	Bacteria; Proteobacteria; Gammaproteobacteria	4.92217	3

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Pectobacterium aquaticum	Bacteria; Proteobacteria; Gammaproteobacteria	4.46724	6
Pectobacterium atrosepticum	Bacteria; Proteobacteria; Gammaproteobacteria	5.10459	11
Pectobacterium betavasculorum	Bacteria; Proteobacteria; Gammaproteobacteria	4.68521	2
Pectobacterium brasiliense	Bacteria; Proteobacteria; Gammaproteobacteria	5.02631	28
Pectobacterium carotovorum	Bacteria; Proteobacteria; Gammaproteobacteria	4.86291	33
Pectobacterium fontis	Bacteria; Proteobacteria; Gammaproteobacteria	4.15156	1
Pectobacterium odoriferum	Bacteria; Proteobacteria; Gammaproteobacteria	5.4726	15
Pectobacterium parmentieri	Bacteria; Proteobacteria; Gammaproteobacteria	5.2273	19
Pectobacterium peruviense	Bacteria; Proteobacteria; Gammaproteobacteria	4.87102	5
Pectobacterium polaris	Bacteria; Proteobacteria; Gammaproteobacteria	5.00842	7
Pectobacterium polonicum	Bacteria; Proteobacteria; Gammaproteobacteria	4.83613	1
Pectobacterium punjabense	Bacteria; Proteobacteria; Gammaproteobacteria	4.73253	1
Pectobacterium versatile	Bacteria; Proteobacteria; Gammaproteobacteria	4.94937	1
Pectobacterium wasabiae	Bacteria; Proteobacteria; Gammaproteobacteria	5.1493	4
Pectobacterium zantedeschiae	Bacteria; Proteobacteria; Gammaproteobacteria	5.094	3
Pedilanthus leaf curl virus	Viruses; Geminiviridae	0.002764	3
Pepper golden mosaic virus	Viruses; Geminiviridae	0.005208	3

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Pepper huasteco yellow vein virus	Viruses; Geminiviridae	0.00522	1
Pepper leaf curl Bangladesh virus	Viruses; Geminiviridae	0.002754	3
Pepper leaf curl Lahore virus	Viruses; Geminiviridae	0.00274	1
Pepper leaf curl Lahore Virus-[Pakistan:Lahore1:2004]	Viruses; Geminiviridae	0.002747	1
Pepper leaf curl virus	Viruses; Geminiviridae	0.00276	3
Pepper leaf curl Yunnan virus-[YN323]	Viruses; Geminiviridae	0.002747	1
Pepper leafroll virus	Viruses; Geminiviridae	0.002568	1
Pepper yellow dwarf virus – Mexico	Viruses; Geminiviridae	0.002971	1
Pepper yellow dwarf virus – New Mexico	Viruses; Geminiviridae	0.002959	1
Pepper yellow leaf curl Indonesia virus	Viruses; Geminiviridae	0.005476	1
Pepper yellow leaf curl Thailand virus	Viruses; Geminiviridae	0.005474	2
Pepper yellow leaf curl virus	Viruses; Geminiviridae	0.006028	2
Pepper yellow leaf curl virus PSSWS-14	Viruses; Geminiviridae	0.002748	1
Pepper yellow vein Mali virus	Viruses; Geminiviridae	0.002786	1
Periwinkle leaf yellowing phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.824596	1
Phytophthora infestans	Eukaryota; Protists; Other Protists	228.544	2
Plantago lanceolata latent virus	Viruses; Geminiviridae	0.002832	1
Plasmodiophora brassicae	Eukaryota; Protists; Other Protists	24.5596	7
Poinsettia branch-inducing phytoplasma	Bacteria; Terrabacteria group; Tenericutes	0.63144	1
Potato yellow mosaic Panama virus	Viruses; Geminiviridae	0.005126	1
Potato yellow mosaic virus	Viruses; Geminiviridae	0.00514	4
Pouzolzia golden mosaic virus	Viruses; Geminiviridae	0.002725	2
Pouzolzia mosaic Guangdong virus	Viruses; Geminiviridae	0.002739	1
Premna leaf curl virus	Viruses; Geminiviridae	0.002753	1
Prunus latent virus	Viruses; Geminiviridae	0.003174	1
Pseudomonas syringae	Bacteria; Proteobacteria; Gammaproteobacteria	6.0937	376
Pseudoperonospora cubensis	Eukaryota; Protists; Other Protists	64.3328	1
Pumpkin yellow mosaic Malaysia virus	Viruses; Geminiviridae	0.002724	1
Pythium aphanidermatum	Eukaryota; Protists; Other Protists	35.8768	1
Pythium arrhenomanes	Eukaryota; Protists; Other Protists	44.6726	1
Pythium brassicum	Eukaryota; Protists; Other Protists	50.0694	1
Pythium guiyangense	Eukaryota; Protists; Other Protists	110.178	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Pythium insidiosum	Eukaryota; Protists; Other Protists	53.239	12
Pythium irregulare	Eukaryota; Protists; Other Protists	42.9681	2
Pythium iwayamai	Eukaryota; Protists; Other Protists	43.1992	1
Pythium oligandrum	Eukaryota; Protists; Other Protists	41.9689	3
Pythium periplocum	Eukaryota; Protists; Other Protists	35.8865	1
Pythium splendens	Eukaryota; Protists; Other Protists	53.361	1
Radish leaf curl virus	Viruses; Geminiviridae	0.002759	2
Ramie mosaic virus	Viruses; Geminiviridae	0.005446	1
Ramie mosaic Yunnan virus	Viruses; Geminiviridae	0.002759	1
Rhynchosia golden mosaic Havana virus-[Cuba:Havana:28:2007]	Viruses; Geminiviridae	0.005151	1
Rhynchosia golden mosaic Sinaloa virus	Viruses; Geminiviridae	0.005103	1
Rhynchosia golden mosaic virus	Viruses; Geminiviridae	0.005174	3
Rhynchosia golden mosaic Yucatan virus	Viruses; Geminiviridae	0.005139	1
Rhynchosia mild mosaic virus	Viruses; Geminiviridae	0.005162	1
Rhynchosia rugose golden mosaic virus	Viruses; Geminiviridae	0.005186	1
Rhynchosia yellow mosaic India virus	Viruses; Geminiviridae	0.005406	1
Rhynchosia yellow mosaic virus	Viruses; Geminiviridae	0.005379	1
Rice latent virus 1	Viruses; Geminiviridae	0.002757	2
Rice latent virus 2	Viruses; Geminiviridae	0.002843	1
Rice orange leaf phytoplasma	Bacteria; Terrabacteriagroup; Tenericutes	0.599264	1
Rose leaf curl virus	Viruses; Geminiviridae	0.002741	1
Saccharum streak virus	Viruses; Geminiviridae	0.002744	1
Sauropus leaf curl virus	Viruses; Geminiviridae	0.002762	1
Senecio yellow mosaic virus	Viruses; Geminiviridae	0.002746	1
Senna leaf curl virus	Viruses; Geminiviridae	0.002742	1
Sida angular mosaic virus	Viruses; Geminiviridae	0.005349	1
Sida bright yellow mosaic virus	Viruses; Geminiviridae	0.005348	1
Sida chlorotic mottle virus	Viruses; Geminiviridae	0.002601	1
Sida chlorotic vein virus	Viruses; Geminiviridae	0.005145	1
Sidaciliaris golden mosaic virus	Viruses; Geminiviridae	0.002638	1
Sida common mosaic virus	Viruses; Geminiviridae	0.002687	1
Sida golden mosaic Braco virus	Viruses; Geminiviridae	0.0026	1
Sida golden mosaic Brazil virus	Viruses; Geminiviridae	0.002659	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Sida golden mosaic Buckup virus-[Jamaica:St. Elizabeth:2004]	Viruses; Geminiviridae	0.005199	1
Sida golden mosaic Costa Rica virus	Viruses; Geminiviridae	0.005192	1
Sida golden mosaic Florida virus	Viruses; Geminiviridae	0.005186	2
Sida golden mosaic Honduras virus	Viruses; Geminiviridae	0.005192	1
Sida golden mosaic Lara virus	Viruses; Geminiviridae	0.002633	1
Sida golden mosaic virus	Viruses; Geminiviridae	0.005227	1
Sida golden mottle virus	Viruses; Geminiviridae	0.005184	1
Sida golden yellow spot virus	Viruses; Geminiviridae	0.002813	1
Sida golden yellow vein virus	Viruses; Geminiviridae	0.002603	1
Sida golden yellow vein virus-[Jamaica:Liguanea2:2008]	Viruses; Geminiviridae	0.00515	1
Sida leaf curl virus	Viruses; Geminiviridae	0.002757	1
Sida micrantha mosaic virus	Viruses; Geminiviridae	0.005331	4
Sida mosaic Alagoas virus	Viruses; Geminiviridae	0.005292	1
Sida mosaic Bolivia virus 1	Viruses; Geminiviridae	0.005348	1
Sida mosaic Bolivia virus 2	Viruses; Geminiviridae	0.005316	1
Sida mosaic Sinaloa virus	Viruses; Geminiviridae	0.005182	1
Sida mottle Alagoas virus	Viruses; Geminiviridae	0.002649	1
Sida mottle virus	Viruses; Geminiviridae	0.002668	1
Sida yellow blotch virus	Viruses; Geminiviridae	0.002664	1
Sida yellow leaf curl virus	Viruses; Geminiviridae	0.002664	1
Sida yellow mosaic Alagoas virus	Viruses; Geminiviridae	0.00269	1
Sida yellow mosaic China virus	Viruses; Geminiviridae	0.002751	1
Sida yellow mosaic virus	Viruses; Geminiviridae	0.002661	1
Sida yellow mosaic Yucatan virus	Viruses; Geminiviridae	0.005197	1
Sida yellow mottle virus	Viruses; Geminiviridae	0.005222	1
Sida yellow net virus	Viruses; Geminiviridae	0.002676	1
Sida yellow vein Madurai virus	Viruses; Geminiviridae	0.002753	1
Sida yellow vein virus	Viruses; Geminiviridae	0.005205	1
Sida strum golden leaf spot virus	Viruses; Geminiviridae	0.002666	1
Siegesbeckia yellow vein Guangxi virus	Viruses; Geminiviridae	0.002784	1
Siegesbeckia yellow vein virus	Viruses; Geminiviridae	0.002768	1
Solanum mosaic Bolivia virus	Viruses; Geminiviridae	0.005196	1
South African cassava mosaic virus	Viruses; Geminiviridae	0.00556	1
Soybean blistering mosaic virus	Viruses; Geminiviridae	0.002605	1
Soybean chlorotic blotch virus	Viruses; Geminiviridae	0.005355	1
Soybean chlorotic spot virus	Viruses; Geminiviridae	0.005208	1
Soybean mild mottle virus	Viruses; Geminiviridae	0.002768	1
Spilanthes yellow vein virus	Viruses; Geminiviridae	0.002761	1
Spinach curly top Arizona virus	Viruses; Geminiviridae	0.00286	1
Spinach severe curly top virus	Viruses; Geminiviridae	0.003065	1
Spinach yellow vein Sikar virus	Viruses; Geminiviridae	0.002753	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Sporisorium scitamineum	Eukaryota; Fungi; Basidiomycetes	20.0676	4
Sporobolus striate mosaic virus 1	Viruses; Geminiviridae	0.002789	1
Sporobolus striate mosaic virus 1	Viruses; Geminiviridae	0.002789	1
Sporobolus striate mosaic virus 2	Viruses; Geminiviridae	0.002716	1
Squash leaf curl China virus	Viruses; Geminiviridae	0.002756	3
Squash leaf curl China virus – [B]	Viruses; Geminiviridae	0.005455	1
Squash leaf curl Philippines virus	Viruses; Geminiviridae	0.005444	1
Squash leaf curl virus	Viruses; Geminiviridae	0.005241	1
Squash leaf curl Yunnan virus	Viruses; Geminiviridae	0.002714	1
Squash mild leaf curl virus	Viruses; Geminiviridae	0.00519	1
Sri Lankan cassava mosaic virus	Viruses; Geminiviridae	0.005466	2
Stachytarpheta leaf curl virus	Viruses; Geminiviridae	0.002749	1
Streptomyces scabiei	Bacteria; Terrabacteria group; Actinobacteria	10.1487	17
Sugarcane chlorotic streak virus	Viruses; Geminiviridae	0.002757	1
Sugarcane streak Egypt virus	Viruses; Geminiviridae	0.002706	1
Sugarcane streak Reunion virus	Viruses; Geminiviridae	0.00274	2
Sugarcane streak virus	Viruses; Geminiviridae	0.002758	2
Sugarcane striate virus	Viruses; Geminiviridae	0.002749	2
Sugarcane white streak virus	Viruses; Geminiviridae	0.00283	1
Sunn hemp leaf distortion virus	Viruses; Geminiviridae	0.002774	1
Sweet potato golden vein associated virus	Viruses; Geminiviridae	0.002824	1
Sweet potato golden vein Korea virus	Viruses; Geminiviridae	0.002807	1
Sweet potato leaf curl Bengal virus	Viruses; Geminiviridae	0.002823	1
Sweet potato leaf curl Canary virus	Viruses; Geminiviridae	0.002837	2
Sweet potato leaf curl China virus	Viruses; Geminiviridae	0.002771	1
Sweet potato leaf curl Georgia virus	Viruses; Geminiviridae	0.002773	1
Sweet potato leaf curl Guangxi virus	Viruses; Geminiviridae	0.002831	1
Sweet potato leaf curl Henan virus	Viruses; Geminiviridae	0.002785	2
Sweet potato leaf curl Lanzarote virus	Viruses; Geminiviridae	0.002814	1
Sweet potato leaf curl Sao Paulo virus	Viruses; Geminiviridae	0.002782	1
Sweet potato leaf curl Shanghai virus	Viruses; Geminiviridae	0.002834	1
Sweet potato leaf curl Sichuan virus 1	Viruses; Geminiviridae	0.002764	1
Sweet potato leaf curl Sichuan virus 2	Viruses; Geminiviridae	0.002786	1
Sweet potato leaf curl South Carolina virus	Viruses; Geminiviridae	0.002782	1
Sweet potato leaf curl Spain virus	Viruses; Geminiviridae	0.00278	1
Sweet potato leaf curl Uganda virus-[Uganda:Kampala:2008]	Viruses; Geminiviridae	0.002799	1
Sweet potato leaf curl virus	Viruses; Geminiviridae	0.002844	15
Sweet potato mosaic virus	Viruses; Geminiviridae	0.002803	2
Sweet potato symptomless virus 1	Viruses; Geminiviridae	0.002886	2

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Sweet potato symptomless virus 1	Viruses; Geminiviridae	0.002886	2
Switchgrass mosaic-associated virus 1	Viruses; Geminiviridae	0.002739	1
Synedrella leaf curl virus	Viruses; Geminiviridae	0.002749	1
Synedrella yellow vein clearing virus	Viruses; Geminiviridae	0.002751	1
Telfairia golden mosaic virus	Viruses; Geminiviridae	0.002742	1
Tilletia caries	Eukaryota; Fungi; Basidiomycetes	29.5409	2
Tobacco curly shoot virus	Viruses; Geminiviridae	0.002743	1
Tobacco leaf curl Comoros virus	Viruses; Geminiviridae	0.002755	1
Tobacco leaf curl Cuba virus	Viruses; Geminiviridae	0.005176	2
Tobacco leaf curl Japan virus	Viruses; Geminiviridae	0.002761	1
Tobacco leaf curl Pusa virus	Viruses; Geminiviridae	0.002707	1
Tobacco leaf curl Thailand virus	Viruses; Geminiviridae	0.002752	1
Tobacco leaf curl virus	Viruses; Geminiviridae	0.002762	1
Tobacco leaf curl Yunnan virus	Viruses; Geminiviridae	0.00275	1
Tobacco leaf curl Zimbabwe virus	Viruses; Geminiviridae	0.002767	1
Tobacco leaf rugose virus	Viruses; Geminiviridae	0.002622	1
Tobacco mosaic virus	Viruses; Virgaviridae	0.006395	1
Tobacco mottle leaf curl virus	Viruses; Geminiviridae	0.002634	1
Tobacco yellow crinkle virus	Viruses; Geminiviridae	0.005154	1
Tobacco yellow dwarf virus	Viruses; Geminiviridae	0.00258	1
Tobacco yellow dwarf virus	Viruses; Geminiviridae	0.00258	1
Tomato bright yellow mosaic virus	Viruses; Geminiviridae	0.002619	1
Tomato bright yellow mottle virus	Viruses; Geminiviridae	0.002639	1
Tomato chino La Paz virus	Viruses; Geminiviridae	0.002632	3
Tomato chlorotic leaf distortion virus-[Venezuela:Zulia:2004]	Viruses; Geminiviridae	0.00523	1
Tomato chlorotic mottle Guyane virus	Viruses; Geminiviridae	0.005234	1
Tomato chlorotic mottle virus	Viruses; Geminiviridae	0.005195	3
Tomato common mosaic virus	Viruses; Geminiviridae	0.005058	1
Tomato curly stunt virus	Viruses; Geminiviridae	0.002766	1
Tomato dwarf leaf virus	Viruses; Geminiviridae	0.005034	1
Tomato enation leaf curl virus	Viruses; Geminiviridae	0.002756	1
Tomato golden leaf distortion virus	Viruses; Geminiviridae	0.00263	1
Tomato golden leaf spot virus	Viruses; Geminiviridae	0.002669	1
Tomato golden mosaic virus	Viruses; Geminiviridae	0.005096	1
Tomato golden mottle virus	Viruses; Geminiviridae	0.005172	1
Tomato golden vein virus	Viruses; Geminiviridae	0.005095	1
Tomato interveinal chlorosis virus	Viruses; Geminiviridae	0.002617	1
Tomato latent virus	Viruses; Geminiviridae	0.002746	1
Tomato leaf curl Anjouan virus	Viruses; Geminiviridae	0.002781	1
Tomato leaf curl Arusha virus	Viruses; Geminiviridae	0.002766	2
Tomato leaf curl Bangalore virus	Viruses; Geminiviridae	0.002759	5

