Manoj Kaushal
Ram Prasad *Editors*

Microbial Biotechnology in Crop Protection

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Preface

Population increase coupled with degradation of agricultural lands aggravates crop protection and production challenges around the globe. The increased farm fragmentations resulted in pest and disease infestations in farms due to increased movement, carrying planting materials and resistance to major chemical fertilizers and pesticides and overexploiting of natural resources. Thus, producing enough crop yields to feed the rapidly growing population by sustaining its nutritional quality and maintaining plant and soil health is the major challenge for growth and development. Furthermore, classical techniques and products used for agriculture farming are also at their threshold limits of effectiveness in fighting emerging pest and disease problems and protecting agricultural productivity. One of the possible ways to deal with these ever-increasing crop protection issues is through microbial biotechnology approach. Crop protection through microbial biotechnology involves the application of microorganisms in farms through the engagements of modern biotechnology techniques for sustaining future agriculture developments. Microorganisms are the natural solution for the emerging crop protection issues without affecting the production and soil fertility. Many research reports suggested that broad application of microbes used in single or consortia is highly effective in crop protection systems compared to synthetic fertilizer and pesticides. Looking at the present need and future scenario, in this book, we are emphasizing the role of microbial communities for crop protection against major pests and diseases (fungal as well as bacterial) through the use of diversified biotechnological approaches such as biofertilizers, biopesticides, and value additions in crops. Further, the book reflects the emerging paradigms of genetic engineering manipulation through beneficial gene transfer from microorganism which might be the other solution for crop protection. The book meets the growing need for a comprehensive and holistic outlook on crop protection issues, underlying principles, important perspectives, and emerging biological approaches and techniques that are the need of today's sustainable agriculture. The chapter focuses on the broad application of microbes in sustainable agriculture, genetic dependency of plants on the beneficial functions, and symbiotic cohabitants.

We are extremely honored to receive chapters from professors and leading scientists with enormous experience and expertise in the field of crop protection, microbiology and biotechnology, and sustainable agriculture development. The

book targets the academicians, researchers, scientists, doctoral and graduate students working on crop improvement approaches.

Our sincere gratitude goes to the contributors for their insights on Microbial Biotechnology in Crop Protection. We sincerely thank Dr Naren Aggarwal, Editorial Director, Springer and Ms Aakanksha Tyagi, Associate Editor for their generous assistance, constant support, and patience in finalizing this book.

Dar es Salaam, Tanzania — Manoj Kaushal
Bihar, India — Ram Prasad

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Ram Prasad, Ph.D. is associated with Department of Botany, Mahatma Gandhi Central University, Motihari, Bihar, India. His research interest includes applied and environmental microbiology, plant–microbe interactions, sustainable agriculture, and nanobiotechnology. Dr. Prasad has more than two hundred publications to his credit, including research papers, review articles, and book chapters, five patents issued or pending, and edited or authored several books. He has twelve years of teaching experience and has been awarded the Young Scientist Award and Prof. J.S. Datta Munshi Gold Medal by the International Society for Ecological Communications; FSAB fellowship by the Society for Applied Biotechnology; the American Cancer Society UICC International Fellowships for Beginning Investigators, USA; Outstanding Scientist Award in the field of Microbiology by Venus International Foundation; BRICPL Science Investigator Award and Research Excellence Award, etc. Previously, he served as Assistant Professor, Amity University,

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Biocontrol: A Sustainable Agricultural Solution for Management of Plant Diseases

1

Abhishek Walia, Chayanika Putatunda, Rashmi Sharma, Samriti Sharma, and Abhishek Thakur

Abstract

Plant diseases are required to be controlled for abundance and quality maintenance of food, feed, and other plant-based products around the world. Among different approaches used by masses to mitigate widespread plant diseases, use of chemical fungicides and pesticides is most prevalent. However, due to their fast and specific action, such inputs have significantly contributed to the environmental pollution and pathogen resistance over a period of time. This has led to considerable changes in people's attitude toward the use of these chemical compounds. Consequently, agronomists have focused their efforts on developing alternative inputs to these chemicals. Among these alternatives includes the deployment of antagonistic microorganisms at the plant infection site before or after infection takes place which is referred to as biological control. The mechanisms employed by biocontrol organisms for waning or killing of plant pathogens include their ability to parasitize the pathogens directly by production of antibiotics or toxins, competition for nutrients and space, production of enzymes that attack pathogen's cell wall components, induction of various defense responses in plants, and possibly others. This chapter will bring an

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important perspective to the biological control of plant pathogens and will outline and discuss the: (1) types and various mechanisms of biocontrol (2) control of soil-borne, aerial parts and postharvest diseases of plants using various bacterial and fungal antagonists (3) hypovirulence factors as a mechanism of biological control.

Keywords

Antibiosis · Mycoparasitism · Entomopathogenic · Arthropods · Nematodes · Viral agents

1.1 Introduction

In the course of the past 20 years, the scenario of world population has drastically changed. To cater the growing need of food and nutrition of population worldwide, the crop production needs to be redefined in a novel way along with sustainable procedures to counter also the menace of global warming and climate change. The present challenge before agriculture industry, farmers, and researchers across the globe is to increase the crop production and maintain the quality and vitality of crops using eco-friendly methodologies. The different crops in the fields and natural habitats are encountered with different types of pathogens and these pathogens destroy the overall crops and lead to decrease in crop production. A plant pathogen is a very wide terminology that refers to any of the organisms, such as bacteria, fungi, protistans, nematodes, viruses, and other pathogens that cause plant infections and diseases. Plant pathogens that cause plant diseases weaken the ability of the farmers or growers to produce good quality and quantity of crops and can infect almost every type of plants. The traditional and conventional methods of control of plant pathogens include use of pesticides, insecticides, fungicides, herbicides, rodenticides, and other chemical formulations. These substances control the plant pathogens to a good amount but their adverse effects are also seen and felt in food chain. The numerous negative health effects that have been associated with chemical pesticides include, among other effects, dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects (WHO 1990; Sanborn et al. 2007; Mnif et al. 2011; Thakur et al. 2014). Furthermore, high occupational, accidental, or intentional exposure to pesticides can result in hospitalization and death (WHO 1990; Gunnell et al. 2007). One such detrimental effect of these chemicals is bioaccumulation which leads to biomagnification. The other method of plant pathogens control includes use of natural parasites or predators of plant pathogens which constitutes biological or natural control. Biocontrol microorganisms are cellular or noncellular entities, capable of replicating or of transferring genetic material. Various soil and rhizospheric microorganisms have been explored as potential antagonists that possess characteristics of a candidate agent. In fact, with increase in the research area related to potential biocontrol microorganisms, it has been found that such microorganisms have a broader range

of activities that are correlated with biological management of plant pathogens apart from antagonism. The other effects of biocontrol agents include increase in plant vitality, pushing out the pathogens through competition for nutritional resources and occupation of ecological habitat and niche, and by inducing systemic resistance in the host through activation of the host defense mechanisms against the invading pathogen.

The potential biocontrol agents explored so far are *Bacillus subtilis, Pseudomonas fluorescens, Gliocladium* spp., *Trichoderma* spp., *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii,* granulosis viruses, nuclear polyhedrosis viruses (NPV), *Nomuraea rileyi*, *Hirsutella* sp., *Verticillium chlamydosporium, Streptomyces griseoviridis, Streptomyces lydicus, Ampelomyces quisqualis, Candida oleophila, Fusarium oxysporum* (nonpathogenic), *Burkholderia cepacia, Coniothyrium minitans, Agrobacterium radiobacter* strain 84, *Agrobacterium tumefaciens, Pythium oligandrum, Erwinia amylovora* (hairpin protein), *Phlebia gigantea, Paecilomyces lilacinus, Penicilliuim islanidicum* (for groundnut), *Alcaligenes* spp., *Chaetomium globosum, Aspergillus niger* strain AN27, VAM fungi, *Myrothecium verrucaria, Photorhabdus luminescens* akhurustii strain K-1, *Serratia marcescens* GPS 5, and *Piriformospora indica*. These biological agents act on plant pathogens through different modes of action. It includes direct antagonisms like parasitism, for instance, *Trichoderma* is a parasite of a range of fungi and oomycetes in the soil, which produce toxic metabolites and cell wall-degrading enzymes and inhibit the growth of others, hyperparasitism e.g. *Hypovirus*, a hyperparasitic virus on *Cryphonectria parasitica*, a fungus causing chestnut blight, commensalism, mixed-path antagonism by synthesis of chemicals like siderophores, antibiotics, volatile compounds like HCN, lytic enzymes and indirect antagonisms like competitive root colonization and plant growth promotion through systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Walia et al. 2013; Mehta et al. 2013a, 2013b). Biocontrol agents are safe both for the environment and for the persons who apply them and avoid environmental pollution (soil, air, and water) by leaving no toxic residues. It is comparatively easier to manufacture biocontrol agents, sometimes less expensive than chemical agents. The biggest advantage of using biocontrol agents is that they can eliminate the specific pathogens effectively from the site of infection and can be used in combination with biofertilizers (Mehta et al. 2013c, 2014). Biocontrol agents avoid problems of resistance and also induce systemic resistance among the crop species. The only negative aspect of these agents is that these agents work slowly and less effectively in comparison with the chemical pesticides, as their efficacy almost completely depends on environmental conditions. However, these constraints can be nullified due to constant research and more effective biocontrol agents can be generated as these are the demands of the present world for safe food.

1.2 Microbial Biocontrol Agents

According to European Union Regulation No 1107/2009, the term microbial biocontrol agents (MBCAs) are surrounded by microorganisms (bacteria, viruses, and fungi) of different nature. They can be used in covered field or in field crops because of their ability to act against large number of pathogens, pests, and weeds which helps in controlling various diseases, agents, and crop pests. The mode of action of microbial biocontrol agents (MBCAs) will vary from species to species, either they directly start a lethal biological process or may suppress the aggression of pathogenic microorganisms by competition. The use of MBCAs in covered field or in field crops has several advantages like low environmental impact, safe to human health, not inducing pesticide resistance, and greater potential of replacing chemical pesticide. The two most successful MBCAs are *Bacillus thuringiensis* (Bt) (microbial insecticide) and *Ampelomyces quisqualis* (Aq) (antagonist to pathogens like powdery mildew agents).

For the application of microbial biocontrol agents, some conditions need to be satisfied:

1. The legal registration is required for all MBCAs, both at EU level for active ingredient and at country level for commercialization for each crop. The Bt is commercially availablein Chile, Germany, Hungary, and France whereas Aq is also currently available in Italy and Switzerland.
2. The advisers and farmers should be trained for the application of MBCAs.
3. The selection of efficient strains against main pests and pathogens.
4. Familiar with suitable environmental conditions.
5. Familiar with the availability of economically competitive products.
6. The suitable registration and regulations procedure must be known.
7. Awareness about environmental and health issues.

1.2.1 Microorganisms as Microbial Biocontrol Agents

1.2.1.1 Bacteria as Biocontrol Agents

The bacteria have been used as a biocontrol agent to control a number of microbial diseases by applying on seeds and roots (Chauhan et al. 2014; Ohike et al. 2018). One of the examples is the use of *Streptomyces* (nonpathogenic strains) strain to control scab of *Solanum tuberosum* L (potato) caused by *Streptomyces scabies* (Thaxter). On the other hand, the growth of soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica* (van Hall) was inhibited by *Pseudomonas fluorescens* (Trevisan) strain by synthesizing antibiotic 2,4-diacetylphloroglucinol (DAPG) (Cronin et al. 1997). Some of the other studies showed that the *P. fluorescens* F113 produces siderophore that may play a major role in controlling the potato soft rot under iron-limiting conditions whereas the major biocontrol determinant appears to be DAPG.

Pseudomonas species have also the potential to control crown gall disease which is caused by *Agrobacterium tumefaciens* in many dicotyledonous plants (Khmel et al. 1998). The most successful and classic bacteria based biocontrol systems is the use *Agrobacterium* strain K84 against *Agrobacterium tumefaciens.* One of the studies where K84 or K1026 was co-inoculated with pathogenic cells showed the survival of pathogens on roots up to 8 months later but pathogen was not able to show any symptoms, providing the evidence that K84 or K1026 was able to prevent the disease expression rather than killing pathogen cells directly.

There are a number of bacterial strains which produce antifungal metabolites (excluding metal chelators and enzymes) in vitro such as HCN, kanosamine 2,4-diacetylphloroglucinol (Ph1), oomycin A, oligomycin A, butyrolactones, ammonia, xanthobaccin, zwittermicin A, viscosinamide, pyoluteorin (Plt), pyrrolnitrin (Pln), and phenazine-1-carboxylic acid (PCA) as well as several uncharacterized moieties. The PCA gene from *Pseudomonas aureofaciens* Kluyver Tx-1 has the capacity to control dollar spot (*Sclerotinia homoeocarpa* F. T. Bennett) on creeping bentgrass (*Agrostis palustris* Hudson).