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Tomato leaf curl Bangladesh virus	Viruses; Geminiviridae	0.002761	1
Tomato leaf curl Barka virus	Viruses; Geminiviridae	0.002753	1
Tomato leaf curl Burkina Faso virus	Viruses; Geminiviridae	0.002784	1
Tomato leaf curl Cameroon virus	Viruses; Geminiviridae	0.002808	1
Tomato leaf curl Cebu virus	Viruses; Geminiviridae	0.002723	1
Tomato leaf curl China virus	Viruses; Geminiviridae	0.002738	4
Tomato leaf curl China virus – OX2	Viruses; Geminiviridae	0.002744	1
Tomato leaf curl Comoros virus	Viruses; Geminiviridae	0.002765	1
Tomato leaf curl Cotabato virus	Viruses; Geminiviridae	0.00275	1
Tomato leaf curl Diana virus	Viruses; Geminiviridae	0.002745	1
Tomato leaf curl Gandhinagar virus	Viruses; Geminiviridae	0.00276	1
Tomato leaf curl Ghana virus	Viruses; Geminiviridae	0.002803	2
Tomato leaf curl Guangdong virus	Viruses; Geminiviridae	0.002744	1
Tomato leaf curl Guangxi virus	Viruses; Geminiviridae	0.002752	1
Tomato leaf curl Gujarat virus	Viruses; Geminiviridae	0.005445	1
Tomato leaf curl Hainan virus	Viruses; Geminiviridae	0.002756	3
Tomato leaf curl Hanoi virus	Viruses; Geminiviridae	0.00274	1
Tomato leaf curl Iran virus	Viruses; Geminiviridae	0.002763	1
Tomato leaf curl Java virus	Viruses; Geminiviridae	0.002752	2
Tomato leaf curl Joydebpur virus	Viruses; Geminiviridae	0.002798	2
Tomato leaf curl Karnataka virus	Viruses; Geminiviridae	0.002772	7
Tomato leaf curl Kerala virus	Viruses; Geminiviridae	0.002767	2
Tomato leaf curl Kumasi virus	Viruses; Geminiviridae	0.002794	1
Tomato leaf curl Laos virus	Viruses; Geminiviridae	0.002748	1
Tomato leaf curl Liwa virus	Viruses; Geminiviridae	0.002761	1
Tomato leaf curl Madagascar virus	Viruses; Geminiviridae	0.002775	1
Tomato leaf curl Madagascar virus-Menabe [Madagascar: Morondova:2001]	Viruses; Geminiviridae	0.002777	1
Tomato leaf curl Malaysia virus	Viruses; Geminiviridae	0.002754	1
Tomato leaf curl Mali virus	Viruses; Geminiviridae	0.002773	1
Tomato leaf curl Mayotte virus	Viruses; Geminiviridae	0.002768	1
Tomato leaf curl Mindanao virus	Viruses; Geminiviridae	0.002761	1
Tomato leaf curl Moheli virus	Viruses; Geminiviridae	0.002756	1
Tomato leaf curl Namakely virus	Viruses; Geminiviridae	0.002772	2
Tomato leaf curl New Delhi virus	Viruses; Geminiviridae	0.005435	8
Tomato leaf curl New Delhi virus 2	Viruses; Geminiviridae	0.002735	1
Tomato leaf curl New Delhi virus 4	Viruses; Geminiviridae	0.002739	1
Tomato leaf curl Nigeria virus-[Nigeria:2006]	Viruses; Geminiviridae	0.002784	1
Tomato leaf curl Oman virus	Viruses; Geminiviridae	0.002763	1
Tomato leaf curl Palampur virus	Viruses; Geminiviridae	0.005481	4

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Tomato leaf curl Patna virus	Viruses; Geminiviridae	0.002752	1
Tomato leaf curl Philippines virus	Viruses; Geminiviridae	0.002755	3
Tomato leaf curl Pune virus	Viruses; Geminiviridae	0.002756	1
Tomato leaf curl purple vein virus	Viruses; Geminiviridae	0.002629	1
Tomato leaf curl Rajasthan virus	Viruses; Geminiviridae	0.002758	1
Tomato leaf curl Ranchi virus	Viruses; Geminiviridae	0.002762	1
Tomato leaf curl Seychelles virus	Viruses; Geminiviridae	0.002742	1
Tomato leaf curl Sinaloa virus	Viruses; Geminiviridae	0.005173	1
Tomato leaf curl Sri Lanka virus	Viruses; Geminiviridae	0.002756	1
Tomato leaf curl Sudan virus	Viruses; Geminiviridae	0.002782	4
Tomato leaf curl Sulawesi virus	Viruses; Geminiviridae	0.002751	1
Tomato leaf curl Taiwan virus	Viruses; Geminiviridae	0.002743	4
Tomato leaf curl Toliara virus	Viruses; Geminiviridae	0.002764	1
Tomato leaf curl Uganda virus	Viruses; Geminiviridae	0.002747	1
Tomato leaf curl Vietnam virus	Viruses; Geminiviridae	0.002745	1
Tomato leaf curl virus	Viruses; Geminiviridae	0.002766	5
Tomato leaf deformation virus	Viruses; Geminiviridae	0.002591	1
Tomato leaf distortion virus	Viruses; Geminiviridae	0.002645	1
Tomato mild mosaic virus	Viruses; Geminiviridae	0.005371	1
Tomato mild yellow leaf curl Aragua virus	Viruses; Geminiviridae	0.005168	1
Tomato mosaic Havana virus	Viruses; Geminiviridae	0.005206	1
Tomato mosaic Trujillo virus	Viruses; Geminiviridae	0.002637	1
Tomato mottle leaf curl virus	Viruses; Geminiviridae	0.005229	2
Tomato mottle Taino virus	Viruses; Geminiviridae	0.005159	1
Tomato mottle virus	Viruses; Geminiviridae	0.005145	1
Tomato mottle wrinkle virus	Viruses; Geminiviridae	0.005124	1
Tomato pseudo-curly top virus	Viruses; Geminiviridae	0.002861	1
Tomato rugose mosaic virus	Viruses; Geminiviridae	0.005194	1
Tomato rugose yellow leaf curl virus	Viruses; Geminiviridae	0.005305	1
Tomato severe leaf curl virus	Viruses; Geminiviridae	0.002755	4
Tomato severe rugose virus	Viruses; Geminiviridae	0.005164	1
Tomato spotted wilt tospovirus	Viruses; Tospoviridae	0.016634	1
Tomato yellow leaf curl Axarquia virus	Viruses; Geminiviridae	0.002763	1
Tomato yellow leaf curl China virus	Viruses; Geminiviridae	0.002741	7
Tomato yellow leaf curl Guangdong virus	Viruses; Geminiviridae	0.002744	1
Tomato yellow leaf curl Indonesia virus-[Lembang]	Viruses; Geminiviridae	0.002762	1
Tomato yellow leaf curl Kanchanaburi virus	Viruses; Geminiviridae	0.005504	1
Tomato yellow leaf curl Malaga virus	Viruses; Geminiviridae	0.002782	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Tomato yellow leaf curl Mali virus	Viruses; Geminiviridae	0.002796	3
Tomato yellow leaf curl Sardinia virus	Viruses; Geminiviridae	0.002773	1
Tomato yellow leaf curl Saudi virus	Viruses; Geminiviridae	0.002775	1
Tomato yellow leaf curl Shuangbai virus – [Y4536]	Viruses; Geminiviridae	0.002748	1
Tomato yellow leaf curl Thailand virus	Viruses; Geminiviridae	0.005488	5
Tomato yellow leaf curl Vietnam virus	Viruses; Geminiviridae	0.002745	1
Tomato yellow leaf curl virus	Viruses; Geminiviridae	0.00279	6
Tomato yellow leaf curl Yunnan virus	Viruses; Geminiviridae	0.002754	1
Tomato yellow leaf distortion virus	Viruses; Geminiviridae	0.005219	1
Tomato yellow margin leaf curl virus	Viruses; Geminiviridae	0.005118	1
Tomato yellow mottle virus	Viruses; Geminiviridae	0.005121	1
Tomato yellow spot virus	Viruses; Geminiviridae	0.0053	1
Tomato yellow vein streak virus	Viruses; Geminiviridae	0.00513	1
Triumfetta yellow mosaic virus	Viruses; Geminiviridae	0.005277	1
Turnip curly top virus	Viruses; Geminiviridae	0.002981	4
Turnip curly top virus	Viruses; Geminiviridae	0.002981	4
Turnip leaf roll virus	Viruses; Geminiviridae	0.002965	1
Turnip leaf roll virus	Viruses; Geminiviridae	0.002965	1
TYLCAxV-Sic1-[IT:Sic2/2:04]	Viruses; Geminiviridae	0.002771	1
Urochloa streak virus	Viruses; Geminiviridae	0.002736	1
Urochloa streak virus	Viruses; Geminiviridae	0.002736	1
Ustilago maydis	Eukaryota; Fungi; Basidiomycetes	19.6644	7
Vaccinium witches'-broom phytoplasma	Bacteria; Terrabacteriagroup; Tenericutes	0.647754	1
Velvet bean golden mosaic virus	Viruses; Geminiviridae	0.002767	1
Velvet bean severe mosaic virus	Viruses; Geminiviridae	0.00539	1
Venturia inaequalis	Eukaryota; Fungi; Ascomycetes	72.7916	85
Vernonia crinkle virus	Viruses; Geminiviridae	0.002791	1
Vernonia yellow vein Fujian virus	Viruses; Geminiviridae	0.002739	1
Vernonia yellow vein virus	Viruses; Geminiviridae	0.002745	1
Verticillium albo-atrum	Eukaryota; Fungi; Ascomycetes	36.4685	1
Verticillium alfalfae	Eukaryota; Fungi; Ascomycetes	32.863	2
Verticillium dahliae	Eukaryota; Fungi; Ascomycetes	33.9003	13
Verticillium isaacii	Eukaryota; Fungi; Ascomycetes	35.6909	1
Verticillium klebahnii	Eukaryota; Fungi; Ascomycetes	36.0824	1

(continued)

Table 32.2 (continued)

Organism name	Organism groups	Size(Mb)	Assemblies
Verticillium longisporum	Eukaryota; Fungi; Ascomycetes	99.1892	2
Verticillium nonalfalfae	Eukaryota; Fungi; Ascomycetes	31.7515	3
Verticillium nubilum	Eukaryota; Fungi; Ascomycetes	37.9116	1
Verticillium tricorpus	Eukaryota; Fungi; Ascomycetes	36.0604	2
Verticillium zaregamsianum	Eukaryota; Fungi; Ascomycetes	37.1319	1
Vigna yellow mosaic virus	Viruses; Geminiviridae	0.002602	1
Vinca leaf curl virus	Viruses; Geminiviridae	0.002776	1
Watermelon chlorotic stunt virus	Viruses; Geminiviridae	0.005498	1
West African Asystasia virus 1	Viruses; Geminiviridae	0.005388	2
West African Asystasia virus 2	Viruses; Geminiviridae	0.002744	1
Wheat blue dwarf phytoplasma	Bacteria; Terrabacteriagroup; Tenericutes	0.611462	1
Wheat dwarf India virus	Viruses; Geminiviridae	0.002783	1
Wheat dwarf virus	Viruses; Geminiviridae	0.00275	6
Wheat dwarf virus	Viruses; Geminiviridae	0.00275	6
Whitefly-associated begomovirus 1	Viruses; Geminiviridae	0.002609	1
Whitefly-associated begomovirus 2	Viruses; Geminiviridae	0.00259	1
Whitefly-associated begomovirus 3	Viruses; Geminiviridae	0.002629	1
Whitefly-associated begomovirus 4	Viruses; Geminiviridae	0.002608	1
Whitefly-associated begomovirus 6	Viruses; Geminiviridae	0.002638	1
Whitefly-associated begomovirus 7	Viruses; Geminiviridae	0.002767	1
Wissadula golden mosaic virus	Viruses; Geminiviridae	0.0052	1
Wissadula yellow mosaic virus	Viruses; Geminiviridae	0.002621	1
Xanthomonas axonopodis	Bacteria; Proteobacteria; Gammaproteobacteria	5.41058	14
Xanthomonas campestris	Bacteria; Proteobacteria; Gammaproteobacteria	5.07619	71

Table 32.3 Plant genome sequence details

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Abrus precatorius	Eukaryota; Plants; Land Plants	GCA_003935025.1	Scaffold	347.23	QYUI01	160	40048
Acer yangbiense	Eukaryota; Plants; Land Plants	GCA_008009225.1	Chromosome	665.888	VAHF01	280	28320
Actinidia chinensis	Eukaryota; Plants; Land Plants	GCA_000467755.1	Contig	604.217	AONS01	26721	0
Actinidia chinensis var. chinensis	Eukaryota; Plants; Land Plants	GCA_003024255.1	Chromosome	553.842	NKQK01	1234	33115
Actinidia eriantha	Eukaryota; Plants; Land Plants	GCA_004150315.1	Chromosome	690.611	QOVS01	1735	0
Aegilops tauschii	Eukaryota; Plants; Land Plants	GCA_000347335.2	Chromosome	4310.35	AOCO02	112210	0
Aegilops tauschii	Eukaryota; Plants; Land Plants	GCA_002105435.1	Chromosome	247.197	LYXL01	1	0
Aegilops tauschii subsp. strangulata	Eukaryota; Plants; Land Plants	GCA_002575655.1	Chromosome	4224.92	NWVB01	109583	0
Aegilops tauschii subsp. tauschii	Eukaryota; Plants; Land Plants	GCA_001957025.1	Contig	4327.32	MCGU01	68538	55713
Aethionema arabicum	Eukaryota; Plants; Land Plants	GCA_000411095.1	Scaffold	192.488	ASZG01	18312	0
Alloteropsis semialata	Eukaryota; Plants; Land Plants	GCA_004135705.1	Chromosome	747.772	QPGU01	688	0
Alnus glutinosa	Eukaryota; Plants; Land Plants	GCA_003254965.1	Scaffold	611.874	QAOD01	167345	0
Amaranthus hypochondriacus	Eukaryota; Plants; Land Plants	GCA_000753965.1	Scaffold	502.148	JPXE01	117340	0
Amaranthus tuberculatus	Eukaryota; Plants; Land Plants	GCA_000180655.1	Contig	4.34798	ACQK01	15440	0

Amborella trichopoda	Eukaryota; Plants; Land Plants	GCA_000471905.1	Scaffold	706.495	AWHE01	5746	31494
Ananas comosus	Eukaryota; Plants; Land Plants	GCA_902162155.1	Scaffold	315.839	CABGUK01	25	0
Ananas comosus	Eukaryota; Plants; Land Plants	GCA_001661175.1	Scaffold	524.07	LSRQ01	8448	23598
Ananas comosus	Eukaryota; Plants; Land Plants	GCA_001540865.1	Chromosome	382.056	LODP01	3129	35775
Ananas comosus var. bracteatus	Eukaryota; Plants; Land Plants	GCA_902506285.1	Scaffold	513.235	CABWKS01	103	0
Anastatica hierochuntica	Eukaryota; Plants; Land Plants	GCA_900406275.1	Scaffold	542.343	OVAN01	72649	0
Andrographis paniculata	Eukaryota; Plants; Land Plants	GCA_004354405.1	Chromosome	269.408	SMLO01	257	0
Apostasia shenzhenica	Eukaryota; Plants; Land Plants	GCA_002786265.1	Scaffold	348.733	PEFY01	2985	21743
Aquilaria agallochum	Eukaryota; Plants; Land Plants	GCA_000696445.1	Scaffold	726.71	JMHV01	27769	0
Aquilaria sinensis	Eukaryota; Plants; Land Plants	GCA_005392925.1	Contig	699.794	SMDT01	3368	0
Aquilegia coerulea	Eukaryota; Plants; Land Plants	GCA_002738505.1	Scaffold	301.98	NXFA01	970	41063
Arabidopsis halleri	Eukaryota; Plants; Land Plants	GCA_003711535.1	Scaffold	164.574	RCNM01	40344	0
Arabidopsis halleri subsp. gemmifera	Eukaryota; Plants; Land Plants	GCA_900078215.1	Scaffold	196.243	FJVB01	2239	0
Arabidopsis halleri subsp. gemmifera	Eukaryota; Plants; Land Plants	GCA_000523005.1	Scaffold	221.14	BASO01	282453	0
Arabidopsis halleri subsp. gemmifera	Eukaryota; Plants; Land Plants	GCA_003118655.1	Scaffold	413.881	BFAE01	344622	0

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Arabidopsis lyrata subsp. lyrata	Eukaryota; Plants; Land Plants	GCA_000004255.1	Scaffold	206.823	ADBK01	696	39161
Arabidopsis lyrata subsp. petraea	Eukaryota; Plants; Land Plants	GCA_900205625.1	Scaffold	175.183	OANL01	1675	0
Arabidopsis lyrata subsp. petraea	Eukaryota; Plants; Land Plants	GCA_000524985.1	Scaffold	202.972	BASP01	281536	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900303355.1	Contig	119.503	OMOL01	62	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_000835945.1	Contig	127.419	JSAD01	378	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900303345.1	Contig	119.75	OMOK01	78	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900243945.1	Contig	119.167	OFAM01	59	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900243935.1	Contig	119.203	OFEF01	40	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_001753755.2	Contig	244.583	MJMM01	411	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900243955.1	Contig	119.128	OFAN01	139	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_001742845.1	Scaffold	116.846	LXSY01	5197	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_000222345.1	Scaffold	98.0662	AFNB01	1740	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_000222325.1	Scaffold	96.5002	AFNA01	2143	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_000222365.1	Scaffold	96.2565	AFMZ01	1261	0

Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_000222385.1	Scaffold	96.694	AFNC01	2408	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234075.1	Contig	0.195283	OCZF01	19	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234235.1	Contig	1.71971	OCYH01	133	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233735.1	Contig	1.87192	OCWZ01	156	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234125.1	Contig	2.02645	OCXO01	164	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234305.1	Contig	2.01361	OCYE01	163	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233785.1	Contig	2.68429	OCWV01	231	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234265.1	Contig	1.60963	OCYD01	141	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234185.1	Contig	2.04223	OCYB01	169	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233775.1	Contig	1.84553	OCWC01	154	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234295.1	Contig	1.88946	OCYF01	171	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234275.1	Contig	1.95856	OCYJ01	167	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233705.1	Contig	1.53762	OCVZ01	126	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234025.1	Contig	1.4141	OCXK01	116	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233685.1	Contig	1.72997	OCWA01	149	0

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233725.1	Contig	2.27092	OCWQ01	190	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234165.1	Contig	2.13757	OCYN01	189	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234135.1	Contig	1.73315	OCXS01	143	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233965.1	Contig	1.78161	OCXD01	170	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234175.1	Contig	1.93182	OCYM01	173	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234115.1	Contig	1.8648	OCXN01	164	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233995.1	Contig	2.27278	OCXQ01	199	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234145.1	Contig	1.83575	OCXW01	156	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234285.1	Contig	2.15462	OCYA01	194	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234105.1	Contig	1.76999	OCXU01	156	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233755.1	Contig	1.9492	OCWR01	183	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233985.1	Contig	1.75614	OCXL01	156	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234215.1	Contig	1.86784	OCYC01	173	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234205.1	Contig	1.83401	OCYL01	182	0

Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234065.1	Contig	1.56864	OCXH01	151	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233765.1	Contig	1.97277	OCWY01	169	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233795.1	Contig	1.65792	OCXE01	153	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233745.1	Contig	1.86401	OCWJ01	172	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234155.1	Contig	1.93139	OCXX01	180	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234045.1	Contig	1.62901	OCXG01	158	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234195.1	Contig	1.93135	OCYG01	176	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233665.1	Contig	1.82675	OCWE01	191	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233875.1	Contig	1.72986	OCWP01	182	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233905.1	Contig	1.37218	OCWL01	132	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233635.1	Contig	1.68821	OCVY01	157	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233835.1	Contig	1.65938	OCWB01	159	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234085.1	Contig	2.05357	OCXM01	194	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234325.1	Contig	1.55025	OCYO01	153	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233645.1	Contig	1.34032	OCWG01	133	0

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234245.1	Contig	1.90115	OCXY01	200	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234095.1	Contig	1.7997	OCXR01	173	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233865.1	Contig	1.33758	OCWN01	133	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233805.1	Contig	1.5272	OCWS01	146	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234035.1	Contig	2.18075	OCXI01	205	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234365.1	Contig	1.60312	OCYP01	153	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233825.1	Contig	1.4618	OCWH01	147	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233855.1	Contig	1.61604	OCXC01	153	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233695.1	Contig	1.80119	OCXF01	169	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234315.1	Contig	1.66717	OCYK01	179	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233915.1	Contig	1.36257	OCXB01	136	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234055.1	Contig	1.76317	OCXV01	182	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233885.1	Contig	1.38144	OCWT01	140	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233655.1	Contig	1.15189	OCWF01	124	0

Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234005.1	Contig	1.33529	OCXJ01	137	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233815.1	Contig	1.71934	OCWO01	188	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234015.1	Contig	1.11018	OCXT01	124	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233675.1	Contig	1.03397	OCWK01	120	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233845.1	Contig	1.15	OCWM01	133	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233895.1	Contig	1.31957	OCWX01	158	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233715.1	Contig	1.14084	OCXA01	138	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233945.1	Contig	1.26394	OCWD01	151	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233925.1	Contig	2.03504	OCWI01	248	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233975.1	Contig	0.844674	OCXP01	119	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900233955.1	Contig	0.838703	OCWU01	109	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234225.1	Contig	0.32515	OCYI01	52	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900234255.1	Contig	0.847842	OCXZ01	146	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_902460285.1	Chromosome	120.338	CABPTM01	105	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_900660825.1	Chromosome	119.627	CAACVU01	109	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_902460305.1	Chromosome	122.202	CABPTJ01	184	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_902460275.1	Chromosome	119.75	CABPTK01	102	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_902460295.1	Chromosome	120.29	CABPTI01	94	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_902460315.1	Chromosome	120.795	CABPTL01	142	0
Arabidopsis thaliana	Eukaryota; Plants; Land Plants	GCA_001651475.1	Chromosome	118.891	LUHQ01	30	30837
Arabis alpina	Eukaryota; Plants; Land Plants	GCA_000612745.1	Contig	171.788	CBTM01	37680	0
Arabis alpina	Eukaryota; Plants; Land Plants	GCA_000733195.1	Chromosome	308.033	JNGA01	27779	23286
Arabis montbretiana	Eukaryota; Plants; Land Plants	GCA_001484125.1	Contig	199.12	LNCH01	28775	0
Arabis nordmanniana	Eukaryota; Plants; Land Plants	GCA_001484925.1	Scaffold	342.307	LNCG01	267228	0
Arachis duranensis	Eukaryota; Plants; Land Plants	GCA_001687015.1	Scaffold	1075.96	MAMN01	20214	0
Arachis duranensis	Eukaryota; Plants; Land Plants	GCA_000817695.2	Chromosome	1084.26	JQIN01	3189	52826
Arachis hypogaea	Eukaryota; Plants; Land Plants	GCA_003086295.2	Chromosome	2557.07	PIVG01	385	100775
Arachis hypogaea	Eukaryota; Plants; Land Plants	GCA_004170445.1	Chromosome	2551.68	SDMP01	29	101330
Arachis ipaensis	Eukaryota; Plants; Land Plants	GCA_000816755.2	Chromosome	1353.5	JQIO01	997	57621

Arachis monticola	Eukaryota; Plants; Land Plants	GCA_003063285.2	Chromosome	2618.65	QBTX01	6909	0
Argania spinosa	Eukaryota; Plants; Land Plants	GCA_003260245.1	Scaffold	670.097	QLOD01	75327	0
Aristotelia chilensis	Eukaryota; Plants; Land Plants	GCA_008921755.1	Scaffold	96.3544	VEXP01	42602	0
Aristotelia chilensis	Eukaryota; Plants; Land Plants	GCA_008126665.1	Scaffold	0.312713	VDCA01	93	0
Artemisia annua	Eukaryota; Plants; Land Plants	GCA_003112345.1	Scaffold	1792.86	PKPP01	39400	213
Artocarpus camansi	Eukaryota; Plants; Land Plants	GCA_002024485.1	Scaffold	631.308	LNSY01	396025	0
Asclepias syriaca	Eukaryota; Plants; Land Plants	GCA_002018285.1	Scaffold	236.77	MSXX01	221855	0
Asparagus officinalis	Eukaryota; Plants; Land Plants	GCA_001876935.1	Chromosome	1187.54	MPDI01	11792	36763
Atalantia buxifolia	Eukaryota; Plants; Land Plants	GCA_002013935.1	Scaffold	315.806	MKYR01	25600	0
Aurinia saxatilis	Eukaryota; Plants; Land Plants	GCA_900406295.1	Scaffold	316.42	OVAP01	76972	0
Avena sativa	Eukaryota; Plants; Land Plants	GCA_002943605.1	Contig	67.3266	PKQH01	16667	0
Azadirachta indica	Eukaryota; Plants; Land Plants	GCA_000439995.3	Contig	261.458	AMWY02	126142	0
Barbarea vulgaris	Eukaryota; Plants; Land Plants	GCA_001920985.1	Scaffold	167.352	LXTM01	7810	0
Bassia scoparia	Eukaryota; Plants; Land Plants	GCA_008642245.1	Scaffold	711.357	SNQN01	19671	0
Begonia fuchsioides	Eukaryota; Plants; Land Plants	GCA_003255005.1	Scaffold	373.914	QAOC01	55006	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Berberis thunbergii	Eukaryota; Plants; Land Plants	GCA_003290165.1	Contig	2240.74	QNQO01	11815	0
Beta patula	Eukaryota; Plants; Land Plants	GCA_005862465.1	Scaffold	633.549	VASJ01	78458	0
Beta vulgaris subsp. maritima	Eukaryota; Plants; Land Plants	GCA_005862445.2	Scaffold	608.27	VASK02	97415	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_002917755.1	Chromosome	540.534	PCNB01	40	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000510975.1	Chromosome	568.609	AYZY01	43635	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000510365.1	Scaffold	484.231	AYZT01	35771	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000510875.1	Scaffold	479.876	AYZX01	47405	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000510485.1	Scaffold	463.706	AYZW01	48733	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000510465.1	Scaffold	539.552	AYZU01	84234	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000397105.1	Scaffold	426.675	ARYA01	260142	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000729925.1	Contig	1.15347	JMBQ01	1287	0
Beta vulgaris subsp. vulgaris	Eukaryota; Plants; Land Plants	GCA_000511025.2	Chromosome	566.55	AYZS02	40406	32874
Betula nana	Eukaryota; Plants; Land Plants	GCA_000327005.1	Scaffold	564.011	CAOK01	551915	0
Betula pendula	Eukaryota; Plants; Land Plants	GCA_900184695.1	Scaffold	435.915	FXXK01	5644	0

Biscutella auriculata	Eukaryota; Plants; Land Plants	GCA_900406285.1	Scaffold	384.77	OVAO01	150640	0
Biscutella laevigata subsp. laevigata	Eukaryota; Plants; Land Plants	GCA_900406315.1	Scaffold	333.609	OVAU01	144912	0
Boechera puberula	Eukaryota; Plants; Land Plants	GCA_900406335.1	Scaffold	182.592	OVAS01	123951	0
Boechera stricta	Eukaryota; Plants; Land Plants	GCA_002079875.1	Scaffold	188.795	MLHT01	1944	0
Boehmeria nivea	Eukaryota; Plants; Land Plants	GCA_002937015.1	Scaffold	344.617	PHNS01	12775	0
Boehmeria nivea	Eukaryota; Plants; Land Plants	GCA_002806895.1	Scaffold	316.026	NHTU01	154955	0
Brachypodium distachyon	Eukaryota; Plants; Land Plants	GCA_002892335.1	Contig	218.676	MXPZ01	60421	0
Brachypodium distachyon	Eukaryota; Plants; Land Plants	GCA_002892295.1	Contig	218.015	MXQA01	65964	0
Brachypodium distachyon	Eukaryota; Plants; Land Plants	GCA_001742125.1	Contig	214.716	LXJM01	68977	0
Brachypodium distachyon	Eukaryota; Plants; Land Plants	GCA_000005505.4	Chromosome	271.299	ADDN03	15	37892
Brassica cretica	Eukaryota; Plants; Land Plants	GCA_003260655.1	Contig	412.521	QGKV01	243461	0
Brassica cretica	Eukaryota; Plants; Land Plants	GCA_003260635.1	Contig	208.354	QGKW01	100644	0
Brassica cretica	Eukaryota; Plants; Land Plants	GCA_003260675.1	Contig	434.935	QGKX01	338759	0
Brassica cretica	Eukaryota; Plants; Land Plants	GCA_003260695.1	Contig	400.212	QGKY01	396633	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Brassica juncea var. tumida	Eukaryota; Plants; Land Plants	GCA_001687265.1	Chromosome	954.861	LFQT01	9746	0
Brassica napus	Eukaryota; Plants; Land Plants	GCA_000751015.1	Scaffold	848.2	CCCW01	20899	61153
Brassica napus	Eukaryota; Plants; Land Plants	GCA_000686985.2	Chromosome	976.191	JMKK02	1471	123467
Brassica nigra	Eukaryota; Plants; Land Plants	GCA_001682895.1	Chromosome	402.145	LFLV01	2545	0
Brassica oleracea	Eukaryota; Plants; Land Plants	GCA_900416815.2	Chromosome	554.977	OWNI02	129	0
Brassica oleracea var. capitata	Eukaryota; Plants; Land Plants	GCA_000604025.1	Scaffold	514.431	AOIX01	1816	0
Brassica oleracea var. oleracea	Eukaryota; Plants; Land Plants	GCA_000695525.1	Chromosome	488.954	JJMF01	32886	56687
Brassica rapa	Eukaryota; Plants; Land Plants	GCA_900412535.2	Chromosome	401.927	OVXL02	304	0
Brassica rapa	Eukaryota; Plants; Land Plants	GCA_000309985.1	Chromosome	284.129	AENI01	40432	52553
Brassica rapa	Eukaryota; Plants; Land Plants	GCA_003434825.1	Chromosome	314.865	QMKI01	7071	43332
Brassica rapa subsp. pekinensis	Eukaryota; Plants; Land Plants	GCA_008629595.1	Chromosome	234.688	VDME01	3421	0
Cajanus cajan	Eukaryota; Plants; Land Plants	GCA_000230855.2	Contig	648.281	AFSP02	360028	0
Cajanus cajan	Eukaryota; Plants; Land Plants	GCA_000340665.1	Chromosome	592.971	AGCT01	36536	41387
Calamus simplicifolius	Eukaryota; Plants; Land Plants	GCA_900491605.1	Scaffold	1960.81	UESW01	5116	0

Calotropis procera	Eukaryota; Plants; Land Plants	GCA_004801955.1	Scaffold	209.215	LVCA01	20519	0
Camelina sativa	Eukaryota; Plants; Land Plants	GCA_000496875.1	Scaffold	547.649	AUUT01	15937	0
Camelina sativa	Eukaryota; Plants; Land Plants	GCA_000633955.1	Chromosome	641.356	JFZQ01	37212	107481
Camellia sinensis var. sinensis	Eukaryota; Plants; Land Plants	GCA_004153795.1	Scaffold	3105.37	SDRB01	14028	76698
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_001865755.1	Contig	585.824	MNPR01	11110	0
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_002090435.1	Contig	512.174	MXBD01	18355	0
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_003660325.2	Contig	1333.38	QVPT02	3372	0
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_001509995.1	Scaffold	285.933	LKUB01	175088	0
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_000230575.5	Chromosome	891.965	AGQN03	12836	0
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_003417725.2	Chromosome	1009.67	QKVJ02	5303	0
Cannabis sativa	Eukaryota; Plants; Land Plants	GCA_900626175.1	Chromosome	876.148	UZAU01	221	33677
Cannabis sativa subsp. indica	Eukaryota; Plants; Land Plants	GCA_001510005.1	Contig	595.358	LKUA01	311039	0
Capsella bursa-pastoris	Eukaryota; Plants; Land Plants	GCA_001974645.1	Scaffold	268.431	MPGU01	8186	0
Capsella rubella	Eukaryota; Plants; Land Plants	GCA_000375325.1	Scaffold	133.064	ANNY01	773	34126

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Capsicum annuum	Eukaryota; Plants; Land Plants	GCA_002878395.2	Chromosome	3212.12	NPHV01	81378	0
Capsicum annuum	Eukaryota; Plants; Land Plants	GCA_000512255.2	Chromosome	3063.86	AYRZ02	35797	35845
Capsicum annuum	Eukaryota; Plants; Land Plants	GCA_000710875.1	Chromosome	2935.88	ASJU01	6478	45410
Capsicum annuum var. glabriusculum	Eukaryota; Plants; Land Plants	GCA_000950795.1	Chromosome	2768.13	ASJV01	16998	0
Capsicum baccatum	Eukaryota; Plants; Land Plants	GCA_002271885.2	Chromosome	3215.61	MLFT02	23260	35853
Capsicum chinense	Eukaryota; Plants; Land Plants	GCA_002271895.2	Chromosome	3070.91	MCIT02	87978	34974
Carica papaya	Eukaryota; Plants; Land Plants	GCA_000150535.1	Scaffold	370.419	ABIM01	17766	26103
Carnegiea gigantea	Eukaryota; Plants; Land Plants	GCA_002740515.1	Scaffold	980.351	NCQR01	57405	0
Carpinus fangiana	Eukaryota; Plants; Land Plants	GCA_006937295.1	Chromosome	381.949	VIBQ01	4602	0
Carthamus tinctorius	Eukaryota; Plants; Land Plants	GCA_001633085.1	Scaffold	661.938	LUCG01	463906	0
Caryocar brasiliense	Eukaryota; Plants; Land Plants	GCA_004918865.1	Scaffold	212.173	STGP01	55248	0
Castanea mollissima	Eukaryota; Plants; Land Plants	GCA_000763605.1	Scaffold	833.241	JRKL01	133589	0
Casuarina equisetifolia subsp. incana	Eukaryota; Plants; Land Plants	GCA_003795335.1	Scaffold	301.458	RDRV01	2936	0
Casuarina glauca	Eukaryota; Plants; Land Plants	GCA_003255045.1	Scaffold	282.811	QAOB01	39787	0

Catharanthus roseus	Eukaryota; Plants; Land Plants	GCA_000949345.1	Scaffold	522.654	JQHZ01	79302	0
Catharanthus roseus	Eukaryota; Plants; Land Plants	GCA_001292525.1	Contig	0.11552	CCXB01	7	0
Catharanthus roseus	Eukaryota; Plants; Land Plants	GCA_001292565.1	Contig	0.114931	CCXA01	32	0
Cenchrus americanus	Eukaryota; Plants; Land Plants	GCA_002174835.2	Chromosome	1816.95	LKME02	52033	0
Cephalotus follicularis	Eukaryota; Plants; Land Plants	GCA_001972305.1	Scaffold	1614.52	BDDD01	16307	36667
Cercis canadensis	Eukaryota; Plants; Land Plants	GCA_003255065.1	Scaffold	329.325	QAOA01	8828	0
Chamaecrista fasciculata	Eukaryota; Plants; Land Plants	GCA_003254925.1	Scaffold	429.103	QANZ01	56674	0
Chenopodium pallidicaule	Eukaryota; Plants; Land Plants	GCA_001687005.1	Scaffold	337.011	MATR01	3013	0
Chenopodium quinoa	Eukaryota; Plants; Land Plants	GCA_001683475.1	Scaffold	1333.55	LPWI01	3487	63173
Chenopodium quinoa	Eukaryota; Plants; Land Plants	GCA_002732095.1	Scaffold	1336.74	NSDK01	3185	0
Chenopodium quinoa	Eukaryota; Plants; Land Plants	GCA_001742885.1	Scaffold	1087.41	BDCQ01	24845	0
Chenopodium suecicum	Eukaryota; Plants; Land Plants	GCA_001687025.1	Scaffold	536.949	MATQ01	11198	0
Chrysanthemum seticuspe	Eukaryota; Plants; Land Plants	GCA_004359105.1	Scaffold	2721.84	BDUE01	354212	0
Cicer arietinum	Eukaryota; Plants; Land Plants	GCA_002896005.2	Scaffold	653.867	PGTT02	13064	0