The antibiotic production in bacteria is mainly a two-component regulated system, i.e. it may be a cytoplasmic response factor and environmental sensor (presumably a membrane protein). Mutation in either of the system causes direct effects on multiple antibiotic production. For example, mutation in gacA gene of *P. fluorescens* CHA0 causes the loss of production of Plt, HCN, Phl, protease, and phospholipase C, whereas mutation in apdA gene of *P. fluorescens* Pf-5 is responsible for the lost ability to produce Pln, HCN, and Plt.

Other two-component signaling mechanism showed the production of PCA by Pseudomonas on roots which inhibits the secondary growth of the pathogenic bacteria. For *Gaeumannomyces graminis* var. *tritici*, *Pseudomonas aureofaciens* 30–84 acts as a biocontrol agent which causes disease in wheat (*Triticium aestivum* L.). In this system, root exudation has increased due to the growth of pathogen in root which results in increased growth of *Pseudomonas aureofaciens* 30–84 and other bacteria in the infection zone. Consequently, production of signal molecule N-acyl-L-homoserine lactone (HSL) has increased which is produced at low levels by phzI gene which is sufficient to switch on phzR gene, responsible for the production of PCA in *P. aureofaciens* 30–84 at rhizosphere. The end product, i.e. PCA, further inhibits the growth of pathogen.

Interestingly, signaling between potential biocontrol bacteria and pathogenic fungi was also detected, i.e. *Pythium ultimum* Trow is responsible for down-regulation of five gene clusters of *P. fluorescens* F113 helps in controlling growth of this pathogenic bacteria in the rhizosphere of sugar beet and yet another example trehalose production from *Pythium debaryanum* up-regulated genes in its biocontrol strain *Pseudomonas fluorescens* ATCC 17400. This finding has major impact on controlling the gene expression of complex microbial communities.

1.2.1.2 Fungi as Biocontrol Agents

Beneficial fungi can prevent the growth of pathogen by colonizing on the shared habitat, i.e. plant tissues, rhizosphere, or phyllosphere for depriving space and

nutrients. All the fungal agents may possess such kind of action to certain extent depending on the adaptation and properties to the environment and host plants. Trichoderma species are ubiquitous in nature and found in all climatic zone (including temperate and tropical regions, tundra, and Antarctic), nearly all soil types (crop fields, desert, and marsh) and unusual niches such as lakes, marine bivalves, air, termites, and shellfish. Certain trichoderma chelates iron compounds by producing siderophores which can inhibit the growth of postharvest pathogens such as *Botrytis cinerea* and soil-borne pathogen.

Mycoparasitism is a process of receiving nutrients from one fungus (host) by another fungus (mycoparasite) in a parasitic manner. Mycoparasitism involves the penetration of mycoparasite into the host hyphae by forming various peculiar organs such as haustoria or secretion of different types of enzymes (endochitinases, β-1,3-glucanases, and proteases) and secondary metabolites which leads to degradation of fungal structure followed by metabolite/nutrient uptake from the host fungus (Lopes et al. 2012; Geraldine et al. 2013; Vos et al. 2015). In the initial stage, pathogen's hyphae are surrounded by *Trichoderma* hyphae which then penetrate into the host cell by breaking chitin through the action of chitinase and glucanase enzymes. The mycoparasitic fungus hyphae then subsequently release antibiotic compounds which permeate the affected hyphae and prevent resynthesis of the cell wall.

Antibiosis is other mechanism which involves antimicrobial compounds produced by different biocontrol agents to reduce or suppress the growth and/or proliferation of the phytopathogens. Antibiosis has been noticed in a number of fungi including *Trichoderma* having cell wall-degrading enzymes such as xylanase, cellulase, glucanase, amylase, arabinase, protease, pectinase, lipase, and various volatile compounds such as 6-n-pentyl-2H-pyran-2-one (6-PAP) and number of antibiotics such as peptaibols, ethylene, pyrones, gliovirin, gliotoxin, herzianolide, viridin, trichodermin, formic aldehyde, and trichodermol (Jelen et al. 2013; Hermosa et al. 2014; Strakowska et al. 2014). *T. atroviride* mycelia ethyl acetate extract has the capacity of inhibiting spore germination of *F. solani* with minimum inhibitory concentration (MIC) of 0.66 mg/ml (Toghueoa et al. 2016). Trichoderma produced highly toxic secondary metabolites, i.e. epipolythiodioxopiperazines (ETPs) which is a diketopiperazine ring. ETPs are produced by certain isolates like gliovirin through P strains of *T. virens* while gliotoxin is produced by Q strain (Vey et al. 2001; Mukherjee et al. 2012; Błaszczyk et al. 2014; Scharf et al. 2016). The biological action has been shown by *Purpureocillium lilacinum* biological agent that are antibacterial, antimalarial, antifungal, antiviral, antitumor and having phytotoxic activities which control phytopathogens, phytophthora infestans and *P. capsici* (Wang et al. 2016). In vitro and field trials of *P. lilacinum* also showed its capacity of parasitizing eggs, inhibiting egg hatching, and mortality in juvenile phase of the *Meloidogyne incognita* (root-knot nematode) (Singh et al. 2013).

1.2.1.3 Viruses as Biocontrol Agents

Pathogenic virus shows their importance to act as biological control for pest/insect by manipulating naturally occurring pathogens. Instead of waiting for virus disease

to appear in populations of pest insects, viruses are collected, mass cultured, formulated, packaged, stored, and applied when needed to control insect pests (Falcon 1982). The number of viruses that were already collected about three decades ago was over 650 entomopathogenic viruses. The entomopathogenic viruses were mainly baculovirus (dsDNA viruses) which belongs to the main group of arthropod viral pathogens. Baculovirus has been available as 60 commercial products which produce characteristic occlusion bodies for better survival in environment and for good insect infestation. After ingestion of occlusion bodies, it gets dissolved due to alkaline midgut and releases the virions for infestation initiation of epithelial cells before contaminating the whole organism.

Soil acts as a major reservoir for occlusion bodies and helps in controlling the insect to complete their life cycle under soil surface. The narrow host specificity is occurred nowadays due to high diversity within baculovirus results from long coevolution with insects. Due to this it has adverse effect on non-target organisms. Despite various biocontrol programs, the viruses for below ground biocontrol remain very less. One of the examples was the use of potato tuberworm granulovirus (PoGV) for controlling potato tuberworm complex. The various government agencies in different countries like North Africa, Asia, South America, and in the Middle East area are working on PoGV against *Phthorimaea operculella*. *Phthorimaea operculella* is responsible for 100% economic losses of potato tubers, worldwide pest of solanaceous crops. The females of *Phthorimaea operculella* lay eggs on leaves and on tubers during growing season. So, based on the life cycle of *P. operculella*, the PoGV was tested against the potato tuber worm and success has been achieved 73% in crops whereas 53% in the stored tubers.

Several new challenges have been led to the growth of agricultural production which needs to be overcome appropriately and timely for making further growth possible. The use of excessive chemical fertilizers and pesticides for growth of agriculture production is becoming a matter of concern where plateau has already reached for increasing crop production through modern farming in most of the countries including India. So, for achieving goal in agriculture microbial biological agents play an important role.

1.3 Biological Mechanisms of Pathogen Inhibition

Plants and pathogens interact throughout their life cycle that notably alter the plant health in many ways (Sharma et al. 2017). In order to have a positive or negative effect the organisms must have some form of direct or indirect contact with the host. By studying the ways in which the organisms interact, one can understand the mechanisms involved in biocontrol of plant pathogens. Consequently, understanding the mechanisms of biological control of plant diseases through the interactions between biocontrol agent and pathogen may allow us to maneuver the soil environment to create conditions favorable for successful biocontrol of plant pathogens (Sharma et al. 2015a, b, 2016; Guleria et al. 2014). The interactions involved between host plant and microorganism can be mutualistic, antagonistic, synergistic,

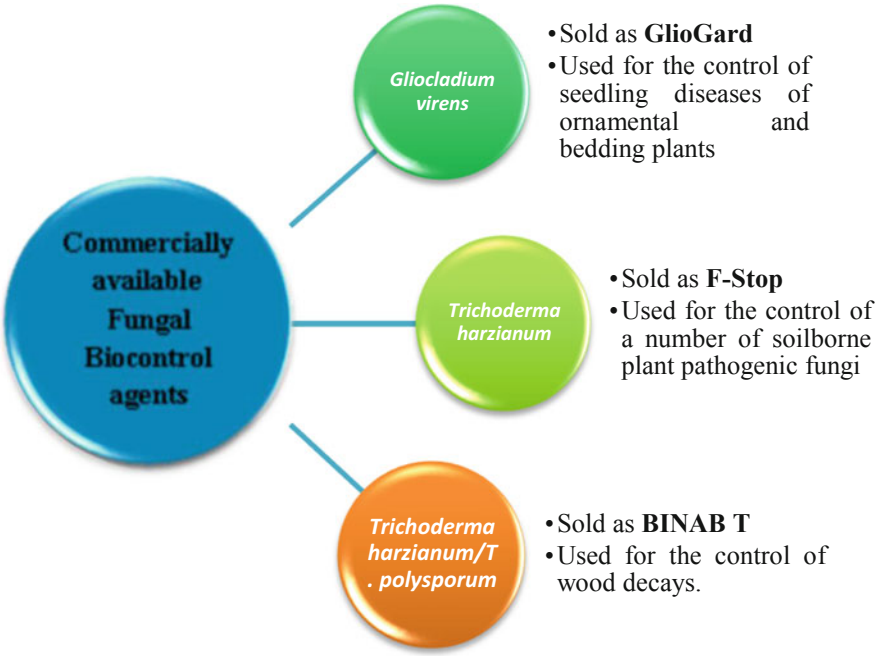

Fig. 1.1 Registered/commercialized fungal biocontrol agents

parasitic, predatory, commensalism, and competition (Bankhead et al. 2004; Mehta et al. 2015; Guleria et al. 2016). Through negative interactions, pathogens are antagonized by the presence and activities of antagonists they encounter.

Although numerous microorganisms have been reported worldwide that antagonize an array of plant pathogens under in vitro as well as under *in planta* conditions, only a few are registered and available commercially for use worldwide. Figures 1.1 and 1.2 depict the most widely reported and commercialized fungal and bacterial biocontrol agents.

Although the actual use of these commercialized products is still limited, it is expected that these and other such bio-products will be accepted for use in the near future. The underlying mechanisms involved in weakening or destroying the plant pathogens by antagonists are:

- Induction of defense responses in the plants they surround primarily
- Their ability to parasitize the pathogens directly through production of antibiotics (toxins)
- Hyperparasites and predation
- Their ability to compete for space and nutrients in the presence of other microorganisms
- Production of enzymes that attack the cell components of the pathogens and possibly others.

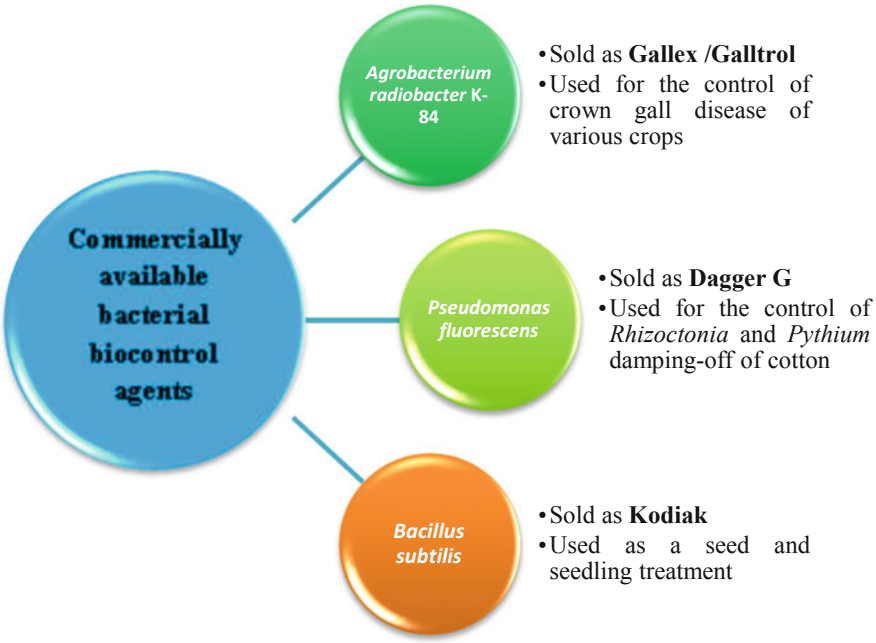

Fig. 1.2 Registered/commercialized bacterial biocontrol agents

The different mechanisms employed by biocontrol agents in controlling the plant diseases are broadly categorized into three types:

- Direct antagonism
- Mixed path antagonism
- Indirect antagonism.