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Cicer arietinum	Eukaryota; Plants; Land Plants	GCA_000347275.4	Chromosome	511.684	AHII03	30401	0
Cicer arietinum	Eukaryota; Plants; Land Plants	GCA_000331145.1	Chromosome	530.894	ANPC01	7545	35754
Cicer echinospermum	Eukaryota; Plants; Land Plants	GCA_002896215.2	Scaffold	657.414	PGTU02	17305	0
Cicer reticulatum	Eukaryota; Plants; Land Plants	GCA_002896235.1	Contig	715.407	PGWS01	38802	0
Cicer reticulatum	Eukaryota; Plants; Land Plants	GCA_003689015.2	Chromosome	416.904	QSLP02	3657	0
Cinnamomum micranthum f. kanehirae	Eukaryota; Plants; Land Plants	GCA_003546025.1	Scaffold	730.416	QPKB01	2150	26531
Cissus quadrangularis	Eukaryota; Plants; Land Plants	GCA_002878655.1	Scaffold	281.704	LLYR01	125206	0
Citrullus lanatus	Eukaryota; Plants; Land Plants	GCA_000238415.2	Chromosome	365.45	AGCB02	113	0
Citrus cavaleriei	Eukaryota; Plants; Land Plants	GCA_002013975.2	Scaffold	357.621	MKYP02	14916	0
Citrus clementina	Eukaryota; Plants; Land Plants	GCA_000493195.1	Scaffold	301.365	AMZM01	1398	32586
Citrus hindsii	Eukaryota; Plants; Land Plants	GCA_004802465.1	Contig	373.17	QWBT01	1331	0
Citrus maxima	Eukaryota; Plants; Land Plants	GCA_002006925.1	Chromosome	345.757	MKYQ01	1612	0
Citrus medica	Eukaryota; Plants; Land Plants	GCA_002013955.2	Scaffold	406.058	MKYO02	32732	0
Citrus reticulata	Eukaryota; Plants; Land Plants	GCA_003258625.1	Scaffold	344.273	NIHA01	67725	0

Citrus sinensis	Eukaryota; Plants; Land Plants	GCA_000695605.1	Scaffold	319.225	JJOQ01	12573	51718
Citrus sinensis	Eukaryota; Plants; Land Plants	GCA_000317415.1	Chromosome	327.83	AJPS01	4995	39056
Citrus unshiu	Eukaryota; Plants; Land Plants	GCA_002897195.1	Scaffold	359.652	BDQV01	20876	37970
Citrus unshiu	Eukaryota; Plants; Land Plants	GCA_001753815.1	Contig	1.1542	BDGO01	507	0
Citrus x paradisi x Citrus trifoliata	Eukaryota; Plants; Land Plants	GCA_001929425.1	Contig	265.534	AZHM01	238488	0
Cochlearia officinalis	Eukaryota; Plants; Land Plants	GCA_900406305.1	Scaffold	164.469	OVAT01	128459	0
Cocos nucifera	Eukaryota; Plants; Land Plants	GCA_006176705.1	Scaffold	2102.42	QRFJ01	7998	0
Cocos nucifera	Eukaryota; Plants; Land Plants	GCA_003604295.1	Scaffold	1839.17	PDMH01	59328	0
Cocos nucifera	Eukaryota; Plants; Land Plants	GCA_008124465.1	Chromosome	2202.46	VOII01	113653	0
Codonopsis pilosula	Eukaryota; Plants; Land Plants	GCA_004523855.1	Scaffold	937.71	SOPR01	1154	0
Coffea arabica	Eukaryota; Plants; Land Plants	GCA_003713225.1	Chromosome	1094.45	RHJU01	2833	67222
Coffea canephora	Eukaryota; Plants; Land Plants	GCA_900059795.1	Chromosome	568.612	CBUE02	13345	25574
Coffea eugenioides	Eukaryota; Plants; Land Plants	GCA_003713205.1	Chromosome	699.904	RHJT01	3530	38150
Conringia planisiliqua	Eukaryota; Plants; Land Plants	GCA_900108845.1	Scaffold	184.156	FNXX01	705	0

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Corchorus capsularis	Eukaryota; Plants; Land Plants	GCA_001974805.1	Scaffold	317.178	AWWV01	16522	29356
Corchorus olitorius	Eukaryota; Plants; Land Plants	GCA_001974825.1	Contig	334.912	AWUE01	24918	35704
Corchorus olitorius	Eukaryota; Plants; Land Plants	GCA_002141455.1	Contig	377.377	LLWS01	52373	0
Crucihimalaya himalaica	Eukaryota; Plants; Land Plants	GCA_004349715.1	Scaffold	234.721	SMJT01	582	0
Cucumis melo	Eukaryota; Plants; Land Plants	GCA_000313045.1	Scaffold	374.928	CAJI01	31464	29798
Cucumis melo	Eukaryota; Plants; Land Plants	GCA_902497455.1	Scaffold	357.857	CABVGI01	13	0
Cucumis melo var. makuwa	Eukaryota; Plants; Land Plants	GCA_005549215.1	Scaffold	358.48	SSTE01	23444	38173
Cucumis melo var. makuwa	Eukaryota; Plants; Land Plants	GCA_005549225.1	Scaffold	347.184	SSTD01	20255	36235
Cucumis sativus	Eukaryota; Plants; Land Plants	GCA_001483825.2	Contig	342.654	LKUO02	8035	0
Cucumis sativus	Eukaryota; Plants; Land Plants	GCA_000224045.1	Scaffold	323.986	ACYN01	13113	0
Cucumis sativus	Eukaryota; Plants; Land Plants	GCA_000004075.2	Chromosome	195.669	ACHR02	190	25668
Cucurbita argyrosperma subsp. argyrosperma	Eukaryota; Plants; Land Plants	GCA_004115005.1	Scaffold	230.034	SDJN01	938	0
Cucurbita maxima	Eukaryota; Plants; Land Plants	GCA_002738345.1	Scaffold	271.413	NEWN01	8299	42777
Cucurbita moschata	Eukaryota; Plants; Land Plants	GCA_002738365.1	Scaffold	269.943	NEWM01	3500	43715

Cucurbita pepo subsp. pepo	Eukaryota; Plants; Land Plants	GCA_002806865.2	Chromosome	261.355	NHTM01	25263	43466
Cuscuta australis	Eukaryota; Plants; Land Plants	GCA_003260385.1	Contig	262.63	NQVE01	218	18157
Cuscuta campestris	Eukaryota; Plants; Land Plants	GCA_900332095.1	Scaffold	476.792	OOIL01	6907	0
Cynara cardunculus var. scolymus	Eukaryota; Plants; Land Plants	GCA_001531365.1	Chromosome	725.198	LEKV01	13588	38406
Dactylis glomerata	Eukaryota; Plants; Land Plants	GCA_007115705.1	Scaffold	1781.32	QXEO01	2117	0
Dactylis glomerata	Eukaryota; Plants; Land Plants	GCA_002892645.1	Scaffold	839.915	MVYT01	1072009	0
Datisca glomerata	Eukaryota; Plants; Land Plants	GCA_003255025.1	Scaffold	688.404	QANY01	13864	0
Daucus carota subsp. sativus	Eukaryota; Plants; Land Plants	GCA_001625215.1	Chromosome	421.539	LNRQ01	4826	44655
Dendrobium catenatum	Eukaryota; Plants; Land Plants	GCA_001605985.2	Scaffold	1104.26	JSDN02	286090	34389
Dianthus caryophyllus	Eukaryota; Plants; Land Plants	GCA_000512335.1	Scaffold	567.662	BAUD01	45088	0
Dichanthelium oligosanthes	Eukaryota; Plants; Land Plants	GCA_001633215.2	Scaffold	589.166	LWDX02	17436	26468
Dioscorea alata	Eukaryota; Plants; Land Plants	GCA_002904275.2	Scaffold	620.909	CZHE02	57706	0
Dioscorea rotundata	Eukaryota; Plants; Land Plants	GCA_002260605.1	Scaffold	594.227	BBQW01	4723	0
Dioscorea rotundata	Eukaryota; Plants; Land Plants	GCA_002260665.1	Scaffold	730.21	BDML01	615107	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Dioscorea rotundata	Eukaryota; Plants; Land Plants	GCA_002260645.1	Scaffold	683.283	BDMK01	641416	0
Dioscorea rotundata	Eukaryota; Plants; Land Plants	GCA_002240015.2	Chromosome	456.675	BDMI01	21	0
Dioscorea sansibarensis	Eukaryota; Plants; Land Plants	GCA_900631875.1	Contig	0.128321	CABFPD01	6	0
Diospyros lotus	Eukaryota; Plants; Land Plants	GCA_000774125.1	Scaffold	1.10419	JRBH01	796	0
Dorcoceras hygrometricum	Eukaryota; Plants; Land Plants	GCA_001598015.1	Scaffold	1521.36	LVEL01	401752	47778
Drosera capensis	Eukaryota; Plants; Land Plants	GCA_001925005.1	Scaffold	263.788	LIEC01	12713	0
Dryas drummondii	Eukaryota; Plants; Land Plants	GCA_003254865.1	Scaffold	225.547	QANW01	13357	0
Durio zibethinus	Eukaryota; Plants; Land Plants	GCA_002303985.1	Scaffold	715.23	NSDW01	677	63007
Echinochloa crus-galli	Eukaryota; Plants; Land Plants	GCA_900205405.1	Scaffold	1486.61	OAMR01	4534	0
Echium plantagineum	Eukaryota; Plants; Land Plants	GCA_003412495.2	Chromosome	349.028	QFAX02	809	0
Eichhornia paniculata	Eukaryota; Plants; Land Plants	GCA_001647135.1	Scaffold	571.388	LTAE01	40286	0
Elaeis guineensis	Eukaryota; Plants; Land Plants	GCA_001672495.1	Scaffold	499.029	JRVM01	218141	0
Elaeis guineensis	Eukaryota; Plants; Land Plants	GCA_002146295.1	Contig	134.97	AXCU01	186862	0
Elaeis guineensis	Eukaryota; Plants; Land Plants	GCA_000442705.1	Chromosome	1535.18	ASJS01	40349	43551

Elaeis oleifera	Eukaryota; Plants; Land Plants	GCA_000441515.1	Scaffold	1402.73	ASIR01	26756	0
Elaeis oleifera	Eukaryota; Plants; Land Plants	GCA_002146275.1	Contig	60.0019	AXCH01	93897	0
Eleusine coracana subsp. coracana	Eukaryota; Plants; Land Plants	GCA_002180455.1	Scaffold	1195.99	LXGH01	525627	0
Eleusine indica	Eukaryota; Plants; Land Plants	GCA_003369855.1	Scaffold	492.27	QEPD01	24072	0
Embelia ribes	Eukaryota; Plants; Land Plants	GCA_001753735.1	Scaffold	660.51	MKEJ01	107000	0
Ensete ventricosum	Eukaryota; Plants; Land Plants	GCA_000818735.3	Scaffold	451.279	JTFG03	45742	58438
Ensete ventricosum	Eukaryota; Plants; Land Plants	GCA_001884845.1	Scaffold	444.842	MKKT01	51525	58998
Ensete ventricosum	Eukaryota; Plants; Land Plants	GCA_001884805.1	Contig	429.48	MKKS01	60129	56086
Ensete ventricosum	Eukaryota; Plants; Land Plants	GCA_000331365.3	Scaffold	437.269	AMZH03	52691	55115
Eragrostis curvula	Eukaryota; Plants; Land Plants	GCA_007726485.1	Chromosome	603.072	RWGY01	1143	55182
Eragrostis tef	Eukaryota; Plants; Land Plants	GCA_000970635.1	Scaffold	607.318	LAPY01	13883	0
Erigeron canadensis	Eukaryota; Plants; Land Plants	GCA_000775935.1	Contig	326.165	JSWR01	20075	0
Erucastrum elatum	Eukaryota; Plants; Land Plants	GCA_900406325.1	Scaffold	362.114	OVBX01	80289	0
Erysimum cheiri	Eukaryota; Plants; Land Plants	GCA_900406345.1	Scaffold	147.321	OVAQ01	37531	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Erysimum pusillum	Eukaryota; Plants; Land Plants	GCA_900406355.1	Scaffold	184.064	OVBW01	47441	0
Erythranthe guttata	Eukaryota; Plants; Land Plants	GCA_000504015.1	Scaffold	322.167	APLE01	2212	31861
Eschscholzia californica subsp. californica	Eukaryota; Plants; Land Plants	GCA_002897215.1	Scaffold	489.065	BEHA01	53253	0
Eucalyptus camaldulensis	Eukaryota; Plants; Land Plants	GCA_000260855.1	Contig	654.922	BADO01	274001	0
Eucalyptus grandis	Eukaryota; Plants; Land Plants	GCA_000612305.1	Scaffold	691.43	AUSX01	4951	52554
Eucalyptus melliodora	Eukaryota; Plants; Land Plants	GCA_004368105.1	Scaffold	643.228	SISH01	423	0
Eucalyptus pauciflora	Eukaryota; Plants; Land Plants	GCA_007663325.1	Scaffold	594.528	VMYD01	415	0
Euclidium syriacum	Eukaryota; Plants; Land Plants	GCA_900116095.1	Scaffold	229.211	FPAK01	160	0
Eugenia uniflora	Eukaryota; Plants; Land Plants	GCA_004012085.1	Contig	3.15213	RQIG01	2601	0
Euphorbia esula	Eukaryota; Plants; Land Plants	GCA_002919075.1	Scaffold	1124.89	PJAD01	1633094	0
Euphorbia esula	Eukaryota; Plants; Land Plants	GCA_002918425.1	Scaffold	639.02	PJAE01	912031	0
Eutrema heterophyllum	Eukaryota; Plants; Land Plants	GCA_002933915.1	Scaffold	348.971	PKMM01	57686	0
Eutrema salsugineum	Eukaryota; Plants; Land Plants	GCA_000478725.1	Scaffold	243.11	ANOA01	638	33637
Eutrema salsugineum	Eukaryota; Plants; Land Plants	GCA_000325905.2	Chromosome	231.893	AHIU01	2663	0

Eutrema yunnanense	Eukaryota; Plants; Land Plants	GCA_002933935.1	Scaffold	415.364	PKML01	78020	0
Fagopyrum esculentum	Eukaryota; Plants; Land Plants	GCA_004303065.1	Scaffold	1087.7	QWEW01	14903	0
Fagopyrum esculentum	Eukaryota; Plants; Land Plants	GCA_001661195.1	Scaffold	1177.69	BCYN01	387594	0
Fagopyrum tataricum	Eukaryota; Plants; Land Plants	GCA_002928575.1	Scaffold	526.768	PKMW01	2564	0
Fagopyrum tataricum	Eukaryota; Plants; Land Plants	GCA_002319775.1	Chromosome	505.883	NCTC01	7020	0
Fagus sylvatica	Eukaryota; Plants; Land Plants	GCA_003347535.1	Scaffold	428.2	QCXR01	8673	0
Ficus carica	Eukaryota; Plants; Land Plants	GCA_002002945.1	Scaffold	247.091	BDEM01	27995	0
Ficus erecta	Eukaryota; Plants; Land Plants	GCA_008635985.1	Contig	595.835	BKCH01	2455	0
Foeniculum vulgare	Eukaryota; Plants; Land Plants	GCA_003724115.1	Scaffold	1010.97	PHNY01	300377	0
Fragaria iinumae	Eukaryota; Plants; Land Plants	GCA_000511975.1	Scaffold	199.628	BATU01	117822	0
Fragaria nipponica	Eukaryota; Plants; Land Plants	GCA_000512025.1	Scaffold	206.415	BATV01	215024	0
Fragaria nubicola	Eukaryota; Plants; Land Plants	GCA_000511995.1	Scaffold	203.686	BATW01	210780	0
Fragaria orientalis	Eukaryota; Plants; Land Plants	GCA_000517285.1	Scaffold	214.184	BATX01	323163	0
Fragaria vesca subsp. vesca	Eukaryota; Plants; Land Plants	GCA_000184155.1	Chromosome	214.373	AEMH01	3048	31387

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Fragaria x ananassa	Eukaryota; Plants; Land Plants	GCA_000511835.1	Scaffold	697.762	BATT01	625966	0
Fragaria x ananassa	Eukaryota; Plants; Land Plants	GCA_000511695.1	Scaffold	173.23	BATS01	211588	0
Fraxinus excelsior	Eukaryota; Plants; Land Plants	GCA_900149125.1	Scaffold	867.455	FTPI01	89515	0
Gastrodiaelata f. glauca	Eukaryota; Plants; Land Plants	GCA_002966915.1	Scaffold	1060.98	PVEL01	3768	0
Genlisea aurea	Eukaryota; Plants; Land Plants	GCA_000441915.1	Scaffold	43.3578	AUSU01	10684	17685
Geum urbanum	Eukaryota; Plants; Land Plants	GCA_900236755.1	Scaffold	1217.04	OEJZ01	170029	0
Glycine max	Eukaryota; Plants; Land Plants	GCA_001269945.2	Contig	927.706	BBNX02	108601	0
Glycine max	Eukaryota; Plants; Land Plants	GCA_003349995.1	Chromosome	1017.57	QKRT01	495	0
Glycine max	Eukaryota; Plants; Land Plants	GCA_002905335.2	Chromosome	1016.28	PELE01	475	0
Glycine max	Eukaryota; Plants; Land Plants	GCA_000004515.4	Chromosome	979.046	ACUP03	1579	71219
Glycine soja	Eukaryota; Plants; Land Plants	GCA_000722935.2	Scaffold	863.568	AZNC01	33170	50399
Glycine soja	Eukaryota; Plants; Land Plants	GCA_004193775.2	Chromosome	1013.77	QZWG01	1120	69277
Glycine soja	Eukaryota; Plants; Land Plants	GCA_002907465.1	Chromosome	985.26	PGFP01	805	0
Glycine tomentella	Eukaryota; Plants; Land Plants	GCA_007407185.1	Scaffold	1694.09	PYAF01	6353	0

Gossypioides kirkii	Eukaryota; Plants; Land Plants	GCA_002818315.1	Chromosome	528.715	PEQG01	745	0
Gossypium arboreum	Eukaryota; Plants; Land Plants	GCA_000787975.1	Scaffold	1862.24	JRRC01	392831	33609
Gossypium arboreum	Eukaryota; Plants; Land Plants	GCA_000612285.2	Chromosome	1694.6	AYOE01	75419	47568
Gossypium australe	Eukaryota; Plants; Land Plants	GCA_005393395.2	Chromosome	1743.39	SMMG02	564	38281
Gossypium barbadense	Eukaryota; Plants; Land Plants	GCA_002928715.1	Scaffold	1394.24	LAGA01	9269	40359
Gossypium barbadense	Eukaryota; Plants; Land Plants	GCA_001856525.1	Scaffold	2566.74	AXCG01	29751	0
Gossypium barbadense	Eukaryota; Plants; Land Plants	GCA_002926015.1	Scaffold	775.252	LAGB01	4265	36871
Gossypium barbadense	Eukaryota; Plants; Land Plants	GCA_008761655.1	Chromosome	2195.8	VKDL01	4748	108363
Gossypium darwinii	Eukaryota; Plants; Land Plants	GCA_007990325.1	Chromosome	2182.96	VKGI01	821	97407
Gossypium hirsutum	Eukaryota; Plants; Land Plants	GCA_006980745.1	Chromosome	2287.87	VCQY01	599	0
Gossypium hirsutum	Eukaryota; Plants; Land Plants	GCA_006980775.1	Chromosome	2308.22	VCQX01	2238	0
Gossypium hirsutum	Eukaryota; Plants; Land Plants	GCA_000987745.1	Chromosome	2189.14	LBLM01	9148	90927
Gossypium mustelinum	Eukaryota; Plants; Land Plants	GCA_007990455.1	Chromosome	2315.09	VKGF01	2146	106487
Gossypium raimondii	Eukaryota; Plants; Land Plants	GCA_000331045.1	Scaffold	773.768	AMOP01	4699	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Gossypium raimondii	Eukaryota; Plants; Land Plants	GCA_003327365.1	Chromosome	761.565	ALYE01	1034	59057
Gossypium thurberi	Eukaryota; Plants; Land Plants	GCA_004027125.1	Chromosome	582.007	RCOT01	15297	0
Gossypium tomentosum	Eukaryota; Plants; Land Plants	GCA_007990485.1	Chromosome	2193.56	VKGE01	749	112713
Handroanthus impetiginosus	Eukaryota; Plants; Land Plants	GCA_002762385.1	Scaffold	503.289	NKXS01	13204	30271
Helianthus annuus	Eukaryota; Plants; Land Plants	GCA_002127325.1	Chromosome	3027.84	MNCJ01	1528	73839
Heliophila coronopifolia	Eukaryota; Plants; Land Plants	GCA_900406365.1	Scaffold	293.602	OVAW01	212649	0
Herrania umbratica	Eukaryota; Plants; Land Plants	GCA_002168275.2	Scaffold	234.039	NHTG01	6074	27748
Hevea brasiliensis	Eukaryota; Plants; Land Plants	GCA_001654055.1	Scaffold	1373.53	LVXX01	7453	58062
Hevea brasiliensis	Eukaryota; Plants; Land Plants	GCA_002003025.1	Scaffold	1256.27	BDHL01	592579	0
Hevea brasiliensis	Eukaryota; Plants; Land Plants	GCA_001907995.1	Scaffold	1550.51	MKXE01	189320	0
Hevea brasiliensis	Eukaryota; Plants; Land Plants	GCA_000340545.1	Scaffold	1301.4	AJJZ01	1150326	0
Hibiscus syriacus	Eukaryota; Plants; Land Plants	GCA_006381635.1	Scaffold	2573.67	VEPZ01	9646	0
Hibiscus syriacus	Eukaryota; Plants; Land Plants	GCA_001696755.1	Scaffold	1748.25	MBGJ01	77488	0
Hordeum bulbosum	Eukaryota; Plants; Land Plants	GCA_900070015.1	Scaffold	1294.87	CBQS01	2883554	0

Hordeum pubiflorum	Eukaryota; Plants; Land Plants	GCA_000582825.1	Scaffold	1425.27	CBMN01	1818420	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_004114815.1	Scaffold	4006.12	SDOW01	1856	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_900075435.2	Scaffold	9788.86	FJWB02	72295	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_900067795.1	Contig	161.166	CEGI01	589134	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_900067805.1	Contig	160.635	CEGJ01	598302	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_900067785.1	Contig	316.11	CEGM01	1201146	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_900067825.1	Contig	207.795	CEGK01	800872	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_900067815.1	Contig	233.129	CEGL01	908607	0
Hordeum vulgare	Eukaryota; Plants; Land Plants	GCA_000947855.1	Contig	98.056	CEGH01	391258	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_000326125.1	Scaffold	1779.49	CAJX01	2077901	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_001077415.1	Scaffold	1645.58	CBLZ01	2280908	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_000326085.1	Scaffold	1868.64	CAJW01	2670738	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_900002345.1	Scaffold	1825.17	CCJR01	2546226	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_002900805.1	Scaffold	2019.37	CAJV01	2742077	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_902500625.1	Scaffold	4129.36	CABVVH01	8	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_000227425.1	Contig	28.016	BACC01	8583	0
Hordeum vulgare subsp. vulgare	Eukaryota; Plants; Land Plants	GCA_002943585.1	Contig	57.9644	PKQG01	14158	0
Humulus lupulus var. cordifolius	Eukaryota; Plants; Land Plants	GCA_000830395.1	Scaffold	2049.21	BBPB01	132476	0
Humulus lupulus var. lupulus	Eukaryota; Plants; Land Plants	GCA_000831365.1	Scaffold	2049.21	BBPC01	132476	0
Iberis amara	Eukaryota; Plants; Land Plants	GCA_900406375.1	Scaffold	361.245	OVAV01	191171	0
Iberis pinnata	Eukaryota; Plants; Land Plants	GCA_900406425.1	Scaffold	686.283	OVBE01	141542	0
Ipomoea batatas	Eukaryota; Plants; Land Plants	GCA_900092185.1	Contig	13.8613	FLTB01	41487	0
Ipomoea batatas	Eukaryota; Plants; Land Plants	GCA_002525835.2	Chromosome	837.013	NXFB01	28461	0
Ipomoea nil	Eukaryota; Plants; Land Plants	GCA_001879475.1	Scaffold	735.231	BDFN01	3418	51054
Ipomoea trifida	Eukaryota; Plants; Land Plants	GCA_000978395.1	Scaffold	512.991	BBOG01	77400	0
Ipomoea trifida	Eukaryota; Plants; Land Plants	GCA_000981105.1	Scaffold	712.155	BBOH01	181194	0
Ipomoea trifida	Eukaryota; Plants; Land Plants	GCA_004706985.1	Chromosome	460.934	SMMV01	4236	0
Isatis lusitanica	Eukaryota; Plants; Land Plants	GCA_900406415.1	Scaffold	203.919	OVBB01	61090	0

Isatis tinctoria	Eukaryota; Plants; Land Plants	GCA_900406385.1	Scaffold	244.177	OVBD01	106900	0
Jaltoma tasinuosa	Eukaryota; Plants; Land Plants	GCA_003996215.1	Scaffold	1443.2	QJPP01	7667	0
Jatropha curcas	Eukaryota; Plants; Land Plants	GCA_000696525.1	Scaffold	318.527	AFEW01	6024	32547
Jatropha curcas	Eukaryota; Plants; Land Plants	GCA_004143595.1	Scaffold	265.767	QFZG01	2959	0
Jatropha curcas	Eukaryota; Plants; Land Plants	GCA_000208675.2	Scaffold	297.661	BABX02	39277	0
Juglans cathayensis	Eukaryota; Plants; Land Plants	GCA_003122765.1	Scaffold	600.151	QEOU01	19972	0
Juglans hindsii	Eukaryota; Plants; Land Plants	GCA_003123825.1	Scaffold	611.109	QEOW01	73433	0
Juglans mandshurica	Eukaryota; Plants; Land Plants	GCA_002916435.1	Scaffold	558.071	PKSJ01	13809	0
Juglans microcarpa	Eukaryota; Plants; Land Plants	GCA_003123845.1	Scaffold	913.972	QEOX01	112570	0
Juglans microcarpa x Juglans regia	Eukaryota; Plants; Land Plants	GCA_004785585.1	Chromosome	534.672	QKZY01	73	0
Juglans microcarpa x Juglans regia	Eukaryota; Plants; Land Plants	GCA_004785595.1	Chromosome	527.896	QKZX01	154	0
Juglans nigra	Eukaryota; Plants; Land Plants	GCA_003123865.1	Scaffold	620.767	QEOV01	90472	0
Juglans nigra	Eukaryota; Plants; Land Plants	GCA_002916485.1	Scaffold	682.557	PKSI01	18575	0
Juglans regia	Eukaryota; Plants; Land Plants	GCA_001411555.1	Scaffold	699.673	LIHL01	105803	55627

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Juglans regia	Eukaryota; Plants; Land Plants	GCA_003122785.1	Scaffold	650.478	QEOZ01	4401	0
Juglans regia	Eukaryota; Plants; Land Plants	GCA_002916465.1	Scaffold	634.748	PKSH01	25789	0
Juglans sigillata	Eukaryota; Plants; Land Plants	GCA_003123805.1	Scaffold	648.117	QEOY01	134300	0
Kalanchoe fedtschenkoi	Eukaryota; Plants; Land Plants	GCA_002312845.1	Scaffold	256.351	NQLW01	1324	0
Kernera saxatilis	Eukaryota; Plants; Land Plants	GCA_900406395.1	Scaffold	143.969	OVAY01	18372	0
Kokia drynarioides	Eukaryota; Plants; Land Plants	GCA_002814295.1	Scaffold	517.43	NTFQ01	15383	0
Lactuca sativa	Eukaryota; Plants; Land Plants	GCA_002870075.1	Scaffold	2384.19	NBSK01	11453	45242
Lactuca sativa	Eukaryota; Plants; Land Plants	GCA_900243165.1	Scaffold	2224.43	OFAD01	161898	0
Lactuca sativa	Eukaryota; Plants; Land Plants	GCA_900198505.1	Scaffold	1975.25	FZNH01	138326	0
Lactuca sativa	Eukaryota; Plants; Land Plants	GCA_000227445.1	Contig	1133.66	AFSA01	876110	0
Lagenaria siceraria	Eukaryota; Plants; Land Plants	GCA_003268545.1	Scaffold	313.387	NHZF01	438	0
Lagenaria siceraria	Eukaryota; Plants; Land Plants	GCA_000466325.1	Scaffold	176.727	ATBX01	305112	0
Lagenaria siceraria	Eukaryota; Plants; Land Plants	GCA_002890555.2	Chromosome	297.879	MIMD02	27	0
Larix sibirica	Eukaryota; Plants; Land Plants	GCA_004151065.1	Scaffold	12342.1	NWUY01	11325800	0

Leavenworthiaalabamica	Eukaryota; Plants; Land Plants	GCA_000411055.1	Scaffold	173.432	ASXC01	11715	0
Leersia perrieri	Eukaryota; Plants; Land Plants	GCA_000325765.3	Chromosome	266.688	ALNV02	12	0
Lepidium africanum	Eukaryota; Plants; Land Plants	GCA_900406405.1	Scaffold	225.262	OVAX01	17445	0
Lepidium aucheri	Eukaryota; Plants; Land Plants	GCA_900406435.1	Scaffold	332.063	OVBA01	31191	0
Lindernia brevidens	Eukaryota; Plants; Land Plants	GCA_004919715.1	Scaffold	266.105	SWDC01	158	0
Liriodendron chinense	Eukaryota; Plants; Land Plants	GCA_003013855.2	Scaffold	1742.42	PVNU02	3710	0
Lolium perenne	Eukaryota; Plants; Land Plants	GCA_001735685.1	Scaffold	481.479	MEHO01	666180	0
Lophocereus schottii	Eukaryota; Plants; Land Plants	GCA_002740545.1	Scaffold	797.926	NCQV01	158704	0
Lotus japonicus	Eukaryota; Plants; Land Plants	GCA_000181115.2	Contig	394.455	BABK02	44464	0
Lupinus angustifolius	Eukaryota; Plants; Land Plants	GCA_000338175.1	Scaffold	523.298	AOCW01	71995	0
Lupinus angustifolius	Eukaryota; Plants; Land Plants	GCA_001865875.1	Chromosome	609.203	MLAU01	14378	52821
Macadamia integrifolia	Eukaryota; Plants; Land Plants	GCA_900631585.1	Scaffold	744.636	UZVR01	4098	0
Macadamia integrifolia	Eukaryota; Plants; Land Plants	GCA_900087525.1	Scaffold	518.49	FLKO01	193493	0
Macleaya cordata	Eukaryota; Plants; Land Plants	GCA_002174775.1	Scaffold	377.834	MVGT01	4547	21911

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Macropodium nivale	Eukaryota; Plants; Land Plants	GCA_900406455.1	Scaffold	300.735	OVBF01	122604	0
Magnolia ashei	Eukaryota; Plants; Land Plants	GCA_003571905.1	Scaffold	284.512	PCNC01	265493	0
Malus baccata	Eukaryota; Plants; Land Plants	GCA_006547085.1	Scaffold	674.412	VIEB01	47473	45900
Malus domestica	Eukaryota; Plants; Land Plants	GCA_004115385.1	Chromosome	660.463	RDQH01	343	42841
Malus domestica	Eukaryota; Plants; Land Plants	GCA_000148765.2	Chromosome	1874.77	ACYM01	1667	0
Malus domestica	Eukaryota; Plants; Land Plants	GCA_002114115.1	Chromosome	703.358	MJAX01	807	52039
Manihot esculenta	Eukaryota; Plants; Land Plants	GCA_000737115.1	Scaffold	292.098	JPQF01	65771	0
Manihot esculenta	Eukaryota; Plants; Land Plants	GCA_003957885.1	Scaffold	1276.89	RSFS01	4440	0
Manihot esculenta	Eukaryota; Plants; Land Plants	GCA_003957995.1	Scaffold	1224.64	RSFT01	5398	0
Manihot esculenta	Eukaryota; Plants; Land Plants	GCA_001659605.1	Chromosome	582.279	LTYI01	2020	43286
Manihot esculenta subsp. flabellifolia	Eukaryota; Plants; Land Plants	GCA_000737105.1	Scaffold	390.836	JPQE01	54016	0
Marchantia inflexa	Eukaryota; Plants; Land Plants	GCA_006177815.1	Scaffold	208.753	QLSQ01	41556	0
Marchantia polymorpha	Eukaryota; Plants; Land Plants	GCA_003032435.1	Scaffold	225.761	PNPG01	2957	24674
Marchantia polymorpha subsp. ruderalis	Eukaryota; Plants; Land Plants	GCA_001641455.1	Scaffold	205.718	LVLJ01	4137	17956

Medicago truncatula	Eukaryota; Plants; Land Plants	GCA_003473485.2	Chromosome	429.612	PSQE01	40	44450
Medicago truncatula	Eukaryota; Plants; Land Plants	GCA_002024945.1	Scaffold	402.065	MWMB01	909	0
Medicago truncatula	Eukaryota; Plants; Land Plants	GCA_002251925.1	Scaffold	427.447	MLKM01	2368	0
Medicago truncatula	Eukaryota; Plants; Land Plants	GCA_002251935.1	Scaffold	394.059	MKZU01	1490	0
Medicago truncatula	Eukaryota; Plants; Land Plants	GCA_002251955.1	Scaffold	426.024	MNAG01	2700	0
Medicago truncatula	Eukaryota; Plants; Land Plants	GCA_000219495.2	Chromosome	412.924	APNO01	2187	41939
Mentha longifolia	Eukaryota; Plants; Land Plants	GCA_001642375.1	Scaffold	353.287	LSBG01	190876	0
Metrosideros polymorpha var. glaberrima	Eukaryota; Plants; Land Plants	GCA_001662345.1	Scaffold	304.366	BCNH01	36376	0
Mimosa pudica	Eukaryota; Plants; Land Plants	GCA_003254945.1	Scaffold	557.202	QANV01	97892	0
Miscanthus sacchariflorus	Eukaryota; Plants; Land Plants	GCA_002993905.1	Chromosome	2074.92	PUID01	137916	0
Momordica charantia	Eukaryota; Plants; Land Plants	GCA_001995035.1	Scaffold	285.614	BDCS01	1052	28666
Momordica charantia	Eukaryota; Plants; Land Plants	GCA_900491585.1	Scaffold	296.263	UESV01	3101	0
Monotropa hypopitys	Eukaryota; Plants; Land Plants	GCA_002855965.1	Contig	2197.49	NMUG01	1259264	0
Morella rubra	Eukaryota; Plants; Land Plants	GCA_003952965.1	Chromosome	313.02	RXIC01	500	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Morusn otabilis	Eukaryota; Plants; Land Plants	GCA_000414095.2	Scaffold	320.379	ATGF01	31301	27648
Mucuna pruriens	Eukaryota; Plants; Land Plants	GCA_003370565.1	Scaffold	397.042	QJKJ01	18487	56019
Musa acuminata subsp. malaccensis	Eukaryota; Plants; Land Plants	GCA_000313855.2	Chromosome	472.231	CAIC01	7512	47707
Musa balbisiana	Eukaryota; Plants; Land Plants	GCA_004837865.1	Chromosome	492.775	PYDT01	2590	33021
Musa itinerans	Eukaryota; Plants; Land Plants	GCA_001649415.1	Scaffold	455.349	LVTN01	28415	0
Musa schizocarpa	Eukaryota; Plants; Land Plants	GCA_900464855.1	Scaffold	525.283	UBIG01	194	0
Nasturtium officinale	Eukaryota; Plants; Land Plants	GCA_900406445.1	Scaffold	216.122	OVAZ01	10793	0
Nelumbo nucifera	Eukaryota; Plants; Land Plants	GCA_000365185.2	Scaffold	804.648	AQOG01	3603	38191
Nelumbo nucifera	Eukaryota; Plants; Land Plants	GCA_000805495.1	Scaffold	790.339	APLB01	14895	0
Nelumbo nucifera	Eukaryota; Plants; Land Plants	GCA_003033685.1	Chromosome	817.268	DLUB01	2341	0
Nelumbo nucifera	Eukaryota; Plants; Land Plants	GCA_003033695.1	Chromosome	799.479	DLUA01	12643	0
Nicotiana attenuata	Eukaryota; Plants; Land Plants	GCA_002018495.1	Scaffold	1827.78	MCOF01	951503	0
Nicotiana attenuata	Eukaryota; Plants; Land Plants	GCA_001879085.1	Chromosome	2365.68	MJEQ01	37194	44491
Nicotiana benthamiana	Eukaryota; Plants; Land Plants	GCA_000723945.1	Contig	61.9511	CBMM01	100480	0