1.3.1 Direct Antagonism

1.3.1.1 Parasitism

Parasitism is an interactive association in which two phylogenetically and physiologically different organisms live together over a prolonged period of time. In this type of relationship, one organism, usually benefitted, called the "parasite" and the other called the "host," is harmed. For example, *Trichoderma* is a parasite of a range of fungi and oomycetes in the soil, which produces toxic metabolites and cell wall-degrading enzymes and inhibits the growth of other fungi and microorganisms.

1.3.1.2 Commensalism

Commensalism is a unidirectional association between two unrelated species by living together, in which one population (commensals) benefits from these

relationships, while the other (the host) is not harmed. Microbes present in the rhizosphere control soil-borne pathogens through competition for nutrients and production of antibiotics and help the plants survive pathogen infection (Kumar et al. 2016a, b). On the other hand, the microbes have an important role in the growth of the plant by increasing solubilization of minerals or by synthesizing amino acids, vitamins, and growth regulators that stimulate the plant growth.

1.3.1.3 Induced Resistance

Induced resistance (IR) is one of the principal mechanisms of biocontrol of major plant pathogens. Resistance may be induced only at the site of infection called local resistance or may be throughout the plant parts called systemic resistance. Induced systemic resistance (ISR) is of prime importance and can protect plants against multiple soil-borne or foliar pathogens. A variety of microbes are involved in resistance induction including nonpathogenic saprophytes, beneficial rhizospheric microorganisms, and avirulent pathogenic strains (Paulitz and Belanger 2001). Besides living microorganisms, certain chemicals, UV exposure, and manure have also been known to induce resistance in host plant (Choudhary et al. 2007; Sang et al. 2010). An avirulent strain of pathogenic fungi *Colletotrichum orbiculare* when applied to the seedlings induces resistance in the older leaves of the plant (Tuzun and Kuc 1985). ISR improvise the secretion of various enzymes involved in plant defense such as chitinases, cellulases, proteinases, and peroxidases; moreover, the cell wall has become more lignified, thus restricting the further entry of pathogen in adjacent healthy tissues (Houston et al. 2016). Certain PGPRs such as *Pseudomonas putida*, *Serratia marcescens*, *Flavimonas oryzihabitans,* and *Bacillus pumilus* potentiate the plant defense response against subsequent pathogen challenge. A number of bacterial determinants act as resistance elicitors of ISR such as pseudobactin, pyoverdin, salicylic acid, polysaccharides, and flagellin proteins reported so far (Annapurna et al. 2013).

Salicylic acid (SA), a signal molecule involved in systemic acquired resistance, was produced by *Pseudomonas aeruginosa* and induced resistance to gray mold fungi *Botrytis cinerea* in beans (De Meyer and Höfte 1997), and exogenously applied SA in tobacco (*Nicotiana tabacum* L.) induced resistance against Tobacco mosaic virus (TMV). In another study by De Meyer et al. (1998) a strain of *Trichoderma harzianum* induces similar resistance effect like *Pseudomonas aeruginosa* in beans inoculated with gray mold. Inoculation of cucumber seedlings with spores of *T. harzianum* resulted in significant increase in peroxidase and chitinase activities in other foliar parts, thus avoiding subsequent pathogen attack (Yedidia et al. 1999).

UV exposure has also been known for induction of resistance as evident earlier when a pathogenic strain of *Colletotrichum magna* was converted to an avirulent mutualistic endophytic strain after UV exposure and gene disruption enabling the mutant avirulent strain to induce resistance in host plants against phytopathogenic fungi such as *Colletotrichum, Fusarium,* and *Phytophthora.* This disease resistance was correlated to a decrease in the time of activation of host defense systems after exposure to the pathogens. This phenomenon was termed as "endophyte associated

resistance" as it did not induce host hypersensitive response and systemically acquired resistance (Redman et al. 1999).

1.3.1.4 Inhibitory Compounds

Antibiotics are the toxic metabolites released by certain microorganisms and at low concentration act and inhibit the other microorganisms. Many microorganisms are known to release one or more types of antibiotics. These antibiotics are particularly effective in antagonizing various plant pathogens and diseases they cause. To be an effective antagonist, these inhibitory compounds must be produced in fair amount near the site of pathogen colonization. In vitro production of various antibiotics by a number of biocontrol agents has been reported; however, the effective concentration is difficult to estimate because of very less amount produced and less toxicity to pathogen. Though there are a number of methods to know the type of microorganisms producing which antibiotic (Notz et al. 2001), detection of their production and action under field conditions is difficult because of the uneven distribution of producer microbes and the pathogens.

A number of antibiotics are produced by PGPRs such as phenazines, pyoluteorin, oomycinA, 2,4 diacetylphloroglucinol by different species of *Pseudomonas* and subtilin, iturin, fengycin, zwittermicin A, bacillomycin by *Bacillus* spp. and many others including butyrolactones, kanosamine, rhamnolipids, cepaciamides, antitumor antibiotics, and antiviral antibiotics. All these antibiotics attack pathogenic bacteria, fungi, insects, and other phytotoxic compounds depending upon their modes of action (Dilantha et al. 2005). Their mechanisms of action include rupturing of cell membrane, disturbing osmotic balance, and inhibitory effect on ribosomes and other cellular components (Reid et al. 2002; Koch et al. 2021). That may be the reason why some antibiotics are effective against certain pathogens and not against others, depending upon the cellular components they attack (McSpadden and Fravel 2002).

1.3.1.5 Hyperparasites and Predation

Hyperparasitism is a phenomenon where the pathogen is killed after direct attack by biocontrol agent. Hyperparasites are divided into four categories: obligate bacterial pathogens, mycoviruses as hypovirulence factors, facultative parasites, and predators. Obligate bacterial pathogens are known to attack nematodes. Tzortzakakis et al. (2003) reported the interaction between bacterial parasite *Pasteuria penetrans* and the root-knot nematode *Meloidogyne* spp. under in vitro and under *in planta* conditions. The bacterial parasite successfully reduced the *Meloidogyne* population in soil over repeated crop cycles. Hypovirulence factors are dsRNA viruses those attack and multiply in fungal pathogens and reduce their virulence. A classic example is the chestnut blight, which is caused by the fungus *Cryphonectria parasitica*, and is controlled through inoculation of cankers caused by the normal pathogenic strain with that of hypovirulent strains of the same fungi. The hypovirulent strains had reduced virulence due to infection of dsRNA virus. These mycoviruses through mycelia fusions pass through hypovirulent to virulent strains of fungi and the latter are rendered hypovirulent, thus the development of canker either slows down or completely inhibited (Milgroom and Cortesi 2004). However, it must

be noted that the interaction between virus, fungus, host plant, and environmental conditions determines the success or failure of biocontrol.

A number of microbial biocontrol agents act as hyperparasites against powdery mildew fungi (Ranković 1997; Sullivan and White 2000). Hyperparasite of the genus *Ampelomyces* was most common and was found to inhibit as many as 33 different species of powdery mildew fungi (Ranković 1997). Another hyperparasite *Verticillium lecanii* inhibiting many pathogenic fungi reduced the disease incidence of *Sphaerotheca fusca* under *in planta* in low vapor pressure deficit (VPD) conditions (Verhaar et al. 1996).

Microbial predation, unlike hyperparasitism is a nonspecific interaction and reportedly less predictable biocontrol success has been known. *Trichoderma harzianum* is the widely known predatory fungi that exhibit predatory behavior under nutrient scarce environment. *Trichoderma* is an active producer of chitinase that is directed against fungal cell wall. In compost as substrate when fresh litter is used, ample amount of readily available cellulose is present, thus *Trichoderma* does not directly attack the pathogenic fungi *Rhizoctonia solani*. However, in decomposed organic matter, the concentration of free cellulose decreases that activates the chitinase activity of *Trichoderma* rendering it to act as a predator of phytopathogen *Rhizoctonia solani* (Benhamou and Chet 1997).

1.3.1.6 Competition

Rhizosphere colonizing bacterial and fungal population can inhibit the phytopathogenic fungi by imposing a competition for nutrients and space. Reduction in the concentration of essential elements like carbon, nitrogen, phosphorus, and other micro-elements often leads to less spore germination and slower germ tube growth, thus inhibiting the pathogen infection. Microbes often live in a nutrient limiting environment such as soil and plant surfaces where readily available nutrients are less available. To successfully colonize these habitats, the pathogen and existing microflora must compete for the available nutrients and space. Host plants release nutrients in the form of exudates, leachates, ward off cells and dead parts. A competition between pathogenic and nonpathogenic microorganisms can effectively reduce the disease incidence and severity. Notably, the soil-borne pathogenic fungi such as *Pythium* and *Fusarium* infect through mycelia fusion and are more prone to nutrient competition from other indigenous microflora and pathogens that germinate on plant surface through germ tube and appressorium formation (Ryder and Talbot 2015). Zimand et al. (1996) also reported the reduction in spore germination of *Botrytis cinerea* on detached leaves containing epiphytic bacterial and fungal population. The control of rot symptoms was attributed to the competition for exuded nutrients on the leaves.

The most abundant nonpathogenic plant-associated microbes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow. For example, effective catabolism of nutrients in the spermosphere has been identified as a mechanism contributing to the suppression of *Pythium ultimum* by *Enterobacter cloacae* (Van Dijk and Nelson 2000; Kageyama and Nelson 2003). At the same

time, these microbes produce metabolites that suppress pathogens. These microbes colonize the sites where water and carbon-containing nutrients are most readily available, such as exit points of secondary roots, damaged epidermal cells, and nectaries and utilize the root mucilage.

Competition for ferric iron, an essential micronutrient is also a mechanism of biocontrol by many BCAs. Iron is used as ferric iron by plants and due to frequent oxidation reduction it is present in limiting concentration in soil (Shahraki et al. 2009). Sometimes the concentration is too low in soil to support microbial growth. Therefore in such situations, microbes secrete iron chelating compounds called siderophores, those have high affinity to chelate ferric iron. Almost all soil microbes produce siderophores but bacterial siderophores have high affinity to chelate iron than fungal siderophores thus rendering fungal pathogen devoid of ferric iron (Neilands 1981). Kloepper et al. (1980a, b) first demonstrated the siderophore mediated biocontrol of *Erwinia carotovora* by plant growth-promoting strains of *Pseudomonas fluorescens*.

1.3.2 Mixed-Path Antagonism

1.3.2.1 Siderophores

Siderophores are ligands with low molecular weight having high affinity to sequester iron from the micro-environment. It has the ability to sequester ferric ion and competitively acquire iron from iron-limiting microenvirons, thereby preventing growth of other microorganisms (Das et al. 2007). Two major classes of siderophores, classified on the basis of their functional group, are catechols and hydroxamate. A mix of carboxylate-hydroxamate group of siderophores is also reported (Hider and Kong 2010; Sharma et al. 2016: Chauhan et al. 2016). Numerous strains of *Streptomyces* spp. have been reported as siderophore producers, namely, *S. pilosus* (Muller et al. 1984; Muller and Raymond 1984), *S. lydicus* (Tokala et al. 2002), and *S. violaceusniger* (Buyer et al. 1989). Biological control of *Erwinia carotovora* by several siderophore-producing and plant growth-promoting *Pseudomonas fluorescens* strains A1, BK1, TL3B1, and B10 was reported for the first time as an important mechanism of biological control (Kloepper et al. 1980a, b). On the other hand, increased efficiency of iron uptake by the commensal microorganisms is thought to dislocate pathogenic microorganisms from the possible infection sites by aggressive colonization in plant rhizosphere. Sneh et al. (1984) and Elad and Baker (1985) showed a direct correlation between in vitro inhibition capacity of chlamydospore germination of *F. oxysporum* and siderophore synthesis in fluorescent pseudomonads.

1.3.2.2 Antibiosis

The term "antibiosis" came from the term antibiotics, which refers to organic substances produced by microorganisms that affect the metabolic activity of other microbes and inhibit the growth (Roshan et al. 2013; Kumar et al. 2015). The result of antibiosis is often death of microbial cells by endolysis and breakdown of the cell

cytoplasm. *Agrobacterium radiobacter* K-84, produced commercially as Agricon 84, was first recognized as a valuable control agent of crown gall since 1973. It is very effective against *A. tumefaciens* attacking stone fruit (e.g., plums and peaches), but not effective against *A. tumefaciens* strains that attack grapes, pome fruit (e.g., apples) and some ornamentals. A variety of antibiotics have been identified, including compounds such as 2,4-diacetylphloroglucinol (DAPG), amphisin, oomycinA, hydrogen cyanide, pyoluteorin, phenazine, tensin, pyrrolnitrin, cyclic lipopeptides and tropolone produced by pseudomonads and kanosamine, oligomycin A, xanthobaccin and zwittermicin A produced by *Streptomyces, Bacillus,* and *Stenotrophomonas* spp. (Kumar et al. 2016a). For instance, antibiotic 2,4-diacetylphloroglucinol is reported to be involved in the suppression of *Pythium* spp., iturin suppresses the pathogens *Botrytis cinerea* and *Rhizoctonia solani,* and phenazine carboxylic acid antagonist the pathogen *Rhizoctonia solani* in rice (Padaria et al. 2016) and phenazines control *Gaeumannomyces graminis* var. tritici in wheat.