Nicotiana glauca	Eukaryota; Plants; Land Plants	GCA_002930595.1	Scaffold	3222.83	PGPE01	514289	0
Nicotiana knightiana	Eukaryota; Plants; Land Plants	GCA_005239525.1	Scaffold	2298.94	MDKJ01	160415	0
Nicotiana obtusifolia	Eukaryota; Plants; Land Plants	GCA_002018475.1	Scaffold	1222.77	MCJB01	53128	0
Nicotiana otophora	Eukaryota; Plants; Land Plants	GCA_000715115.1	Scaffold	2689.35	AWOL01	929607	0
Nicotiana paniculata	Eukaryota; Plants; Land Plants	GCA_005239505.1	Scaffold	2190.56	MDKI01	181977	0
Nicotiana rustica	Eukaryota; Plants; Land Plants	GCA_005239535.1	Scaffold	4231.29	MDKG01	337581	0
Nicotiana sylvestris	Eukaryota; Plants; Land Plants	GCA_000393655.1	Scaffold	2221.99	ASAF01	253918	48160
Nicotiana tabacum	Eukaryota; Plants; Land Plants	GCA_000715135.1	Scaffold	3643.47	AYMY01	168247	84255
Nicotiana tabacum	Eukaryota; Plants; Land Plants	GCA_000715075.1	Scaffold	3732.64	AWOJ01	582565	0
Nicotiana tabacum	Eukaryota; Plants; Land Plants	GCA_000715095.1	Scaffold	3735.82	AWOK01	643545	0
Nicotiana tabacum	Eukaryota; Plants; Land Plants	GCA_002210045.1	Scaffold	4646.65	NCAA01	937112	0
Nicotiana tomentosiformis	Eukaryota; Plants; Land Plants	GCA_000390325.2	Scaffold	1688.47	ASAG01	159548	48963
Nicotiana undulata	Eukaryota; Plants; Land Plants	GCA_005239495.1	Scaffold	1914.3	MDKH01	117566	0
Nissolia schottii	Eukaryota; Plants; Land Plants	GCA_003254905.1	Scaffold	466.099	QANU01	116213	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Noccaea caerulescens	Eukaryota; Plants; Land Plants	GCA_900406465.1	Scaffold	140.792	OVBC01	19808	0
Noccaea goesingensis	Eukaryota; Plants; Land Plants	GCA_900406475.1	Scaffold	150.323	OVBG01	183017	0
Nothapodytes nimmoniana	Eukaryota; Plants; Land Plants	GCA_002091855.1	Contig	1.36527	BDGC01	2301	0
Nymphaea colorata	Eukaryota; Plants; Land Plants	GCA_902499525.1	Scaffold	409.931	CABVML01	806	0
Nymphaea colorata	Eukaryota; Plants; Land Plants	GCA_008831285.1	Chromosome	408.397	VYXN01	799	0
Nyssa sinensis	Eukaryota; Plants; Land Plants	GCA_008638375.1	Chromosome	1001.45	VIRR01	654	36241
Ochetophila trinervis	Eukaryota; Plants; Land Plants	GCA_003254975.1	Scaffold	309.116	QANX01	8237	0
Ocimum tenuiflorum	Eukaryota; Plants; Land Plants	GCA_001278415.1	Contig	332.617	AYJT01	121993	0
Ocimum tenuiflorum	Eukaryota; Plants; Land Plants	GCA_001748785.1	Contig	311.125	JQCZ01	230018	0
Odon tarrhena argentea	Eukaryota; Plants; Land Plants	GCA_900406245.1	Scaffold	183.186	OVAF01	32097	0
Oenanthe javanica	Eukaryota; Plants; Land Plants	GCA_008931105.1	Scaffold	1278.51	QRFB01	149923	0
Olea europaea subsp. europaea	Eukaryota; Plants; Land Plants	GCA_900603015.1	Scaffold	1318.65	UWJE01	11038	0
Olea europaea var. sylvestris	Eukaryota; Plants; Land Plants	GCA_002742605.1	Chromosome	1141.15	MSRW01	41226	58334
Oropetium thomaeum	Eukaryota; Plants; Land Plants	GCA_001182835.1	Contig	243.175	LFJQ01	625	0

Oryza barthii	Eukaryota; Plants; Land Plants	GCA_002926215.1	Scaffold	295.586	PQXR01	46663	0
Oryza barthii	Eukaryota; Plants; Land Plants	GCA_002926235.1	Scaffold	294.191	PQXQ01	52511	0
Oryza barthii	Eukaryota; Plants; Land Plants	GCA_003020155.1	Scaffold	292.235	PTLQ01	66245	0
Oryza barthii	Eukaryota; Plants; Land Plants	GCA_000182155.3	Chromosome	308.272	ABRL02	12	0
Oryza brachyantha	Eukaryota; Plants; Land Plants	GCA_000710545.1	Chromosome	14.4404	JNWF01	1	0
Oryza brachyantha	Eukaryota; Plants; Land Plants	GCA_000231095.2	Chromosome	259.908	AGAT01	2491	26803
Oryza glaberrima	Eukaryota; Plants; Land Plants	GCA_000147395.2	Scaffold	303.295	ADWL01	25599	0
Oryza glumipatula	Eukaryota; Plants; Land Plants	GCA_000576495.1	Chromosome	372.86	ALNU02	12	0
Oryza longistaminata	Eukaryota; Plants; Land Plants	GCA_000789195.1	Scaffold	326.443	AMDW01	60198	0
Oryza longistaminata	Eukaryota; Plants; Land Plants	GCA_001514335.2	Chromosome	362.064	LQBC01	11745	0
Oryza meridionalis	Eukaryota; Plants; Land Plants	GCA_001551795.1	Contig	354.611	LONC01	3249	0
Oryza meridionalis	Eukaryota; Plants; Land Plants	GCA_000338895.2	Chromosome	335.668	ALNW02	12	0
Oryza meyeriana var. granulata	Eukaryota; Plants; Land Plants	GCA_005223365.1	Scaffold	736.649	SPHZ01	2389	0
Oryza meyeriana var. granulata	Eukaryota; Plants; Land Plants	GCA_003991445.1	Contig	776.957	RYFJ01	4618	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Oryza meyeriana var. granulata	Eukaryota; Plants; Land Plants	GCA_000325645.2	Scaffold	35.2457	ALNT01	1	0
Oryza minuta	Eukaryota; Plants; Land Plants	GCA_000632695.1	Chromosome	45.1659	JJNN01	2	0
Oryza officinalis	Eukaryota; Plants; Land Plants	GCA_008326285.1	Scaffold	584.134	BDMV01	91	0
Oryza officinalis	Eukaryota; Plants; Land Plants	GCA_000717455.1	Chromosome	26.1885	JJMQ01	1	0
Oryza punctata	Eukaryota; Plants; Land Plants	GCA_000710525.1	Chromosome	22.4654	JNWE01	1	0
Oryza punctata	Eukaryota; Plants; Land Plants	GCA_000573905.1	Chromosome	393.817	AVCL01	12	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_000817225.1	Scaffold	339.177	CBQP01	3818	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_001551805.1	Contig	384.518	LONB01	2582	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609075.1	Contig	0.391959	UXAX01	64	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609175.1	Contig	0.439665	UXBI01	116	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609365.1	Contig	0.435203	UXBZ01	67	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609345.1	Contig	0.430311	UXBX01	112	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609235.1	Contig	0.431171	UXBN01	130	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609435.1	Contig	0.460371	UXCI01	141	0

Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609385.1	Contig	0.522775	UXCD01	399	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609605.1	Contig	0.479941	UXCZ01	272	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609155.1	Contig	0.451183	UXBF01	185	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609195.1	Contig	0.444689	UXBK01	168	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609375.1	Contig	0.418576	UXCA01	72	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609635.1	Contig	0.437219	UXDC01	115	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609335.1	Contig	0.426264	UXBY01	80	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609565.1	Contig	0.416343	UXCV01	99	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609455.1	Contig	0.437726	UXCL01	172	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609145.1	Contig	0.4324	UXBJ01	128	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609205.1	Contig	0.429589	UXBM01	129	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609535.1	Contig	0.425584	UXCS01	176	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609445.1	Contig	0.428734	UXCJ01	87	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609595.1	Contig	0.445408	UXDA01	162	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609645.1	Contig	0.448673	UXDD01	155	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609285.1	Contig	0.420471	UXBV01	143	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609165.1	Contig	0.426734	UXBG01	121	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609325.1	Contig	0.456272	UXCH01	231	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609355.1	Contig	0.427134	UXCB01	114	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609415.1	Contig	0.439065	UXCE01	125	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609575.1	Contig	0.426419	UXCW01	113	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609125.1	Contig	0.461807	UXBC01	223	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609475.1	Contig	0.435078	UXCN01	159	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609215.1	Contig	0.433062	UXBO01	157	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609395.1	Contig	0.399871	UXCF01	66	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609585.1	Contig	0.433896	UXCY01	137	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609555.1	Contig	0.423266	UXCT01	170	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609295.1	Contig	0.403327	UXBR01	109	0

Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609545.1	Contig	0.436432	UXCU01	221	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609185.1	Contig	0.434658	UXBH01	155	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609525.1	Contig	0.44213	UXCQ01	233	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609105.1	Contig	0.42703	UXBD01	130	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609615.1	Contig	0.4457	UXCX01	188	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609505.1	Contig	0.49038	UXCR01	321	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609135.1	Contig	0.424383	UXBE01	108	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609495.1	Contig	0.429759	UXCM01	130	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609305.1	Contig	0.531837	UXBS01	488	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609625.1	Contig	0.442979	UXDB01	201	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609265.1	Contig	0.430376	UXBQ01	146	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609245.1	Contig	0.441818	UXBP01	94	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609405.1	Contig	0.466007	UXCC01	262	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609225.1	Contig	0.428872	UXBL01	138	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609465.1	Contig	0.420063	UXCK01	121	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609255.1	Contig	0.551867	UXBT01	457	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609515.1	Contig	0.438855	UXCP01	218	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609085.1	Contig	0.467544	UXAY01	265	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609275.1	Contig	0.552388	UXBU01	559	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609095.1	Contig	0.4166	UXBA01	216	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609315.1	Contig	0.576561	UXBW01	564	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609485.1	Contig	0.512907	UXCO01	440	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609425.1	Contig	0.5568	UXCG01	519	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609115.1	Contig	0.416778	UXBB01	188	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_900609665.1	Contig	0.521122	UXDG01	471	0
Oryza rufipogon	Eukaryota; Plants; Land Plants	GCA_000700045.1	Chromosome	12.7409	JNHC01	1	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_001648735.1	Scaffold	307.225	LVCG01	55637	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_001648745.1	Scaffold	295.39	LVCH01	64800	0

Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609875.1	Contig	0.418428	UXEA01	94	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609755.1	Contig	0.412758	UXDO01	77	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900610885.1	Contig	0.415805	UXHM01	101	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609945.1	Contig	0.420498	UXEH01	97	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609745.1	Contig	0.412413	UXDL01	77	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609855.1	Contig	0.424341	UXDZ01	111	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609765.1	Contig	0.426436	UXDS01	105	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609895.1	Contig	0.420731	UXED01	102	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609705.1	Contig	0.413182	UXDI01	83	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609835.1	Contig	0.414506	UXDX01	86	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609655.1	Contig	0.4229	UXDE01	95	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609715.1	Contig	0.421534	UXDK01	109	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609735.1	Contig	0.412833	UXDM01	71	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609915.1	Contig	0.417059	UXEE01	85	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609955.1	Contig	0.41464	UXEI01	83	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609885.1	Contig	0.415361	UXEC01	81	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900610895.1	Contig	0.414252	UXHO01	91	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609905.1	Contig	0.416009	UXEB01	104	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609865.1	Contig	0.4183	UXDY01	102	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609845.1	Contig	0.424074	UXDW01	114	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609685.1	Contig	0.415611	UXDH01	101	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609925.1	Contig	0.412137	UXEF01	98	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609695.1	Contig	0.414261	UXDJ01	76	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609775.1	Contig	0.411428	UXDP01	78	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609825.1	Contig	0.415893	UXDV01	83	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609725.1	Contig	0.413692	UXDN01	95	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609805.1	Contig	0.411297	UXDU01	88	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609675.1	Contig	0.413512	UXDF01	83	0

Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609935.1	Contig	0.415952	UXEG01	100	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609815.1	Contig	0.405341	UXDT01	71	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609785.1	Contig	0.42347	UXDQ01	148	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_900609795.1	Contig	0.422231	UXDR01	125	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_003865215.1	Chromosome	395.354	PKRX01	127	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_004007595.1	Chromosome	377.604	RPSM01	615	0
Oryza sativa	Eukaryota; Plants; Land Plants	GCA_004348155.2	Chromosome	415.393	QQAJ01	367	0
Oryza sativa aus subgroup	Eukaryota; Plants; Land Plants	GCA_001952365.2	Chromosome	372.203	LWDA01	1312	0
Oryza sativa f. spontanea	Eukaryota; Plants; Land Plants	GCA_006942195.1	Scaffold	377.675	QKSA01	350	0
Oryza sativa f. spontanea	Eukaryota; Plants; Land Plants	GCA_000710535.2	Chromosome	19.4244	JNWG02	1	0
Oryza sativa f. spontanea	Eukaryota; Plants; Land Plants	GCA_000576065.1	Chromosome	337.95	AWHD01	12	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001623365.2	Chromosome	387.424	LNNK02	19	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001623345.2	Chromosome	387.326	LNNJ02	20	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001889745.1	Chromosome	389.088	MPPV01	66	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001618795.1	Chromosome	386.486	LBBA01	8481	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_000725085.2	Chromosome	389.753	AZTA02	15907	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001618785.1	Chromosome	398.762	LBAZ01	11486	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_000004655.2	Chromosome	426.337	AAAA02	10627	37358
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_003449045.1	Contig	388.772	QWGD01	410	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001611195.1	Scaffold	351.225	LQHG01	39669	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001611255.1	Scaffold	331.819	LQHF01	75421	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_001611235.1	Scaffold	352.227	LQHE01	116212	0
Oryza sativa Indica Group	Eukaryota; Plants; Land Plants	GCA_006992885.1	Scaffold	281.326	SWLY01	91264	0
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_003449065.1	Contig	378.097	QWGC01	144	0
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_002573525.1	Contig	418.901	PDFQ01	588	0
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_003865235.1	Chromosome	379.626	PKRW01	115	0
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_000817635.1	Chromosome	337.74	JSUG01	1739	0
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_000817615.1	Chromosome	342.028	JSUF01	1160	0

Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_000149285.1	Chromosome	391.148	AACV01	7777	35394
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_000321445.1	Chromosome	382.627	BACJ01	12	0
Oryza sativa Japonica Group	Eukaryota; Plants; Land Plants	GCA_000164945.1	Chromosome	382.151	BABO01	12	0
Pachycereus pringlei	Eukaryota; Plants; Land Plants	GCA_002740445.1	Scaffold	629.656	NCQS01	171584	0
Panicum hallii	Eukaryota; Plants; Land Plants	GCA_002211085.2	Chromosome	535.889	NCQW02	1027	37612
Panicum hallii var. hallii	Eukaryota; Plants; Land Plants	GCA_003061485.1	Chromosome	487.474	QAVV01	144	42523
Panicum miliaceum	Eukaryota; Plants; Land Plants	GCA_002895445.2	Chromosome	848.352	PPDP02	466	0
Panicum miliaceum	Eukaryota; Plants; Land Plants	GCA_003046395.2	Chromosome	854.793	PQIB02	1306	55964
Papaver somniferum	Eukaryota; Plants; Land Plants	GCA_003573695.1	Chromosome	2715.53	PUWZ01	34381	84179
Parasponia andersonii	Eukaryota; Plants; Land Plants	GCA_002914805.1	Scaffold	475.834	JXTB01	2732	37227
Passiflora edulis	Eukaryota; Plants; Land Plants	GCA_002156105.1	Scaffold	165.657	MUZT01	234012	0
Penstemon barbatus	Eukaryota; Plants; Land Plants	GCA_003313485.1	Contig	696.306	QOIQ01	18827	0
Penstemon centranthifolius	Eukaryota; Plants; Land Plants	GCA_000737435.1	Contig	4.47159	JPFH01	6761	0
Penstemon cyananthus	Eukaryota; Plants; Land Plants	GCA_000281005.1	Contig	4.62226	AKKG01	9712	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Penstemon davidsonii	Eukaryota; Plants; Land Plants	GCA_000280985.1	Contig	2.37523	AKKI01	4880	0
Penstemon dissectus	Eukaryota; Plants; Land Plants	GCA_000280965.1	Contig	2.62809	AKKH01	5361	0
Penstemon fruticosus	Eukaryota; Plants; Land Plants	GCA_000281025.1	Contig	2.31904	AKKJ01	4770	0
Penstemon grinnellii	Eukaryota; Plants; Land Plants	GCA_000737425.1	Contig	3.66352	JPFI01	5523	0
Pereskia humboldtii	Eukaryota; Plants; Land Plants	GCA_002740485.1	Scaffold	414.047	NCQU01	126352	0
Perilla citriodora	Eukaryota; Plants; Land Plants	GCA_004303085.1	Scaffold	618.797	SDAM01	29924	0
Persea americana	Eukaryota; Plants; Land Plants	GCA_008087245.1	Contig	912.698	SDSS01	8135	0
Persea americana	Eukaryota; Plants; Land Plants	GCA_002908915.1	Contig	446.756	NXHZ01	5000	0
Persea americana var. drymifolia	Eukaryota; Plants; Land Plants	GCA_008033785.1	Scaffold	820.369	SDXN01	43777	0
Phalaenopsis aphrodite	Eukaryota; Plants; Land Plants	GCA_003013225.1	Scaffold	1025.1	NEWO01	13732	0
Phalaenopsis equestris	Eukaryota; Plants; Land Plants	GCA_001263595.1	Scaffold	1064.2	APLD01	89584	29894
Phalaenopsis hybrid cultivar	Eukaryota; Plants; Land Plants	GCA_002079205.1	Scaffold	2687.66	JXCR01	149149	0
Phaseolus coccineus subsp. coccineus	Eukaryota; Plants; Land Plants	GCA_003122825.1	Scaffold	371.086	QBDZ01	192921	0
Phaseolus vulgaris	Eukaryota; Plants; Land Plants	GCA_001517995.1	Chromosome	549.748	LPQZ01	68335	0

Phaseolus vulgaris	Eukaryota; Plants; Land Plants	GCA_000499845.1	Chromosome	521.077	ANNZ01	708	32720
Phoenix dactylifera	Eukaryota; Plants; Land Plants	GCA_000413155.1	Scaffold	556.481	ATBV01	80317	40634
Phoenix dactylifera	Eukaryota; Plants; Land Plants	GCA_000181215.2	Scaffold	381.563	ACYX02	57277	0
Phoenix dactylifera	Eukaryota; Plants; Land Plants	GCA_007821505.1	Scaffold	454.367	PEFZ01	252335	0
Physaria acutifolia	Eukaryota; Plants; Land Plants	GCA_900406485.1	Scaffold	199.442	OVBH01	276837	0
Physaria fendleri	Eukaryota; Plants; Land Plants	GCA_900406525.1	Scaffold	331.342	OVBV01	99932	0
Physaria ovalifolia	Eukaryota; Plants; Land Plants	GCA_900406505.1	Scaffold	290.267	OVBY01	335399	0
Physcomitrella patens	Eukaryota; Plants; Land Plants	GCA_000002425.2	Chromosome	472.081	ABEU02	359	48022
Picea abies	Eukaryota; Plants; Land Plants	GCA_900067695.1	Scaffold	11961.4	CBVK01	11340369	0
Picea abies var. abies	Eukaryota; Plants; Land Plants	GCA_900491625.1	Scaffold	42.7831	UETF01	41150	0
Picea glauca	Eukaryota; Plants; Land Plants	GCA_000411955.5	Scaffold	24633.1	ALWZ04	3033322	6445
Picea glauca	Eukaryota; Plants; Land Plants	GCA_000966675.1	Scaffold	26936.2	JZKD01	3353683	0
Picea glauca	Eukaryota; Plants; Land Plants	GCA_001687225.1	Contig	258.272	LDPM01	222034	0
Pinus lambertiana	Eukaryota; Plants; Land Plants	GCA_001447015.2	Scaffold	27602.7	LMTP01	4253097	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Pinus sylvestris	Eukaryota; Plants; Land Plants	GCA_900143225.1	Contig	0.985624	FRDG01	224	0
Pinus taeda	Eukaryota; Plants; Land Plants	GCA_000404065.3	Scaffold	22103.6	APFE03	1760464	0
Pistacia vera	Eukaryota; Plants; Land Plants	GCA_008641045.1	Scaffold	671.28	VTWI01	1865	82
Pisum sativum	Eukaryota; Plants; Land Plants	GCA_003013575.1	Scaffold	4275.93	PUCA01	5449423	0
Platycodon grandiflorus	Eukaryota; Plants; Land Plants	GCA_004681165.1	Scaffold	680.178	SPEA01	4816	0
Pleurozium schreberi	Eukaryota; Plants; Land Plants	GCA_006891605.1	Contig	220.032	VACF01	2689	0
Pogostemon cablin	Eukaryota; Plants; Land Plants	GCA_003675935.1	Scaffold	1916.69	QKXD01	41698	0
Populus alba	Eukaryota; Plants; Land Plants	GCA_005239225.1	Contig	416.961	RCHU01	1287	32959
Populus euphratica	Eukaryota; Plants; Land Plants	GCA_000495115.1	Scaffold	496.033	AOFL01	9615	49760
Populus simonii	Eukaryota; Plants; Land Plants	GCA_007827005.2	Chromosome	441.407	VJNQ02	686	0
Populus trichocarpa	Eukaryota; Plants; Land Plants	GCA_000002775.3	Chromosome	434.29	AARH03	1694	51717
Primula veris	Eukaryota; Plants; Land Plants	GCA_000788445.1	Scaffold	309.693	JTKG01	8756	0
Primula vulgaris	Eukaryota; Plants; Land Plants	GCA_001077355.1	Scaffold	1.50478	CDJJ02	229	0
Primula vulgaris	Eukaryota; Plants; Land Plants	GCA_001403715.1	Scaffold	1.50478	CYSU01	229	0

Prosopis alba	Eukaryota; Plants; Land Plants	GCA_004799145.1	Contig	707.162	SMJV01	6087	57572
Prunus avium	Eukaryota; Plants; Land Plants	GCA_002207925.1	Scaffold	272.362	BDGV01	10148	35009
Prunus avium	Eukaryota; Plants; Land Plants	GCA_003946875.1	Contig	287.192	QXJJ01	1540	0
Prunus dulcis	Eukaryota; Plants; Land Plants	GCA_902201215.1	Chromosome	227.599	CABIKO01	691	32556
Prunus mume	Eukaryota; Plants; Land Plants	GCA_000346735.1	Chromosome	234.03	AOHF01	8626	29705
Prunus persica	Eukaryota; Plants; Land Plants	GCA_000218175.1	Scaffold	214.225	AEJG01	30834	0
Prunus persica	Eukaryota; Plants; Land Plants	GCA_000218215.1	Scaffold	207.185	AEKV01	43890	0
Prunus persica	Eukaryota; Plants; Land Plants	GCA_000218195.1	Scaffold	211.308	AEKW01	35219	0
Prunus persica	Eukaryota; Plants; Land Plants	GCA_000346465.2	Chromosome	227.569	AKXU02	192	32595
Prunus yedoensis	Eukaryota; Plants; Land Plants	GCA_005406145.1	Contig	690.106	BJCG01	4571	0
Prunus yedoensis var. nudiflora	Eukaryota; Plants; Land Plants	GCA_002966975.2	Scaffold	319.21	PJQY01	4016	41294
Prunus yedoensis var. nudiflora	Eukaryota; Plants; Land Plants	GCA_900382725.1	Scaffold	319.21	OSDV01	4016	0
Pseudotsuga menziesii	Eukaryota; Plants; Land Plants	GCA_001517045.1	Scaffold	14673.2	LPNX01	1236665	0
Pseudoturritis turrita	Eukaryota; Plants; Land Plants	GCA_900406555.1	Contig	321.563	OVBL01	1111	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Pseudoturritis turrita	Eukaryota; Plants; Land Plants	GCA_900406515.1	Scaffold	263.513	OVBK01	25008	0
Psidium guajava	Eukaryota; Plants; Land Plants	GCA_002914565.1	Contig	386.852	NTGF01	4728	0
Pterocarya stenoptera	Eukaryota; Plants; Land Plants	GCA_003123785.1	Scaffold	955.601	QEOT01	124315	0
Punica granatum	Eukaryota; Plants; Land Plants	GCA_002864125.1	Scaffold	274.043	MTJX01	2117	0
Punica granatum	Eukaryota; Plants; Land Plants	GCA_002201585.1	Scaffold	296.383	MTKT01	17405	29127
Punica granatum	Eukaryota; Plants; Land Plants	GCA_002837095.1	Scaffold	380.178	PGOL01	45308	50476
Punica granatum	Eukaryota; Plants; Land Plants	GCA_007655135.2	Chromosome	320.336	MABG02	473	0
Purshia tridentata	Eukaryota; Plants; Land Plants	GCA_003254885.1	Scaffold	175.971	QANT01	9353	0
Pyrus betulifolia	Eukaryota; Plants; Land Plants	GCA_007844245.1	Chromosome	532.747	VDML01	139	0
Pyrus x bretschneideri	Eukaryota; Plants; Land Plants	GCA_000315295.1	Scaffold	508.551	AJSU01	2182	47086
Quercus lobata	Eukaryota; Plants; Land Plants	GCA_001633185.2	Chromosome	846.07	LRBV02	2010	53228
Quercus robur	Eukaryota; Plants; Land Plants	GCA_900291515.1	Scaffold	814.336	OLKR01	550	0
Quercus robur	Eukaryota; Plants; Land Plants	GCA_003013145.1	Scaffold	719.602	PVWZ01	84416	0
Quercus suber	Eukaryota; Plants; Land Plants	GCA_002906115.1	Scaffold	953.299	PKMF01	23344	59614

Quillaja saponaria	Eukaryota; Plants; Land Plants	GCA_003338715.1	Contig	248.908	PVLG01	48349	0
Raddia distichophylla	Eukaryota; Plants; Land Plants	GCA_005191435.1	Scaffold	580.833	SPJY01	38257	0
Raparia bulbosa	Eukaryota; Plants; Land Plants	GCA_900406535.1	Scaffold	152.784	OVBJ01	13874	0
Raphanus raphanistrum subsp. raphanistrum	Eukaryota; Plants; Land Plants	GCA_000769845.1	Contig	253.834	JRQH01	64732	0
Raphanus sativus	Eukaryota; Plants; Land Plants	GCA_000801105.2	Scaffold	426.614	JRUI02	10676	61216
Raphanus sativus	Eukaryota; Plants; Land Plants	GCA_000715565.1	Scaffold	402.328	BAUK01	76592	0
Raphanus sativus	Eukaryota; Plants; Land Plants	GCA_001047155.1	Scaffold	383.105	BAOO01	40123	0
Raphanus sativus	Eukaryota; Plants; Land Plants	GCA_002197605.1	Chromosome	382.79	JSDR01	44239	0
Rhamnella rubrinervis	Eukaryota; Plants; Land Plants	GCA_007844105.1	Chromosome	245.336	VOIH01	133	0
Rhazya stricta	Eukaryota; Plants; Land Plants	GCA_001752375.1	Scaffold	274.354	MEJB01	979	0
Rhizophora apiculata	Eukaryota; Plants; Land Plants	GCA_900174605.1	Scaffold	232.055	FWPW01	142	0
Rhizophora apiculata	Eukaryota; Plants; Land Plants	GCA_900004065.1	Scaffold	232.431	CELW01	45996	0
Rhodamnia argentea	Eukaryota; Plants; Land Plants	GCA_900635035.1	Scaffold	414.816	CAAAGQ01	15781	42570
Rhodoleia championii	Eukaryota; Plants; Land Plants	GCA_008932045.1	Contig	105.726	VMOD01	278658	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Ricinus communis	Eukaryota; Plants; Land Plants	GCA_000151685.2	Scaffold	350.622	AASG02	25763	28584
Rosa chinensis	Eukaryota; Plants; Land Plants	GCA_002994745.1	Chromosome	513.854	PDCK01	45	45097
Rosa lucieae	Eukaryota; Plants; Land Plants	GCA_006954505.1	Scaffold	786.105	RQIQ01	500476	0
Rosa multiflora	Eukaryota; Plants; Land Plants	GCA_002564525.1	Scaffold	739.638	BDJD01	83189	0
Rosa x damascena	Eukaryota; Plants; Land Plants	GCA_001662545.1	Scaffold	711.72	LYNE01	307872	0
Ruellia speciosa	Eukaryota; Plants; Land Plants	GCA_001909325.1	Contig	740.036	MAYD01	794288	0
Saccharum hybrid cultivar	Eukaryota; Plants; Land Plants	GCA_900465005.1	Scaffold	530.66	UBIK01	5708	0
Saccharum hybrid cultivar SP80-3280	Eukaryota; Plants; Land Plants	GCA_008692665.1	Scaffold	4014.93	QPEU01	398353	0
Saccharum hybrid cultivar SP80-3280	Eukaryota; Plants; Land Plants	GCA_002018215.1	Contig	1169.95	JXQF01	199028	0
Saccharum hybrid cultivar SP80-3280	Eukaryota; Plants; Land Plants	GCA_009173535.1	Scaffold	49.3852	PYBL01	461	0
Saccharum spontaneum	Eukaryota; Plants; Land Plants	GCA_900500655.1	Contig	3924.19	UINE01	75981	0
Saccharum spontaneum	Eukaryota; Plants; Land Plants	GCA_003544955.1	Chromosome	3133.29	QVOL01	15303	0
Salix brachista	Eukaryota; Plants; Land Plants	GCA_009078335.1	Chromosome	339.588	VDCV01	30	30209
Salvia splendens	Eukaryota; Plants; Land Plants	GCA_004379255.1	Scaffold	809.16	PNBA01	1525	53354

Santalum album	Eukaryota; Plants; Land Plants	GCA_002911635.1	Contig	196.101	NXEK01	180	0
Santalum album	Eukaryota; Plants; Land Plants	GCA_002925775.1	Scaffold	220.961	LOCJ01	12821	0
Schrenkiella parvula	Eukaryota; Plants; Land Plants	GCA_000218505.1	Chromosome	137.073	AFAN01	1463	0
Scutellaria baicalensis	Eukaryota; Plants; Land Plants	GCA_005771605.1	Chromosome	386.674	VALJ01	114	0
Secale cereale	Eukaryota; Plants; Land Plants	GCA_900079665.1	Scaffold	1684.93	FKKI01	1581707	0
Secale cereale	Eukaryota; Plants; Land Plants	GCA_900002355.1	Scaffold	1684.93	CCJQ01	1581707	0
Sedum album	Eukaryota; Plants; Land Plants	GCA_006409495.1	Contig	302.251	QZGG01	6038	0
Selaginella kraussiana	Eukaryota; Plants; Land Plants	GCA_001021135.1	Scaffold	114.503	LDJE01	105914	0
Selaginella moellendorffii	Eukaryota; Plants; Land Plants	GCA_000143415.2	Scaffold	212.315	ADFJ01	757	45247
Selaginella tamariscina	Eukaryota; Plants; Land Plants	GCA_003024785.1	Scaffold	300.729	PUQB01	1391	0
Sequoia sempervirens	Eukaryota; Plants; Land Plants	GCA_007258455.1	Scaffold	26537.2	VDFB01	517852	0
Sequoia dendrongiganteum	Eukaryota; Plants; Land Plants	GCA_007115665.1	Scaffold	8122.13	VCHN01	39798	0
Sesamum indicum	Eukaryota; Plants; Land Plants	GCA_001692995.1	Scaffold	210.758	MBSK01	5868	0
Sesamum indicum	Eukaryota; Plants; Land Plants	GCA_003268515.1	Scaffold	242.679	LUAT01	48805	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Sesamum indicum	Eukaryota; Plants; Land Plants	GCA_000975565.1	Scaffold	340.464	JPLX01	76023	0
Sesamum indicum	Eukaryota; Plants; Land Plants	GCA_000512975.1	Chromosome	275.059	APMJ01	16369	35410
Setaria italica	Eukaryota; Plants; Land Plants	GCA_001652605.1	Chromosome	477.542	LWRS01	2689	0
Setaria italica	Eukaryota; Plants; Land Plants	GCA_000263155.2	Chromosome	405.868	AGNK02	337	35844
Setaria viridis	Eukaryota; Plants; Land Plants	GCA_005286985.1	Chromosome	395.732	SNSE01	75	52459
Silene latifolia	Eukaryota; Plants; Land Plants	GCA_003260165.1	Scaffold	1185.09	QBIE01	319506	0
Silene latifolia	Eukaryota; Plants; Land Plants	GCA_900095335.1	Contig	36.0486	FMHP01	46178	0
Silene latifolia subsp. alba	Eukaryota; Plants; Land Plants	GCA_001412135.1	Scaffold	665.279	LHUT01	307720	0
Silphium perfoliatum	Eukaryota; Plants; Land Plants	GCA_900538075.1	Contig	121.712	UXAI01	1197534	0
Silybum marianum	Eukaryota; Plants; Land Plants	GCA_001541825.1	Contig	1477.57	LMWD01	258575	0
Sisymbrium altissimum	Eukaryota; Plants; Land Plants	GCA_900406495.1	Scaffold	178.647	OVBI01	14597	0
Sisymbrium irio	Eukaryota; Plants; Land Plants	GCA_000411075.1	Scaffold	245.55	ASZH01	21357	0
Solanum americanum	Eukaryota; Plants; Land Plants	GCA_900188915.1	Contig	9.01369	FYFB01	837	0
Solanum americanum	Eukaryota; Plants; Land Plants	GCA_900188785.1	Contig	7.74921	FYHF01	1085	0