1.3.2.3 Volatile Substances

Apart from the production of antibiotics, some biocontrol agents are also known to produce volatile compounds as tools for pathogen inhibition. Common volatile compounds are hydrocyanic acid (HCN), certain acids, alcohols, ketones, aldehydes and sulfides (Bouizgarne 2013). HCN production is reported to play a role in disease suppression (Wei et al. 1991), for instance, Haas et al. (1991) reported HCN production by strains of *P. fluorescens* that helped in the suppression of black root rot of tobacco. Reports on the production of HCN by beneficial microbes in order to minimize the deleterious effect of pathogenic fungi and bacteria are available (Ahmad et al. 2008; Gopalakrishnan et al. 2011a, b, 2014).

1.3.2.4 Cell Wall Lysing Enzymes

Inhibition of fungi by various microorganisms relies on production of various hydrolytic enzymes to a greater extent (Walia et al. 2014; Sharma et al. 2015a, b). These enzymes include chitinases, proteases, amylases, cellulases, β 1–3 glucanases and many more those impart in the biocontrol mechanisms of antagonists. Cell wall-degrading enzymes produced by antagonists affect pathogenic fungi (Chernin et al. 1995) by lysing cell wall or other cellular constituents. Secretion of cellulase enzyme by *Bacillus subtilis* strain CKTR significantly reduced the disease severity of collar rot in tomato caused by *Phytophthora capsici*, an oomycete with cell wall composed of cellulose (Sharma et al. 2015a, b). A synergistic effect was observed due to combination of two enzymes endochitinase and chitobiosidase produced by *Trichoderma harzianum*, which was much more prominent than the activity of *Enterobacter cloacae* chitinase (Lorito et al. 1993).

Two isolates of *Trichoderma*, *T. reesei* and *T. harzianum* were reported to produce a series of lytic enzymes such as proteinase, mannanase, laminarinase, and chitinase which were actively involved in biocontrol mechanism of the two fungi (Labudova and Gogorova 1988). Wisniewski et al. (1991) also reported release

of cell wall-degrading enzymes as the possible mechanism of biocontrol by postharvest biocontrol yeast, *Pichia guilliermondii*.

In addition to the cell wall-degrading enzymes, there are other microbial by-products which play important role in plant disease control (Phillips et al. 2004). One such by-product is hydrogen cyanide (HCN) that blocks electron transport chain by inhibiting cytochrome oxidase and is highly toxic to aerobic microorganisms even at picomolar concentrations (Ramette et al. 2003). The fact is supported by earlier studies where a strain of *P. fluorescens* CHA0, produced antibiotics, siderophores and cyanides, but the inhibition of black rot fungus *Thielaviopsis basicola* was primarily due to HCN production. Similarly ammonia secretion by *Enterobacter cloacae* was involved in the biocontrol of cotton seedling damping-off caused by *Pythium ultimum* (Howell et al. 1988).

1.3.2.5 Unregulated Waste Products

Few soil microbes release a range of unregulated waste products or harmful gases, e.g. ethylene, methane, nitrite, ammonia, hydrogen sulfide, other volatile sulfur compounds, carbon dioxide, etc., and suppress the growth of other pant pathogenic bacteria. This interaction between two species is called amensalism. *Bacillus megaterium* produces ammonia and has an inhibitory effect on the growth of *Fusarium oxysporum* (Shobha and Kumudini 2012).

1.3.2.6 Detoxification and Degradation of Virulence Factor

Biological control by detoxification involves production of a protein that binds with the pathogen toxin and detoxifies pathogen virulence factors, either reversibly or irreversibly, ultimately decreasing the virulence potential of pathogen toxin. For example, the biocontrol agents *Alcaligenes denitrificans* and *Pantoea dispersa* are able to detoxify albicidin toxin produced by *Xanthomonas albilineans.* Similarly, strains like *B. cepacia* and *Ralstonia solanacearum* can hydrolyze fusaric acid, a phytotoxin produced by various *Fusarium* spp. The protein has the ability to bind reversibly with the toxins of both *Klebsiella oxytoca* and *Alcaligenes denitrificans,* as well as irreversibly with the toxin albicidin in *Pantoea dispersa.*

1.3.3 Indirect Antagonism

1.3.3.1 Competitive Root Colonization

From the microbial perspective, living plant surfaces and soils are often nutrient restricted environments. Nutrient limitation is an important mode of action of some biological control agents. Carbon plays an important role in competition of root colonization for nutrients such as *Trichoderma* spp. (Sivan and Chet 1989). Carbon competition between pathogenic and nonpathogenic strains of *F. oxysporum* is one of the main mechanisms in the suppression of Fusarium wilt (Alabouvette et al. 2009). The disease suppression of bacterium *Erwinia amylovora* causes fireblight by the closely related saprophytic species *E. herbicola* due to competition of the nutrient on the leaf surface. Competition between rhizosphere bacteria and *Pythium*

ultimum, a common cause of seedling damping-off for the same carbon source, has resulted in an effective biological control of the latter organism in several crops.

Germination of the conidia of *Botrytis cinerea* is inhibited by *Pseudomonas* species due to competition for amino acids. This mechanism may not be useful in suppressing biotrophs such as powdery mildews and rusts, because they do not require exogenous nutrients for host infection.

1.3.3.2 Plant Growth Promotion Through SAR and ISR

Chemical stimuli are produced by some biocontrol agents, i.e. nonpathogenic plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF), or by soil- and plant-associated microbes. Such stimuli can either induce a sustained change in the plants which increase the capacity of tolerance to infection by pathogens or induce the local and/or systemic host defenses of the whole plant against broad-spectrum pathogens. This phenomenon is known as induced resistance. Two types of induced resistance are distinguished in plants, systemic acquired resistance (SAR) and induced systemic resistance (ISR). The first of the two pathways is mediated by salicylic acid (SA) which is frequently produced after pathogen infection and induces the expression of pathogenesis-related (PR) proteins that include a variety of enzymes. The second method is mainly jasmonic acid (JA) and/or ethylene mediated following the applications of some nonpathogenic rhizobacteria. The SAR-induced resistant was observed when *Trichoderma harzianum* was inoculated in roots and leaves of grapes, and it provides control of diseases caused by *Botrytis cinerea* from the site of application of *T. harzianum* (Deshmukh et al. 2006). It was found that the biocontrol agent *P. fluorescens* strain CHAO induces accumulation of salicylic acid and by inducing SAR-associated proteins it confers systemic resistance to a vira1 pathogen in tobacco. Colonization of *Glomus intraradices* on the roots of *Oryza sativa* conferred resistance through induction of defense-related genes (Campos-Soriano et al. 2012). *Penicillium simplicissimum* enhanced the resistance of barley to *Colletotrichum orbiculare* by inducing salicylic acid accumulation, formation of active oxygen species, lignin deposition and activation of defense genes. In addition, *Fusarium equiseti* and Phoma spp. elicited *Arabidopsis thaliana* systemic resistance against *Pseudomonas syringae* pv. tomato and *Pythium oligandrum* against *Ralstonia solanacearum.*

However, different ISR elicitors like secondary metabolites and proteins involved in mycoparasitism and antibiosis have also been identified. Secondary metabolites like trichokinin, alamethicin, harzianopyridone, harzianolide, and 6-pentyl-α-pyrone have antagonist effects at high doses but in low doses act as ISR inducers. Expression of endochitinase Ech42 of *Trichoderma atroviride* was found to act as an ISR inducer in barley, resulting in an increased resistance to *Fusarium* spp. infection. Similarly, chitinase Chit42 of *T. harzianum* expression increased resistance in potato and tobacco against the foliar pathogens, *B. cinerea, Alternaria solani,* and *A. alternata,* and soil-borne pathogen, *Rhizoctonia solani.* The detailed aspects of these strategies need to be explored more for better results in the fields.

1.3.3.3 Combination of Modes of Action

Biological control of plant pathogens is an intricate process, which is a result of multiple mechanisms of action by antagonists. Synergism between different antagonistic strains can occur, as reflected in the *in planta* inhibition of *Phytophthora capsici* by a consortium of four *Bacillus* strains (Sharma et al. 2015a, b). This inhibition was due to a multitude of mechanisms including release of antibiotics, cellulase and protease production combined with successful colonization of antagonists in the rhizosphere. Nonpathogenic *Fusarium oxysporum* reduced the fusarium wilt by successful competition for space (infection sites) and nutrients in the soil (Schneider 1984; Allabouvette et al. 1996).

To efficiently use biocontrol agents, it is important to understand the underlying mechanisms of action for effective biocontrol, to develop safe application processes; this is also an important background to select new and efficient strains. Basic information must be generated at both, the biochemical and the molecular level, contributing in this way, in the elucidation of effects such as antibiosis, competition for nutrients, and induction of resistance (Zak et al. 2003).

In the course of last 20 years, the scenario of world population has drastically changed. To cater the growing need of food and nutrition of population worldwide, the crop production needs to be redefined in a novel way along with sustainable procedures to counter also the menace of global warming and climate change. The present challenge before agriculture industry, farmers, and researchers across the globe is to increase the crop production and maintain the quality and vitality of crops using eco-friendly methodologies. The different crops in the fields and natural habitats are encountered with different types of pathogens and these pathogens destroy the overall crops and lead to decrease in crop production. A plant pathogen is a very wide terminology that refers to any of the organisms, such as bacteria, fungi, protistans, nematodes, viruses, and other pathogens that cause plant infections and diseases. Plant pathogens that cause plant diseases weaken the ability of the farmers or growers to produce good quality and quantity of crops and can infect almost every type of plants. The traditional and conventional methods of control of plant pathogens include use of pesticides, insecticides, fungicides, herbicides, rodenticides, and other chemical formulations. These substances control the plant pathogens to a good amount but their adverse effects are also seen and felt in food chain. The numerous negative health effects that have been associated with chemical pesticides include, among other effects, dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects (WHO 1990; Sanborn et al. 2007; Mnif et al. 2011; Thakur et al. 2014). Furthermore, high occupational, accidental, or intentional exposure to pesticides can result in hospitalization and death (WHO 1990; Gunnell et al. 2007). One such detrimental effect of these chemicals is bioaccumulation which leads to biomagnification. The other method of plant pathogens control includes use of natural parasites or predators of plant pathogens which constitutes biological or natural control. Biocontrol microorganisms are cellular or noncellular entities, capable of replication or of transferring genetic material. Various soil and rhizospheric microorganisms have been explored as potential antagonists that possess characteristics of a candidate

agent. In fact, with increase in the research area related to potential biocontrol microorganisms, it has been found that such microorganisms have a broader range of activities that are correlated to biological management of plant pathogens apart from antagonism. The other effects of biocontrol agents include increase in plant vitality, pushing out the pathogens through competition for nutritional resources and occupation of ecological habitat and niche, and by inducing systemic resistance in the host through activation of the host defense mechanisms against the invading pathogen.

The potential biocontrol agents explored so far are *Bacillus subtilis, Pseudomonas fluorescens, Gliocladium* spp., *Trichoderma* spp., *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii,* granulosis viruses, nuclear polyhedrosis viruses (NPV), *Nomurea rileyi*, *Hirsutella* species, *Verticillium chlamydosporium, Streptomyces griseoviridis, Streptomyces lydicus, Ampelomyces quisqualis, Candida oleophila, Fusarium oxysporum* (nonpathogenic), *Burkholderia cepacia, Coniothyrium minitans, Agrobacterium radiobacter* strain 84, *Agrobacterium tumefaciens, Pythium oligandrum, Erwinia amylovora* (hairpin protein), *Phlebia gigantean, Paecilomyces lilacinus, Penicilliuim islanidicum* (for groundnut), *Alcaligenes* spp., *Chaetomium globosum, Aspergillus niger* strain AN27, VAM fungi, *Myrothecium verrucaria, Photorhabdus luminescens* sakhurustii strain K-1, *Serratia marcescens* GPS 5 and *Piriformospora indica*. These biological agents act on plant pathogens through different modes of action. It includes direct antagonisms like parasitism, for instance, *Trichoderma* is a parasite of a range of fungi and oomycetes in the soil, which produce toxic metabolites and cell wall-degrading enzymes and inhibit the growth of others, hyperparasitism, e.g. *Hypovirus*, a hyperparasitic virus on *Cryphonectria parasitica*, a fungus causing chestnut blight, commensalism, mixed-path antagonism by synthesis of chemicals like siderophores, antibiotics, volatile compounds like HCN, lytic enzymes, and indirect antagonisms like competitive root colonization and plant growth promotion through systemic acquired resistance (SAR) and induced systemic resistance (ISR). Biocontrol agents are safe both for the environment and for the persons who are applying them and avoid environmental pollution (soil, air, and water) by leaving no toxic residues. It is comparatively easier to manufacture biocontrol agents, sometimes less expensive than chemical agents. The biggest advantage of using biocontrol agents is that they can eliminate the specific pathogens effectively from the site of infection and can be used in combination with biofertilizers. Biocontrol agents avoid problems of resistance and also induce systemic resistance among the crop species. The only negative aspect of these agents is that these agents work slowly and less effectively in comparison with the chemical pesticides, as their efficacy almost completely depends on environmental conditions. However, these constraints can be nullified due to constant research and more effective biocontrol agents can be generated as these are the demand of present world for safe food.

1.4 Biocontrol of Plant Pathogens

1.4.1 Biocontrol of Bacterial Plant Pathogens

A broad array of food crops and ornamental plants are susceptible to bacterial infection. Many a times, abrupt onset of bacterial infections leads to major economic losses to farmers. Every types of vegetable crops, vines, fruit trees, and ornamental plants has been distressed by bacterial infections (Amusa and Ojo 2002; Amusa and Muhammad 2003). Among major bacterial diseases, bacterial wilt caused by *Ralstonia solanacearum* and bacterial blight caused by *Xanthomonas campestris* are most devastating. *Pseudomonas syringae* pv. syringae is unique pathovar of *P. syringae* that is known to cause disease in nearly 180 different plant species (Bradbury 1986). This pathogen causes bacterial canker and blast disease in stone fruit trees and affects nearly all commercially grown *Prunus* species in the USA (Ogawa and English 1991). Other bacterial disease of economic important crops includes *Erwinia carotovora* var. *carotovora* causing fruit and vegetable rot under field and postharvest practices. *Xanthomonas citri* is a known bacterial pathogen causing citrus canker, leaf spots, and oozes from infected stems. *X. citri* has a wide host range causing necrotic lesions and canker in tangelo, sweet orange, grape, and lime (Orce et al. 2015).

Since the effective bactericides are very scarce and most fungicides available do not control bacterial disease. McMullen and Arthur Lamey (2000) reported that antibacterial antibiotic streptomycin inhibit blight bacteria only at the surface and not in internal tissues. Due to non-availability of synthetic chemicals for controlling bacterial diseases of crops, farmers often incur huge losses on crops infected with these pathogens. He et al. (2020) identified several metabolites produced by *Streptomyces* sp. A217, which proved to be effective in inhibiting the growth of several plant pathogenic bacteria and fungi. Since agricultural chemicals are chiefly concerned with environmental pollution, deposition of residues in food chain and rendering the pathogens resistance, there is a strong need for considering more environmentally friendly control measures. Biological control of bacterial diseases has overcome the many drawbacks of using chemical inputs. Table 1.1 shows some known bacterial diseases and their reported biocontrol agents. In developed countries, BCAs have been successfully introduced in commercial markets for controlling certain diseases such as crown gall, caused by *Agrobacterium tumefaciens* (Vicedo et al. 1993), and fire blight of pear, caused by *Erwinia amylovora* (Özaktan and Bora 2004). However these commercialized biocontrol agents have found little applicability worldwide more specifically in developing nations. Reason for this emanate as the lack of knowledge, facilities and funds required to conduct research on biological control of plant diseases. In cases where technologies have been adopted, subsequent problems like inconsistent performances leading to farmer's rejection are needed to be addressed.

Numerous saprophytic bacteria belong to genera *Pseudomonas*, *Xanthomonas*, *Pantoea*, *Bacillus*, *Lactobacillus* and certain actinobacteria live as epiphytes in early growing stages of plants. Some pathogenic bacteria, such as *Pseudomonas syringae*,

Table 1.1 List of bacterial pathogens and their biocontrol agents

Bacterial pathogen	Host/ disease involved	Biocontrol agent	Reference
Agrobacterium tumefaciens	Peach/ crown gall	*Agrobacterium radiobacter* K84	Vicedo et al. (1993)
Xanthomonas spp.	Tomato/ bacterial spot	*Hrp* mutant strain of *Xanthomonas campestris* pv. *Vesicatoria*	Moss et al. (2007)
Xanthomonas spp.	Citrus/ bacterial canker	*Pseudomonas* spp.	Khodakaramian et al. (2008)
Ralstonia solanacearum	Tomato/ bacterial wilt	*Acinetobacter* and *Enterobacter*	Xue et al. (2009)
Xanthomonas euvesicatoria	Tomato/ bacterial spot	Mutant strain of *Xanthomonas perforans*	Hert et al. (2009)
Ralstonia solanacearum	Tomato/ bacterial wilt	*Bacillus* spp.	Almoneafy et al. (2012)
Ralstonia solanacearum	Tomato/ bacterial wilt	*Bacillus amyloliquefaciens*	Tan et al. (2013)
Erwinia carotovora ssp. *Carotovora*	Potato/ soft rot	*Streptomyces diastatochromogenes, Streptomyces gramineareus*	Doolotkeldieva et al. (2016)
Erwinia amylovora	Pear/fire blight	*Pseudomonas* and *Pantoea* spp.	Sharifazizi et al. (2017)
Ralstonia pseudosolanacearum; R. syzygii subsp. *Indonesiensis*	Tobacco/ bacterial wilt	Bacteriophage P4282	Alvarez and Biosca (2017)

Erwinia amylovora, *E. carotovora,* and *Xanthomonas* also live epiphytically on many plant tissues before invading and causing plant infection. It has been reported that foliar spray of these saprophytic bacteria and avirulent strains of pathogenic bacteria effectively reduced the subsequent pathogenic attack by other pathogenic bacteria and fungi. For example, foliar spray of *Erwinia herbicola* formulation partially inhibited the fire blight of apple blossoms, caused by *E. amylovora*; and spraying suspensions of *Erwinia* and of *Pseudomonas* inhibited the bacterial leaf streak of rice, caused by *Xanthomonas translucens* spp. *Oryzicola* (Agrios 1997).

Biocontrol of bacteria-mediated frost injury has also been reported. Frost-sensitive plants are injured due to ice crystal formation below 0 °C. However ice crystals will not form if no catalyst centers or cell nuclei are present to influence ice formation even at temperature as low as −10 °C. Three strains of epiphytic bacteria, *P. syringae*, *P. fluorescens*, and *E. herbicola*, serve as ice nucleation-active catalysts

for ice formation at low temperature. However spraying non-ice nucleation-active bacteria antagonistic to above reported ice nucleation-active bacteria will significantly reduce the ice forming bacterial population on the plant surfaces. This treatment protects frost-sensitive plants from injury at temperatures at which untreated plants may be severely injured (Agrios 1997).

Another strategy which is gaining prominence in the recent times for screening out potential biocontrol agents is to look for quorum quenching microbes. Alymanesh et al. (2016) isolated several bacteria belonging to the genus *Pseudomonas*, which possessed quorum quenching and were also observed to be effective in controlling several bacterial and non-bacterial phytopathogens. Similarly, Alinejad et al. (2020) have also reported that several quorum quenching bacteria like *Pseudomonas fluorescens*, *Bacillus pumilus,* etc. were capable of controlling *Pectobacterium carotovorum* subsp. *carotovorum*.

1.4.2 Biocontrol of Fungal Plant Pathogens

Fungal plant diseases are quite diverse and affect nearly all plant parts such as root, stem, leaf, fruit, etc. Besides chemical control of fungal pathogens, biological control has also been in practice. Studies have highlighted several bacterial and fungal biocontrol agents to combat fungal diseases. These BCAs may be applied as soil application or seed treatment mostly against soil-borne fungal pathogens (Sharma et al. 2015a, b; Hussain 2018; Smolińska and Kowalska 2018). Since many soil-borne pathogenic fungi can spread readily in aerial plant parts, control of these pathogens requires suppression of primary inoculum in soil and reduction in the infection rate (Lo et al. 1997). Table 1.2 shows the major fungal pathogens, their diseases and biocontrol agents involved in their management.

Biocontrol agents are often employed with additives to improve their efficacy against fungal pathogens. Lo et al. (1997) reported the seed treatment with 10% Pelgel primed with solid matrix which enhances biocontrol efficacy of *Trichoderma* spp. against *Pythium* spp. Use of certain surfactants such as Triton 100 as additive along with *Trichoderma* gave effective biocontrol as chemical fungicides. Here surfactant enhanced the biocontrol activity by acting against pathogen's cell membrane and by enhancing wetting and attachment of *Trichoderma* spore suspension to infection site (Lo et al. 1997).

Trichoderma, an effective biocontrol agent of several soil-borne and foliar fungal pathogens of crop plants, is applied as granular suspension in soil and as spray suspension. Although population of *Trichoderma* strain 1295–22 in soils treated with each separate suspension was found to be equivalent, granular suspension resulted in more effective decrease in disease incidence of Pythium blight, root rot, and brown patch when compared to spray application (Lo et al. 1995, 1996, 1997). This may reflect the inoculum potential of two separate applications and inferred that efficacy of *Trichoderma* as BCA is chiefly determined by method of application. The granules were several millimeter-diameter particles colonized by *Trichoderma*.

Table 1.2 List of selected fungal pathogens and their biocontrol agents

Fungal pathogen	Host/disease involved	Biocontrol agent	Reference
Fusarium oxysporum f. sp. *lycopersici*	Tomato/ vascular wilt	Nonpathogenic *Fusarium* spp., *Trichoderma* spp., *Gliocladium virens*, *Pseudomonas fluorescens*, *Burkholderia cepacia*	Larkin and Fravel (1998)
Phytophthora capsici	Pepper/ crown root rot	*Bacillus* spp. and *Trichoderma harzianum*	Sid Ahmed et al. (2003)
Fusarium oxysporum f. sp. *melonis*	Musk melon/ vascular wilt	*Pseudomonas putida*	Bora et al. (2004)
Magnaporthe oryzae and *Rhizoctonia solani*	Rice/Rice blast and sheath blight	*Pseudomonas fluorescens*	Reddy et al. (2009)
Pythium spp.	Tomato/root diseases	*Pseudomonas fluorescens*	Khalil and Alsanius (2010)
Phytophthora capsici	Pepper/ crown root rot	*Bacillus cereus* and *Chryseobacterium* spp.	Yang et al. (2012)
Colletotrichum Gloeosporioides	Grape berries/ anthracnose	*Bacillus amyloliquefaciens* S13-3	Mochizuki et al. (2012)
Bipolaris maydis	Maize/ southern corn leaf blight	*Bacillus amyloliquefaciens*	Deng et al. (2014)
Rhizoctonia cerealis	Wheat/wheat sharp eyespot	*Bacillus amyloliquefaciens*	Deng et al. (2014)
Phytophthora capsici	Tomato/ crown rot	*Bacillus subtilis*	Sharma et al. (2015a, b)
Fusarium oxysporum	Tomato/ wilting	*Bacillus* spp./*Pseudomonas* spp.	Verma et al. (2018)

However, conidial suspension applied as spray, on the other hand, was much smaller and would therefore be expected to contain lower inoculum potential.

Fusarium, Phytophthora, Pythium, *Rhizoctonia,* and *Alternaria* are important soil-borne phytopathogenic fungi those live as saprophytes on plant debris in soil and serve as primary inoculum. Therefore, in order to combat the subsequent infection, suppression of the primary inoculum is required in soil (Lo et al. 1997). Besides drenching of soil with granular application of BCA, monthly spray application is required to inhibit foliar infection of these pathogens from soil. Inhibition of the secondary inoculum and its dissemination is also important for fungal disease management (Agrios 1997).

In addition to *Trichoderma* and other fungal BCAs, many antagonistic bacteria including species of genus *Pseudomonas*, *Bacillus*, *Burkholderia*, *Bacillus* have also been used successfully for biocontrol of various soil-borne fungal pathogens (Heydari and Misaghi 1998; Haas and Defago 2005; Heydari and Pessarakli 2010; Sharma et al. 2015a, b). By application of these bacterial antagonists, various fungal pathogens such as *Rhizoctonia solani*, *Fusarium solani*, *Pythium, Verticillium dahliae*, *Phytophthora capsici,* and other soil-borne diseases caused by them such as root rot, damping-off, collar rot, and vascular wilt have been biologically controlled on major agricultural crops including cotton, wheat, rice, capsicum, and other crops of economic importance. Shan et al. (2019) have reported that *Alcaligenes faecalis* displayed antifungal activities against *Botrytis cinerea*. Liu et al. (2020) have reported that lipopeptides produced by *Bacillus velezensis* HC6 were effective in controlling *Fusarium* and *Aspergillus*.