Solanum americanum	Eukaryota; Plants; Land Plants	GCA_900188895.1	Contig	7.74473	FYHB01	1085	0
Solanum americanum	Eukaryota; Plants; Land Plants	GCA_900188885.1	Contig	7.6066	FYHD01	1085	0
Solanum americanum	Eukaryota; Plants; Land Plants	GCA_900198685.1	Contig	8.308	FZPQ01	1460	0
Solanum americanum	Eukaryota; Plants; Land Plants	GCA_900188835.1	Contig	9.83576	FYHH01	1483	0
Solanum arcanum	Eukaryota; Plants; Land Plants	GCA_000612985.1	Contig	665.187	CBYQ01	46594	0
Solanum chilense	Eukaryota; Plants; Land Plants	GCA_006013705.1	Scaffold	913.881	RXGB01	81304	19
Solanum commersonii	Eukaryota; Plants; Land Plants	GCA_001239805.1	Scaffold	729.603	JXZD01	63664	0
Solanum habrochaites	Eukaryota; Plants; Land Plants	GCA_000577655.1	Contig	724.285	CBYS01	42990	0
Solanum lycopersicum	Eukaryota; Plants; Land Plants	GCA_000181095.1	Scaffold	540.589	BABP01	100783	0
Solanum lycopersicum	Eukaryota; Plants; Land Plants	GCA_000325825.1	Scaffold	0.575198	AFYB01	195	0
Solanum lycopersicum	Eukaryota; Plants; Land Plants	GCA_000188115.3	Chromosome	828.349	AEKE03	3150	37660
Solanum melongena	Eukaryota; Plants; Land Plants	GCA_000787875.1	Scaffold	833.081	BAUE01	33873	0
Solanum pennellii	Eukaryota; Plants; Land Plants	GCA_000577875.1	Contig	720.458	CBYR01	57205	0
Solanum pennellii	Eukaryota; Plants; Land Plants	GCA_000820945.1	Contig	720.458	CCXL01	57205	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Solanum pimpinellifolium	Eukaryota; Plants; Land Plants	GCA_003660305.1	Scaffold	748.694	NRDK01	48863	0
Solanum pimpinellifolium	Eukaryota; Plants; Land Plants	GCA_000230315.1	Contig	688.247	AGFK01	309180	0
Solanum tuberosum	Eukaryota; Plants; Land Plants	GCA_000226075.1	Scaffold	705.934	AEWC01	14854	37966
Solanum tuberosum	Eukaryota; Plants; Land Plants	GCA_900004685.1	Contig	90.4582	CVMJ01	39446	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185145.1	Scaffold	730.142	FYAA01	224100	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185165.1	Scaffold	667.086	FXZQ01	2343	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185285.1	Contig	659.291	FXZO01	2461	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185275.1	Scaffold	662.264	FXZP01	1331	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185325.1	Scaffold	659.429	FXZS01	1377	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185305.1	Scaffold	716.55	FXZW01	4814	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185245.1	Contig	715.934	FXZV01	5571	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185335.1	Scaffold	749.835	FXZU01	5164	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185175.1	Scaffold	764.063	FXZT01	7840	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185215.1	Contig	722.285	FXZR01	8138	0

Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185185.1	Scaffold	751.585	FYAB01	321725	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185195.1	Scaffold	740.946	FYAC01	170623	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185295.1	Scaffold	728.86	FYAD01	228374	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185265.1	Scaffold	729.31	FYAF01	224108	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185155.1	Scaffold	690.417	FXZY01	22476	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185205.1	Scaffold	688.737	FXZX01	22492	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185315.1	Scaffold	710.407	FYAG01	246082	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185225.1	Scaffold	730.903	FXZZ01	182474	0
Solanum verrucosum	Eukaryota; Plants; Land Plants	GCA_900185235.1	Scaffold	759.168	FYAE01	569872	0
Sorghum bicolor	Eukaryota; Plants; Land Plants	GCA_003482435.1	Scaffold	666.155	QWKM01	308	0
Sorghum bicolor	Eukaryota; Plants; Land Plants	GCA_008000285.1	Contig	374.252	VOIB01	2657	0
Sorghum bicolor	Eukaryota; Plants; Land Plants	GCA_000236765.2	Contig	0.015475	AHAO01	16	16
Sorghum bicolor	Eukaryota; Plants; Land Plants	GCA_000236725.2	Contig	0.018494	AHAQ01	20	22
Sorghum bicolor	Eukaryota; Plants; Land Plants	GCA_000236745.2	Contig	0.021299	AHAP01	35	35

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Sorghum bicolor	Eukaryota; Plants; Land Plants	GCA_000003195.3	Chromosome	709.345	ABXC03	869	39248
Spatholobus suberectus	Eukaryota; Plants; Land Plants	GCA_004329165.1	Chromosome	798.47	QUWT01	816	31106
Spinacia oleracea	Eukaryota; Plants; Land Plants	GCA_002007265.1	Scaffold	869.946	LZYP01	78263	32794
Spinacia oleracea	Eukaryota; Plants; Land Plants	GCA_000510995.2	Scaffold	493.772	AYZV02	103502	23522
Spirodela polyrhiza	Eukaryota; Plants; Land Plants	GCA_900492545.1	Scaffold	138.592	UIDA01	20	0
Spirodela polyrhiza	Eukaryota; Plants; Land Plants	GCA_008360905.1	Scaffold	138.536	SWLF01	134	0
Spirodela polyrhiza	Eukaryota; Plants; Land Plants	GCA_900536055.1	Scaffold	142.661	UNPA01	2585	0
Spirodela polyrhiza	Eukaryota; Plants; Land Plants	GCA_000504445.1	Contig	132.009	ATDW01	16051	0
Sporobolus alterniflorus	Eukaryota; Plants; Land Plants	GCA_008808055.1	Contig	365.573	VSTD01	52184	0
Stenocereus thurberi	Eukaryota; Plants; Land Plants	GCA_002740465.1	Scaffold	853.348	NCQT01	159477	0
Striga asiatica	Eukaryota; Plants; Land Plants	GCA_008636005.1	Scaffold	471.563	BKCP01	13846	33426
Syzygium oleosum	Eukaryota; Plants; Land Plants	GCA_900635055.1	Scaffold	431.291	CAAAGS01	19039	38158
Tarenaya hassleriana	Eukaryota; Plants; Land Plants	GCA_000463585.1	Scaffold	249.93	AOUI01	12249	41094
Theobroma cacao	Eukaryota; Plants; Land Plants	GCA_000403535.1	Chromosome	345.994	ALXC01	814	44186

Theobroma cacao	Eukaryota; Plants; Land Plants	GCA_000208745.2	Chromosome	324.88	FLSQ01	431	30854
Thlaspi arvense	Eukaryota; Plants; Land Plants	GCA_000956625.1	Scaffold	343.012	AZNP01	6768	0
Trema orientale	Eukaryota; Plants; Land Plants	GCA_002914845.1	Scaffold	387.958	JXTC01	2756	35849
Trichopus zeylanicus subsp. travancoricus	Eukaryota; Plants; Land Plants	GCA_005019695.1	Scaffold	713.407	RXID01	22601	0
Trifolium medium	Eukaryota; Plants; Land Plants	GCA_003490085.1	Scaffold	492.653	LXQA01	1471389	0
Trifolium pratense	Eukaryota; Plants; Land Plants	GCA_900292005.1	Chromosome	351.622	OMTE01	38479	0
Trifolium pratense	Eukaryota; Plants; Land Plants	GCA_900079335.1	Chromosome	345.991	FKJA01	39051	0
Trifolium pratense	Eukaryota; Plants; Land Plants	GCA_000583005.2	Contig	304.972	ASHM01	267372	63850
Trifolium subterraneum	Eukaryota; Plants; Land Plants	GCA_001742945.1	Scaffold	471.834	BCLP01	27424	42059
Trifolium subterraneum	Eukaryota; Plants; Land Plants	GCA_002003065.1	Contig	392.71	BBPR01	968279	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_002220415.2	Contig	15344.7	NMPL02	279439	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900241085.1	Scaffold	13916.9	OETA01	519179	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900067645.1	Scaffold	13427.4	FAOM01	735943	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900000045.1	Scaffold	9134.02	CCYC01	6870110	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900067735.1	Scaffold	10058.1	CBTL01	11673940	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_001077335.1	Scaffold	58.5022	CBUC01	1450	250
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_002158495.1	Scaffold	567.21	MOLT01	10339	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_000818885.1	Scaffold	65.1111	JROL01	15144	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_002999095.1	Scaffold	574.295	PKRY01	9044	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900236235.1	Scaffold	452.948	OEIT01	23433	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_002780475.1	Contig	240.199	NTGG01	127921	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_002780545.1	Contig	609.488	NTGH01	523543	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_002780565.1	Contig	564.3	NTGI01	565551	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900235945.1	Scaffold	839.076	OEIJ01	767884	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_900235935.1	Scaffold	804.001	ODGO01	749802	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_001485685.1	Contig	44.4015	FAOV01	50000	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_000334095.1	Contig	3800.33	CALP01	5321847	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_000188135.1	Contig	159.087	AEOM01	311945	0

Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_000334135.1	Contig	437.106	CALO01	945079	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_001889245.1	Contig	2.42226	LOLC01	9834	0
Triticum aestivum	Eukaryota; Plants; Land Plants	GCA_001889205.1	Contig	0.942499	LOLD01	3746	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323645.1	Scaffold	392.684	OOGV01	252227	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323695.1	Scaffold	552.916	OOHG01	360242	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323615.1	Scaffold	477.083	OOGX01	350258	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323635.1	Scaffold	434.779	OOGY01	363434	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323595.1	Scaffold	431.061	OOHA01	427274	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323585.1	Scaffold	288.316	OOGW01	327364	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323625.1	Scaffold	518.951	OOHB01	592030	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323565.1	Scaffold	353.054	OOGZ01	455525	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323665.1	Scaffold	441.177	OOHC01	572876	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323605.1	Scaffold	497.42	OOHD01	649826	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323575.1	Scaffold	585.067	OOHE01	730770	0

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Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323655.1	Scaffold	624.303	OOHF01	905302	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323675.1	Scaffold	388.595	OOHH01	638342	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900323685.1	Scaffold	700.417	OOHP01	1181661	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_002162155.2	Chromosome	10677.9	LSYQ02	148396	0
Triticum dicoccoides	Eukaryota; Plants; Land Plants	GCA_900184675.1	Chromosome	10495	FXXJ01	149145	0
Triticum urartu	Eukaryota; Plants; Land Plants	GCA_000347455.1	Scaffold	3747.05	AOTI01	499221	24169
Triticum urartu	Eukaryota; Plants; Land Plants	GCA_003073215.1	Chromosome	4851.9	MKGO01	10284	0
Turritis glabra	Eukaryota; Plants; Land Plants	GCA_900406565.1	Contig	171.13	OVBN01	250	0
Turritis glabra	Eukaryota; Plants; Land Plants	GCA_900406545.1	Scaffold	157.63	OVBM01	6441	0
Urochloa ruziziensis	Eukaryota; Plants; Land Plants	GCA_003016355.1	Scaffold	732.531	PVZT01	102577	0
Utricularia gibba	Eukaryota; Plants; Land Plants	GCA_002189035.1	Chromosome	100.689	NEEC01	518	0
Vaccinium macrocarpon	Eukaryota; Plants; Land Plants	GCA_000775335.2	Scaffold	414.622	JOTO01	200203	0
Vachellia collinsii	Eukaryota; Plants; Land Plants	GCA_006871305.1	Scaffold	461.065	QFDD01	122260	0
Vanilla planifolia	Eukaryota; Plants; Land Plants	GCA_004338375.1	Scaffold	2203.64	SDXO01	794534	0

Vicia faba	Eukaryota; Plants; Land Plants	GCA_001375635.1	Contig	80.3627	CSVX01	74659	0
Vigna angularis	Eukaryota; Plants; Land Plants	GCA_001190045.1	Chromosome	467.301	JZJH01	37727	37769
Vigna angularis var. angularis	Eukaryota; Plants; Land Plants	GCA_000465365.1	Scaffold	291.824	AUGG01	14501	0
Vigna angularis var. angularis	Eukaryota; Plants; Land Plants	GCA_001723775.1	Chromosome	444.439	JRFV01	3387	0
Vigna radiata	Eukaryota; Plants; Land Plants	GCA_000180895.1	Contig	10.1012	BABL01	46645	0
Vigna radiata var. radiata	Eukaryota; Plants; Land Plants	GCA_001584445.1	Scaffold	454.907	LJIH01	2418	0
Vigna radiata var. radiata	Eukaryota; Plants; Land Plants	GCA_000741045.2	Chromosome	463.638	JJMO01	2499	42284
Vigna unguiculata	Eukaryota; Plants; Land Plants	GCA_004118075.1	Chromosome	519.067	NBOW01	682	41173
Vigna unguiculata subsp. unguiculata	Eukaryota; Plants; Land Plants	GCA_001687525.1	Scaffold	695.046	MATU01	224035	0
Viola pubescens var. scabriuscula	Eukaryota; Plants; Land Plants	GCA_002752925.1	Scaffold	318.366	NBIL01	157716	0
Vitis aestivalis	Eukaryota; Plants; Land Plants	GCA_001562795.1	Contig	432.755	LOML01	756125	0
Vitis cinerea x Vitis riparia	Eukaryota; Plants; Land Plants	GCA_001282645.1	Scaffold	539.624	CCJE01	210444	0
Vitis riparia	Eukaryota; Plants; Land Plants	GCA_004353265.1	Chromosome	500.106	SJAQ01	174	0
Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_002923105.1	Scaffold	427.211	BDSR01	21	0

(continued)

Table 32.3 (continued)

Organism name	Organism groups	Assembly	Level	Size (Mb)	WGS	Scaffolds	CDS
Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_002922885.1	Scaffold	427.171	BDSO01	21	0
Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_002923015.1	Scaffold	427.04	BDSQ01	21	0
Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_002923165.1	Scaffold	426.616	BDSS01	21	0
Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_004011995.1	Contig	868.043	QGNW01	2737	112320
Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_000003745.2	Chromosome	486.197	CAAP03	2061	41208
Vitis x labruscana x Vitis vinifera	Eukaryota; Plants; Land Plants	GCA_008326845.1	Scaffold	490.143	BKBX01	8696	0
Xanthoceras sorbifolium	Eukaryota; Plants; Land Plants	GCA_003430845.1	Chromosome	504.383	QUWJ01	2297	0
Xerophyta viscosa	Eukaryota; Plants; Land Plants	GCA_002076135.1	Scaffold	295.462	MJHO01	896	0
Zea mays	Eukaryota; Plants; Land Plants	GCA_000223545.1	Scaffold	177.051	AECO01	196697	0
Zea mays	Eukaryota; Plants; Land Plants	GCA_000275765.1	Contig	1.33507	AHID01	1844	0
Zea mays	Eukaryota; Plants; Land Plants	GCA_003185045.1	Chromosome	2182.61	NCVQ01	2203	46530
Zea mays	Eukaryota; Plants; Land Plants	GCA_003704525.1	Chromosome	2198.5	RAQR01	797	0
Zea mays	Eukaryota; Plants; Land Plants	GCA_003709335.1	Chromosome	2288.19	RAQT01	972	0
Zea mays	Eukaryota; Plants; Land Plants	GCA_000005005.6	Chromosome	2135.08	LPUQ01	598	58411

Zea mays subsp. mays	Eukaryota; Plants; Land Plants	GCA_002813505.1	Scaffold	2041.55	LMUZ01	48268	0
Zea mays subsp. mays	Eukaryota; Plants; Land Plants	GCA_001990705.1	Chromosome	2392.8	MTTB01	62610	0
Zea mays subsp. mays	Eukaryota; Plants; Land Plants	GCA_001984235.2	Chromosome	2455.26	MTTA01	60567	0
Zea mays subsp. mays	Eukaryota; Plants; Land Plants	GCA_001644905.2	Chromosome	2133.88	LWRW02	191	0
Zea mays subsp. mays	Eukaryota; Plants; Land Plants	GCA_002682915.2	Chromosome	2197.97	NWUM01	3538	0
Zea mays subsp. mays	Eukaryota; Plants; Land Plants	GCA_002237485.1	Chromosome	2155.82	NKIA01	43301	0
Zea mays subsp. mexicana	Eukaryota; Plants; Land Plants	GCA_002813485.1	Scaffold	1204.28	LMVA01	107418	0
Zizania latifolia	Eukaryota; Plants; Land Plants	GCA_000418225.1	Scaffold	603.989	ASSH01	4522	0
Ziziphus jujuba	Eukaryota; Plants; Land Plants	GCA_001835785.1	Scaffold	351.097	LPXJ01	36119	0
Ziziphus jujuba	Eukaryota; Plants; Land Plants	GCA_000826755.1	Chromosome	437.754	JREP01	5897	43574
Zostera marina	Eukaryota; Plants; Land Plants	GCA_001185155.1	Scaffold	203.914	LFYR01	2228	20648
Zoysia japonica	Eukaryota; Plants; Land Plants	GCA_001602275.1	Scaffold	334.384	BCLF01	11786	0
Zoysia matrella	Eukaryota; Plants; Land Plants	GCA_001602295.1	Scaffold	563.439	BCLG01	13609	0
Zoysia pacifica	Eukaryota; Plants; Land Plants	GCA_001602315.1	Scaffold	397.01	BCLH01	11428	0

32.6 Conclusion

The applications of bioinformatics to plant pathology have been pivotal role in understanding of host and pathogen evolution and molecular interactions between host and pathogen. Availability of next-generation sequencing data of candidate model organisms of all kingdom through high-throughput technology is convenient to deal with biological systems and understand the biological sequence–structure–function correlation using in-silico biology tools, technology and databases. Genome annotation, assembly, bioproject, biosample submission, sequence data submission, retrieval of data, data analysis, variation analysis, conserved domain analysis, gene identification, regulatory elements analysis, gene expression analysis, structure prediction, structure visualization, structure analysis, structure classification, molecular modeling, epitope identification and mapping using 3D, drug designing, active site analysis and molecular docking, etc. play an important role to achieve biological function and understand the sequence–structure–function relationship. These all in-silico biology techniques will be further helpful in genomics-assisted crop improvement and development of designer crops with high yield and super quality.

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