1.4.3 Biocontrol of Plant Parasitic Nematodes

Nematodes are extremely abundant and diverse eukaryotic, multicellular, invertebrate organisms belonging to the kingdom Animalia. These organisms have tube-within-a-tube body plan. Although most of the nematodes are free-living and feed on microorganisms like bacteria, fungi, protozoans as well as other nematodes, many are parasites of animals (including human beings) and plants. Needham (1743) is credited for the discovery of first described plant parasitic nematode in wheat seeds. Later, root-knot nematodes on cucumber and cyst nematodes causing "beet-tired" disease on sugar beets were reported by Berkeley in 1855 and Schacht in 1859, respectively (Lambert and Bekal 2002). Pioneering work done by Cobb and Carter went a long way in proving the significance of agricultural nematology. Sasser and Freckman (1987) estimated annual crop losses amounting to 77 billion dollars worldwide due to plant parasitic nematodes. The crop losses in 2015, due to nematodes were projected to be worth 157 billion dollars worldwide, out of which $40.3 million was reported from India alone (Singh et al. 2015).

All plant parts, including roots, stems, leaves, flowers, and seeds are susceptible to nematode attacks. However, root associated soil-borne nematodes are quite commonly encountered. Nematodes make use a specialized spear called a stylet for feeding on plant cell. The nematode feeding may result into cell death leading to formation of lesions. On the other hand, there are some nematodes which do not cause immediate cell death rather they result in enlargement and growth of plant cells, thus leading to development of nutrient-rich feeding cells for the nematode. These feeding cells (like giant cells) may result from repeated nuclear division without complete cell division or by the fusion of adjacent cells into syncytia by the breakdown of neighboring cell walls. Such feeding cells enable long-term feeding associations. Some plant nematodes spend most of their time in the soil (ectoparasites) and others are mostly contained within the plant tissue (endoparasites). On the basis of modes of lifestyle, the plant parasitic nematodes show seven major types of strategies (Perry and Maurice 2011) (Table 1.3).

Table 1.3 Major categories of plant parasitic nematodes

S. no.	Nematode type	Main plant organ affected	Example
1	Ectoparasitic nematode	Roots	*Xiphinema* (dagger nematode)
2	Semi-endoparasitic nematode	Roots	*Rotylenchulus reniformis* (reniform nematode), *Tylenchulus semipenetrans* (citrus nematode)
3	Migratory endoparasitic nematode	Roots	*Pratylenchus* (lesion nematode), *Radopholus* (burrowing nematodes), *Hirschmanniella* (rice root nematode)
4	Sedentary endoparasitic nematode	Roots	Cyst nematodes (*Heterodera* and *Globodera*) Root-knot nematodes (*Meloidogyne*)
5	Stem and bulb nematode	Stem, bulb	*Ditylenchus* *Bursaphelenchus xylophilus*
6	Seed gall nematode	Leaves, seeds	*Anguina*
7	Foliar nematode	Leaves, foliage, buds	*Aphelenchoides*

Ectoparasites**:** These nematodes do not enter into the host plant but penetrate their stylets into the host plant roots. These nematodes can affect large number of host plants. Moreover, since they are ectoparasitic, if conditions become unfavorable on one host plant, these nematodes tend to move to new host plant. These can result in formation of root galls in the roots as well as stunting of the root system and are also responsible for transmission of plant viruses; e.g., *Xiphinema* or dagger nematode.

Semi-endoparasites: Nematodes that feed as semi-endoparasites are able to partially penetrate the plant and feed at some point in their life cycle. These nematodes' heads become inserted into the plant root and the nematode forms a permanent feeding cell. When such nematodes switch to endoparasitic mode, they tend to swell up and become non-motile. Nematodes like *Rotylenchulus reniformis*, the reniform nematode, and *Tylenchulus semipenetrans*, and the citrus nematode fall into this category.

Migratory endoparasites: As the name suggests, such nematodes move on migrating through root tissues and causing destruction of the plant tissue by feeding on them. These nematodes cause massive plant tissue necrosis because of their migration and feeding (Jones et al. 2013). Due to such tissue damage, the plants become susceptible to secondary bacterial and fungal infections which in turn cause further damage to the plant roots. For example, *Pratylenchus* or lesion nematode, *Radopholus* or burrowing nematode, *Hirschmanniella* or rice root nematode.

Sedentary endoparasites: This group includes two major kinds of nematodes, viz. cyst nematodes (e.g., *Heterodera, Globodera*) and root-knot nematodes (e.g., *Meloidogyne* spp.). As the name suggests, these nematodes have a sedentary mode of lifestyle and in fact in the earlier stages of life these nematodes remain completely

embedded in the roots of the host plant (Gheysen and Mitchum 2011). However, the cyst nematodes tend to protrude from the root at later stage of life. These nematodes cause the formation of large feeder cells like giant cells and syncytia. Female sedentary endoparasites enlarge considerably into a sac-like shape and are capable of laying large number of eggs. Eggs are typically laid outside the nematode in a gelatinous egg mass (Perry and Maurice 2011), but in cyst nematodes most eggs are retained inside the female body. The cyst nematodes are extremely problematic because they have the ability to persist for a long period of time in a field by forming the dormant cysts. Having a resistant or dormant nematode stage enables nematodes to survive in non-optimal conditions. Resistant stages also aid the nematode in dispersal. Unlike the cyst nematodes, the root-knot nematodes do not show formation of any resistant structure to tide over the unfavorable conditions. However, these nematodes have much broader host range, thus they are able to survive on one host or the other.

Stem and bulb nematodes: As is indicated by the name, these nematodes (like *Ditylenchus* spp.) damage the upper and lower parts of plants. These nematodes are able to move up the plant stems with the help of water films and hence tend to result in greater infestation and damages during moist environmental conditions. These nematodes enter the shoots via buds, petioles, or stomata and then they behave like the migratory nematodes and cause damage to the host plant by constant feeding and migration. They tend to form dried fluffy masses (known as "nematode wool.") during unfavorable conditions and resume their activity on return of favorable conditions. Another nematode *Bursaphelenchus xylophilus* (pine wood nematode) causing pine wilt disease can be disseminated by insects (Mamiya 1983).

Seed gall nematodes: Seed gall nematodes (*Anguina* spp.) migrate to the leaves of plants where they feed as ectoparasites at the tips, causing distortion of the leaves. Once the plant starts to flower, it penetrates the floral primordia and starts to feed on the developing seed eventually killing the seed to form a blackened "cockle" (seed gall).

Foliar nematodes: Foliar nematodes belong to the genus *Aphelenchoides*. They migrate in water films on the stems to the leaves of their host plant and penetrate the leaves through stomata and destructively feed on plant cells resulting in characteristic interveinal chlorosis and necrosis of the leaf, ultimately resulting in death. In the winter the adult nematodes persist in the dead leaves until favorable conditions arise in the spring. There are several instances where plant-parasitic nematodes assist plant-pathogenic bacteria and viruses as carriers as well as specific vectors of bacterial plant pathogens (Hao et al. 2012). A few examples include the "tundu" or yellow ear-rot disease of wheat, annual ryegrass toxicity and cauliflower disease of strawberry are such diseases transmitted by nematodes (Khan and Pathak 1993).

The idea of using biocontrol for controlling the plant parasitic nematodes has been put forth by Cobb (1917). The efforts put in the direction of nematode biocontrol using microbial agents have been reviewed by several workers, Thorne (1927), Linford (1937), Drechsler (1941), Duddington (1962), Pramer (1964), Rodriguez-Kabana et al. (1965), Mankau (1980), Sayre (1986), Siddiqui and Mahmood (1996).

1.4.4 Fungal Agents for Nematode Biocontrol

Kuhn (1877, 1881) was the first person to observe and report the parasitism of female nematodes belonging to *Heterodera schachtii* by a fungus (named as *Tarichum auxiliare)*. The life cycle of nematodes basically comprises of the egg stage, larval stage, and adult stage. Apart from that some nematodes produce dormant structures like the cysts which help them in tiding over the unfavorable environmental conditions. Several investigations carried out across the globe indicate the fact that all these stages of nematode life cycles are susceptible to attacks by various fungi. For example, Barron (1977) reported *Rhopalomyces elegans* as a nematode egg parasite. Kerry et al. (1980), working in England, reported *Nematophthora gynophila* was capable of parasitizing the adult females and cysts of *Heterodera avenae* (cereal cyst nematode). Stirling and Mankau (1978), working in California (North America), found a fungal species (*Dactylella oviparasidca*) that attacked eggs and adults of *Meloidogyne javanica*. Jatala et al. (1979), working in Peru, isolated *Paecilomyces lilacinus,* which parasitized the eggs and adults of *M. incognita* and *Globodera pallida.* Also, the fungi which have been found to possess capability to control nematodes can be broadly grouped into predacious (or nematophagous) fungi, endoparasitic fungi, opportunistic fungi, arbuscular mycorrhizal fungi.

The nematode-trapping fungi have drawn the attention of scientists as a potential nematode biocontrol agent from the 1930s (Linford 1937). These soil fungi possess unique features (like trap cells, hyphal coils, extracellular polymers, adhesive network, adhesive knob, adhesive column, nonconstricting ring and constricting ring) for ensnaring prey (Niu and Zhang 2011). Most of such fungi can be easily cultured and hence easy to produce at a large scale. However, there are certain constraints in utilizing these fungi. These are nonspecific parasites, not aggressive toward nematodes, also their trapping activity is mainly restricted to the initial stages of growth (Siddiqui and Mahmood 1996). But in spite of these problems, some favorable reports of successful use of *Arthrobotrys* spp. are there (Slepetiene et al. 1993; Dias and Ferraz 1994). *Arthrobotrys* based nematode biocontrol agents have been developed for use in mushroom culture and tomato (Cayrol and Frankowski 1979). Some later reports have also indicated *A. oligospora* as a potential biocontrol agent (Hashmi and Connan 1989; Grønvold et al. 1993; Bird and Herd 1995; Chandrawathani and Omar Jand Waller 1998; Jaffee 2004; Yan et al. 2007).

Another category of fungi includes the endoparasitic fungi. These are mostly obligate parasites generally lack saprophytic phase. They produce motile zoospores and belong to Chytridiomycetes and Oomycetes (Persmark et al. 1992; Li et al. 2000; Bordallo et al. 2002). The spores of such fungi tend to get attached to nematode cuticle (Siddiqui and Mahmood 1996). However, certain inherent problems like limited growth in culture conditions, poor competitiveness, and susceptibility to mycostasis hamper their establishment in the soil environment, thus making them relatively less suitable candidates for nematode biocontrol.

The opportunistic fungi or facultatively parasitic fungi include those fungi which normally have a saprophytic mode of living but can switch to parasitizing the

nematodes (Jansson and Lopez-Llorca 2001). This group is represented by *Paecilomyces lilacinus* and *Pochonia chlamydosporia* (*Verticillium chlamydosporium*) (Lopez-Liorca et al. 2002; Khan et al. 2004). A lot of research work has been undertaken on the nematode controlling activity of these fungi in different plants. Reduction in nematode population was found in cucumber (Stephan et al. 1991) and watermelon (Vicente et al. 1991) by using *Paecilomyces lilacinus.* This fungus has been found to be effective against the nematodes belonging to the *Heteroderid* group like *M. incognita, M. javanica, Heterodera cajani, R. reniformis* (Ekanayake and Jayasundara 1994; Gautam et al. 1995; Siddiqui et al. 1996; Walters and Barker 1994; Kiewnick and Sikora 2006). Similarly, *Pochonia chlamydosporia* has been reported to be effective against nematodes like *Globodera, Heterodera, Rotylenchulus, Meloidogyne, Nacobbus,* etc. (De Leij and Kerry 1990; Muller 1992; Manzanilla-López et al. 2013). They attack the nematodes within the host plant roots, on the root surface, or in the soil. These fungi can colonize nematode reproductive structures, penetrating the cuticle barrier (by using extracellular hydrolytic enzymes) to infect and kill the nematode hosts. Once in contact with cysts or egg masses of nematodes, these fungi also grow rapidly and eventually parasitize all eggs that are in the early embryonic stages of development (Manzanilla-López et al. 2013). Certain other common fungi like *Cylindrocarpon, Fusarium, Penicillium* have been found to be associated with nematodes (Crump 1987; Ruanpanun et al. 2010).

Another category of fungi which have inhibitory effect on plant parasitic nematodes include the arbuscular mycorrhizal fungi. These are obligate root symbionts associated with majority of land plants. They possess many activities which enhance the plant growth including transportation of water and nutrients as well as protection from environmental stress and pathogens and parasites like nematodes (Gianinazzi et al. 2010; Singh et al. 2011; Vos et al. 2012a; Kaushal and Wani 2016). There are many reports of suppression of plant parasitic nematodes by AMF (Veresoglou and Rillig 2012; Pinochet et al. 1996; Hol and Cook 2005). The protective effects against nematodes have been demonstrated in many plants like banana, coffee, and tomato (Calvet et al. 2001; Vos et al. 2012b; Alban et al. 2013).

Several mechanisms can be put forth to explain the protective action of AMF against parasitic nematodes. These include direct competition (for space or nutrients) (Schouteden et al. 2015) or inhibition, enhanced or altered plant growth, morphology and nutrition, biochemical changes associated with plant defense mechanisms and induced systemic resistance (Elsen et al. 2008), development of an antagonistic microbiota possibly by altered root exudates (Whipps 2004). The biocontrol probably results from a combination of different mechanisms (Vierheilig et al. 2008; Cameron et al. 2013). Better understanding of the mechanisms involved will probably result into the development of a commercial product based on AMF for fungal biocontrol in the near future.

1.4.5 Bacterial Agents for Nematode Biocontrol

Due to inherent advantages of bacteria like rapid growth, better adaptability, easier genetic manipulations, etc. many efforts were made to ascertain their possible use for nematode control. The bacteria which can be used for such purpose include obligate parasitic bacteria, opportunistic parasitic bacteria, plant growth-promoting rhizobacteria, parasporal Cry protein-forming bacteria, endophytic bacteria, and symbiotic bacteria (Tian et al. 2007).

The group of obligate parasitic bacteria is represented by the genus *Pasteuria*. Mankau (1975) recognized *Bacillus penetrans* as a possible candidate for nematode control. Sayre and Starr (1985) later designated the bacterium as *Pasteuria penetrans.* Members of the genus *Pasteuria* are mycelial, endospore-forming bacteria parasitizing nematodes and water fleas (Sayre and Starr 1985; Bekal et al. 2001). *Pasteuria* has been found to be capable of infecting nematodes of 116 genera, including both many economically important plant-parasitic nematodes and free-living nematodes (Chen and Dickson 1998; Bird et al. 2003). *Pasteuria penetrans* primarily attacks root-knot nematodes such as *Meloidogyne* spp., *Pratylenchus thornei* infects root-lesion nematodes such as *Pratylenchus* spp., and *Pasteuria nishizawae* mainly parasitizes cyst nematodes belonging to the genera *Heterodera* and *Globodera* (Atibalentja et al. 2000). *Pasteuria penetrans* was found to attach to the nematode cuticle, then penetrating *M. incognita* and establishing microcolonies in the nematode pseudocoelom ultimately killing the nematode and releasing endospores in the soil (Mankau et al. 1976; Sayre and Wergin 1977). Econem is a commercially available preparation made using *Pasteuria* sp. (Abd-Elgawad and Vagelas 2015).

Opportunistic parasitic bacteria include those bacteria which are generally saprophytic but can switch to nematophagous mode. This category is represented by *Brevibacillus laterosporus* and *Bacillus* sp. *B. laterosporus* has been reported to show deleterious effect on four nematode species, viz., *Heterodera glycines, Trichostrongylus colubriformis, Bursaphelenchus xylophilus*, *Panagrellus redivivus* (Oliveira et al. 2004; Huang et al. 2005). The bacteria has been found to attach to the epidermis, propagate rapidly, and secrete hydrolytic enzymes (Decraemer et al. 2003), resulting in circular holes in nematode cuticle. Subsequently, the microbe penetrates inside and digests tissue of host body (Huang et al. 2005).

The plant growth-promoting rhizobacteria have also been found to possess nematode controlling properties. Mostly bacteria belonging to the genera *Bacillus* and *Pseudomonas* have been found to possess antagonistic activity toward nematodes. Members of the genus *Bacillus* have been found to be active against plant-parasitic nematodes like *Meloidogyne, Heterodera*, and *Rotylenchulus* (Gokta and Swarup 1988; Siddiqui and Mahmood 1999; Kokalis-Burelle et al. 2002; Meyer 2003; Giannakou and Prophetou-Athanasiadou 2004; Li et al. 2005). Similarly, *Pseudomonas* strains have also been reported to display antagonistic activity toward nematodes using a variety of mechanisms including production of antibiotics, metabolites, extracellular enzymes and the induction of systemic resistance (Spiegel et al. 1991; Cronin et al. 1997; Siddiqui and Shaukat 2002, 2003; Siddiqui et al.

2005). Other rhizobacteria reported to show antagonistic effects against nematodes include members of the genera *Actinomycetes, Agrobacterium, Arthrobacter, Alcaligenes, Azotobacter, Beijerinckia, Burkholderia, Clostridium, Corynebacterium, Desulforibtio, Enterobacter, Flavobacterium, Gluconobacter, Klebsiella, Methylobacterium, Paenibacillus polymyxa, Rhizobium, Streptomycetes, Serratia,* etc. (Tian et al. 2007; Kaur et al. 2016).

Several commercial products based on such bacteria are available. For example, Deny is a commercial biocontrol product containing *Burkholderia cepacia* (Meyer and Roberts 2002). Another product called Bio Yield™ containing *Paenibacillus macerans* and *Bacillus amyloliquefaciens* is available (Meyer 2003). Another product BioNem contains 3% lyophilized *Bacillus firmus* sporesis used in Israel (Giannakou and Prophetou-Athanasiadou 2004).

The next category includes *Bacillus thuringiensis* (Bt) which produces parasporal crystal inclusions (Cry or δ-endotoxins), possessing toxic activity toward insects and nematodes. Some Cry proteins are also toxic to other invertebrates such as nematodes, mites, and protozoans (Feitelson et al. 1992). Six Cry proteins (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21) have been reported to be toxic to larvae of various nematodes (Alejandra et al. 1998; Crickmore et al. 1998; Wei et al. 2003; Kotze et al. 2005). Cry protein exerts tend to result in formation of lytic pores in the cell membrane of gut epithelial cells ultimately resulting in the eventual degradation of the intestine (Crickmore 2005; Marroquin et al. 2000). Also some strains of *B. thuringiensis israelensis*, *B. thuringiensis kurstaki,* and *B. sphaericus* have been reported to show detrimental effect on the eggs and larvae of parasitic nematode *Trichostrongylus colubriformis* (Bottjer et al. 1986; Bowen and Tinelli 1987; Meadows et al. 1989).

A few endophytic bacteria present inside root tissue have been shown to promote plant growth and to inhibit disease development and nematode pests (Sturz and Matheson 1996; Shaukat et al. 2002; Sturz and Kimpinski 2004). For example, Munif et al. (2000) reported 21 endophytic bacterial isolates obtained from tomato roots to possess antagonistic properties toward *M. incognita.* Endophytic bacteria are thought to inhibit nematodes by competition, production of inhibitory chemicals, and induction of systemic resistance (Hallmann 2001; Compant et al. 2005).

A new category of bacteria possessing anti-nematode properties has been identified in case of bacteria like *Xenorhabdus* spp. and *Photorhabdus* spp., which show symbiosis with entomopathogenic nematodes *Steinernema* spp. and *Heterorhabdus* spp., respectively. It has been reported that these bacteria have some antagonistic activity toward plant parasitic nematodes (Bird and Bird 1986; Perry et al. 1998; Lewis et al. 2001). These bacteria produce some inhibitory compounds (Samaliev et al. 2000) toxic to larvae of *M. incognita* and pine wood nematode *B. xylophilus* (Hu et al. 1999).

1.4.6 Biocontrol of Insects and Mites

Insects form a very diverse group of invertebrate organisms belonging to phylum Arthropoda and class Insect with hard chitinaceous exo-skeleton. Their body is segmented into head, thorax, and abdomen and they generally possess three pairs of jointed legs. They occupy little more than two thirds of the known species of animals in the world (Ujagir and Oonagh 2009). All types of plants including food crops, fruit and vegetable plants, oilseeds plants, fiber plants, forest trees, medicinal plants, weeds, etc. are susceptible to attack by insects. Some insects also attack the stored food articles, and hence can result in severe crop losses and even food scarcity (Manosathiyadevan et al. 2017). As per some estimates provided by FAO, insects cause at least one fifth of worldwide crop losses every year. As per Ujagir and Oonagh (2009), "Insects that cause less than 5 % damage are not considered as pests. The insects which cause damage between 5 and 10% are called minor pests and those that cause damage above 10% are considered as major pests." Depending on the type of mouth parts, the insects can be broadly said to be chewing type and sucking type. Not only this, many insects also act as vectors for various plant diseases. Also, the damage caused by biting, chewing, piercing (during sucking) by the insect, results into plant injury, thus making them more susceptible to microbial infection. The insect pests include leaf hopper, leaf miner, stem borer, whitefly, weevil, aphids, scarab beetles, caterpillars, locusts, midge, etc. Some of the major crops and their insect pests are provided in Table 1.4.

Mites form another very significant group of arthropods which can affect agricultural crops as well as stored products. Mites are microscopic arthropods belonging to class Arachnida and sub-class Acarina. These organisms differ from the insects mainly due to the fact that they have un-segmented (entire) body and bear four pairs of legs (Baker and Wharton 1952). There are several plant parasitic mites associated with the various crops like cereals, pulses, oilseeds, fruits and vegetables, etc. Some of the commonly encountered mites include *Tetranychus*, *Brevipalpus, Eotetranycus, Eutetranychus, Tarsonemus, Tenuipalpus, Oligonychus,* etc. (Putatunda et al. 2002). The major phytophagus mites belong to the families

Table 1.4 A few common insect pests of selected crops/plants

S. no.	Crop/plant	Insect pests
1	Rice	Rice stem borer, rice gall midge, green rice leaf hoppers, brown plant hopper, rice leaf folder, rice earhead bug, whorl maggot
2	Wheat	Grain aphid, termites, armyworm, American pod borer, jassid
3	Sugarcane	Early shoot borer, internode borer, sugarcane top borer, sugarcane leaf hopper, woolly aphid
4	Cotton	Cotton aphid, cotton leaf hopper, cotton whitefly, spotted bollworm, pink bollworm, American bollworm
5	Cruciferous vegetables	Diamond back moth, leaf Webber, cabbage butterfly, cabbage aphid, mustard sawfly, painted bug
6	Mango	Mango hoppers, flower Webber, gall midges, fruit fly, stone weevil, mango stem borer, red tree ant, mealybug

Tetranychidae (spider mites), Tenuipalpidae (false spider mite), and Eriophyidae (gall mites) (Singh et al. 2016). Some of the common symptoms in plants associated with mite infestation include dusty appearance (especially in case of Tetranychidae), white spots (chlorosis), mosaic like appearance, formation of galls (in case of Eriophyidae members) ultimately resulting in drying and shriveling of plants. Also, just like the insect infestation, the mite infestation also enhances the probability of microbial infection.

1.4.7 Bacterial Agents for Insect and Mite Biocontrol

The use of bacterial agents for biocontrol of insect pests is not a new phenomenon. One of the most commonly used biopesticides is based on the bacteria *Bacillus thuringiensis* (Federici 2007). Several commercially available biopesticides include Biobit, Dipel, and Thuricide based on Bt strain *kurstaki* HD1. Some other biopesticides make use of other Bt strains like *kurstaki* SA-11, *kurstaki* SA-12, *israelensis,* and *tenebrionis* (Kaur 2000). The insecticidal property of the microbe is primarily associated with the Cry protein present in the parasporal body. The toxin remains insoluble in the environment and is converted into the active toxin only inside the insect gut, thus making it safe for the humans and animals. Moreover, the toxin shows high level of specificity and hence, it is not expected to impact the non-target and beneficial insects (Lacey and Siegel 2000; Lacey et al. 2015). The Cry proteins have been classified into six major groups, out of which five groups are effective against various insects. Group 1 is effective against lepidopteran insects, Group 2 targets both lepidopteran and dipteran insects, Group 3 works against the coleopterans, Group 4 against dipterans, and Group 5 is functional against both lepidopteran and coleopteran insects (Crickmore et al. 1998).

The commercial potential of Bt also led to cloning of cry genes into other organisms including bacteria as well as plants. Schnepf and Whiteley (1981) cloned a cry gene from *Bt* subsp. *Kurstaki* into *E. coli.* Widespread application of Bt led to emergence of resistance in insects, so targeted delivery systems making use of non-Bt bacterial systems have been tried. Some of such bacteria which have been investigated for production and delivery of the Bt toxins include *Clavibacter xyli, Bacillus cereus*, *Pseudomonas, Rhizobium, Azospirillum* spp. (Lampel et al. 1994; Mahaffee et al. 1994; Obukowicz et al. 1986a, b; Skot et al. 1990; Udayasuryian et al. 1995). Although the conventional *Bt* microbial biopesticides have been found to be effective and eco-friendly, their use is limited by the fact that they tend to get washed off or get inactivated due to sunlight (Federici and Siegel 2008). Such types of problems led to development of transgenic crops containing genes encoding *Bt* toxins. As per Koch et al. (2015), Bt cotton and Bt corn form the majority of the commercially approved *Bt* crops. Apart from these *Bt* soybean and Bt rice have also been approved in a few countries.

B. thuringiensis subsp. *Israelensis* (*Bti*) has been used for controlling mosquito and black fly larvae across the globe. Another member of the genus *Bacillus,* i.e. *B. sphaericus* is also used commercially for mosquito biocontrol (Charles et al.

1996; Lacey et al. 2001). Apart from this, *Paenibacillus popilliae* for controlling Japanese beetle and *Serratia entomophila* for control of white grubs have also been reported. However, *P. popilliae* production can only occur under in vivo conditions, which makes it a relatively less popular biocontrol agent (Klein and Kaya 1995). Entomopathogenic activities of certain other bacteria have also been reported. Commare et al. (2002) reported a formulation containing two strains of *Pseudomonas fluorescens* to be effective in controlling sheath blight disease as well as leaf-folder insect in rice plant. A new strain of *Serratia marcescens* has been reported to show activity against diamondback moth (Jeong et al. 2010). Similarly, a formulation containing two strains of *P. fluorescens* and a fungal strain of *Beauveria bassiana* proved to be capable of controlling leaf-miner insect and collar root pathogen (Senthilraja et al. 2013).

Another bacterial species *Yersinia entomophaga* has been recently reported to be highly pathogenic to an array of insects belonging to Coleoptera, Lepidoptera, and Orthoptera (Hurst et al. 2011a) and has been shown to be effective against porina caterpillar (*Wiseana* spp.) (Brownbridge et al. 2008), *Scopula rubraria* (Jones et al. 2015), *C. zealandica*, diamond back moth, *Pieris rapae*, locust (*Locusta migratoria*), and cotton bollworm (*Helicoverpa armigera*) (Hurst et al. 2011a). It produces an orally active proteinaceous toxin complex (Yen-Tc) composed of ABC toxins (Hurst et al. 2011b). Spinosad, obtained from the bacterium *Saccharopolyspora spinosa*, is used in commercially available insecticide for controlling insect pests in vegetables like cole, spinach, lettuce, tomato (Natwick et al. 2010a, b, c; Koike et al. 2009; Natwick et al. 2013). Constant research efforts are being done for identifying more and more bacterial species with potential insecticidal properties. Hiebert et al. (2020) have reported *Leuconostoc pseudomesenteroides* to possess insecticidal activity against Drosophilid and aphids.

There are several bacterially derived acaricides are available for controlling plant parasitic mites. Abamectin is an effective acaricide, containing Avermectin B1 as the active component obtained from the actinomycete *Streptomyces avermitilis* (Lasota and Dybas 1990). Rahman et al. (2016) found abamectin to be quite effective in controlling *Polyphagotarsonemus latus* (jute yellow mite) in jute plants. Biopesticide based on *Chromobacterium subtsugae* strain PRAA4–1^T has been found to be quite effective against several insect pests as well as mites including two-spotted spider mites. In a field study, biopesticides based on fermentation by-products of *C. subtsugae* strain PRAA4–1 and heat-killed *Burkholderia* spp. strain A396 showed efficacy similar to that of chemical miticides against the two-spotted spider mite, *Tetranychus urticae*, in strawberries (Dara 2015).

1.4.8 Fungal Agents for Insect and Mite Biocontrol

Entomopathogenic fungi (EPF) include those fungi which can cause infection in arthropods including insects, mites, ticks, spiders, etc. About 1000 species of such entomopathogenic fungi are recognized. As per Chandler (2017) fungi cause death of the arthropod host due to physical damage and loss of normal functioning

resulting from fungal colonization of arthropod of tissues and organs, activity of fungal metabolites, loss of water, and starvation. Most of the commercially available EPF biopesticides are based on the genera *Beauveria* and *Metarhizium*, and few make use of *Isaria*, *Lecanicillium,* and others (Faria and Wraight 2007). The target pests include members of Hemiptera, Coleoptera, Lepidoptera, Diptera, Orthoptera, and Acari (mites). In Brazil, a vast range of land is under the use of *M. anisopliae* based biopesticide against spittlebug pests on sugarcane and pasture. The fungus is mass-produced on rice grains and is sold as conidia powder or as fungus-colonized substrate (Li et al. 2010). Another example of successful application of EPF is the use of *Beauveria brongniartii* applied against European cockchafer beetles in central Europe. McCoy et al. (1971) tested *Hirsutella thompsonii* against a rust mite infestation on citrus trees and found very encouraging results which resulted in development of commercial product known as Mycar in 1976 (McCoy and Couch 1982). *Hirsutella thompsonii* was also shown to be effective against the two-spotted spider mite (*Tetranychus urticae*) in laboratory bioassay (Gardner et al. 1982). A biopesticide based on *Lecanicillium muscarium* is registered as "Mycotal" for the control of whitefly larvae, thrips, and spider mites (Arthurs and Brucks 2017).

Several research efforts have indicated the potential use of fungal agents for controlling arthropod pests of various crops. Sánchez-Peña et al. (2007) reported greater than 70% mortality of fall armyworm larvae under lab conditions by using *M. brunneum* (earlier known as *M. anisopliae*) or *B. bassiana. Beauveria bassiana* was also shown in laboratory bioassays to be effective against the citrus rust mite (Alves et al. 2005). It was also found to possess ovicidal activity on two-spotted spider mite (Shi et al. 2008). *Lecanicillium lecanii* was found to be effective in controlling the brown stink bug which is a major soybean pest in Indonesia (Prayogo 2014). Carrillo et al. (2015) reported entomopathogenic fungi (*Isaria fumosorosea* 3581 and PFR97) and *B. bassiana* (GHA) to be effective in killing redbay ambrosia beetle. Maniania et al. (2002) reported that *M. brunneum* strain ICIPE 69 achieved good control of adult thrips infesting *Chrysanthemum*.

EPF not only plays a crucial role in pest management but could also lead to betterment of plant growth and health (Liao et al. 2014). Dara (2014a) conducted a field study and found that when *B. bassiana* was added to the roots of strawberry transplants, there was improvement in the plant growth and health during the next few months. Similar results were also obtained on adding *B. bassiana* in strawberry fields (Dara 2014b).

1.4.9 Viral Agents for Insect and Mite Biocontrol

Viruses offer another avenue for insect biocontrol. The greatest number of insect viruses belongs to the Baculoviridae family. Being highly specific toward the insects, these viruses do not pose any threat to humans or animals and hence are regarded to be safe. About 30 baculovirus species have either been used to develop biopesticides or are at a developmental stage for biopesticide development (Lacey et al. 2015).

The family Baculoviridae comprises viruses with double-stranded DNA circular genomes packaged in rod-shaped infective particles or nucleocapsids which in turn are found within crystal-like proteinaceous bodies called occlusion bodies (OBs) (Miller and Ball 1998; Harrison and Hoover 2012). These are divided into four genera on the basis of genome sequence and host specificity (Jehle et al. 2006; Herniou et al. 2012). Alphabaculoviruses are Lepidoptera-specific nucleopolyhedroviruses (NPVs), with polyhedral occlusion bodies containing multiple virions. The Betabaculoviruses include Lepidoptera-specific granuloviruses (GVs) with rod-shaped occlusion containing only a single virion. The Gammabaculoviruses comprised of Hymenoptera-specific NPVs with polyhedral occlusion bodies containing multiple singly enveloped nucleocapsids. The Deltabaculoviruses are Diptera-specific NPVs with occlusion bodies containing many virions.

The process of infection in baculoviruses is generally divided into two stages: a primary infection and amplification stage in the midgut followed by a systemic infection phase culminating in massive production of occlusion bodies, ultimately being released from dead insects (Federici 1997; Lacey et al. 2008). In Gammabaculoviruses and Deltabaculoviruses, production of progeny OBs is much lower. The infection cycle is initiated by ingestion of Baculoviral occlusion bodies, which in turn get dissolved in the alkaline pH of larval midgut region, thus leading to release of virions. These pass through the peritrophic membrane using various mechanisms and reach the epithelial cells (Del Rincón-Castro and Ibarra 2005; Slavicek 2012) where the DNA replication occurs (Lapointe et al. 2012). The progeny virus bud through the basal lamellar membrane and show rapid cell-to-cell transmission leading to rapid spreading of infection throughout the host body (Wang et al. 2014) ultimately bringing about the larval death. Larvae in the late stage of infection climb to the top of the plant/tree, become anchored, and die hanging in the distinctive V-shaped head-down posture (Federici 1997). The dead bodies rupture releases the viral occlusion bodies on to foliage and which in turn can be ingested by insect larvae.

Baculoviruses have been used in a wide variety of crops (Glare et al. 2012) and have been applied against pests like *Helicoverpa/Heliothis* spp. and *Spodoptera* spp. which impact multiple crops (Gowda 2005). The Baculovirus HearNPV based bioinsecticide is available for controlling insect pests like *Helicoverpa armigera*, cotton bollworm, pod borer, Old World bollworm in a wide range of crops including maize, soy, cotton, vegetables, legumes (Rabindra and Jayaraj 1995; Buerger et al. 2007). Similarly, HzSNPV based biopesticide is used for biocontrol of *Helicoverpa zea*, corn earworm, tomato fruitworm, tobacco budworm in a variety of crops like corn, cotton, tomato, tobacco (Ignoffo 1999). Also, the viruses SpexMNPV and SeMNPV have been used to restrict the crop losses caused by *Spodoptera exempta* and *Spodoptera exigua* beet on various plants (Grzywacz et al. 2008; Kolodny-Hirsch et al. 1997; Lasa et al. 2007). One of the earlier uses of Baculoviruses for large-scale insect control can be observed in case Codling Moth Granulovirus for controlling the codling moth. The virus has been widely used in Europe (Tanada 1964; Payne 1982; Lacey and Shapiro-Ilan 2008). In Brazil, velvet bean caterpillar

(*Anticarsia gemmatalis*) has been successfully controlled by the use of *A. gemmatalis* NPV (AgMNPV) at a comparatively lower rate of application, thus offering a very economical method for controlling the insect pest (Grzywacz 2017).

Relatively less number of viral pathogens has been found to be associated with mites. These include citrus red mite and the European red mite (Van der Geest et al. 2000). Although these viruses must be impacting under natural conditions but commercial application of these is yet to be achieved.

1.5 Applications of Biocontrol Agents

In order to meet the requirements of the growing population, increase in food grains production is required up to 250 million tonnes by the year 2020. For better horticultural and agronomic practices, growers often rely on excessive use or misuse of agrochemicals caused environmental pollution. The toxic chemicals uses have been increased for the management of increased pest and diseases. But excessive use causes increase in the resistance to pesticides and fungicides. The World Trade Organization general agreement on trade and tariff emphasizes more on the use of pesticide in an eco-friendly way which is least toxic, causing low residual problem and low levels of disease resistance for crop production. The biocontrol agents alone or in combination are used to control the number of pest and diseases. The general properties of biocontrol agents are:

- Cheaper and less costlier than other methods
- Crop protection throughout the crop period
- No toxicity to the plants
- Safer to the environment and person who applies them
- Easy multiplication in soil and no left residue
- Control disease along with root and plant growth enhancement by the way of promoting beneficial soil micro flora.
- Increased yield
- Easy to handle and apply to the target
- Biological agents can be combined with bio-fertilizer
- Easy to manufacture
- Not harmful for human being and animals.

The biological control agents promote the growth of plants and suppress the deleterious pathogens by producing growth hormones like cytokinin, auxins, and gibberellins, etc. with simultaneous increase in the yield. The analyses on mechanisms of biological control agents represented that these agents promote plant growth directly by producing plant growth regulators or indirectly by producing siderophores or antibiotics (soil-borne pathogens) and increase nutrient uptake. List of bacteria and fungal based biocontrol agents are given in Tables 1.5 and 1.6. Life cycle of insect virus is given in Fig. 1.3.

Table 1.5 List of microorganisms effective against plant pathogens and their status

S. no.	Substance	Effective	Status	Date of approval	Date of Expiry
1.	*Aureobasidium pullulans* (strains DSM 14940 and DSM 14941)	Fungi and bacteria	Approved	01/02/2014	31/01/2024
2.	*Bacillus amyloliquefaciens* subsp. *plantarum* D747	Bacteria	Approved	01/04/2015	31/03/2025
3.	*Bacillus firmus* I-1582	Nematodes	Approved	01/10/2013	30/09/2023
4.	*Bacillus pumilus* QST 2808	Fungi	Approved	01/09/2014	31/08/2024
5.	*Bacillus subtilis* str. QST 713	Fungi and bacteria	Approved	01/02/2007	30/04/2018
6.	*Candida oleophila* strain O	Fungi	Approved	01/10/2013	30/09/2023
7.	*Phlebiopsis gigantea* (several strains)	Fungi	Approved	01/05/2009	30/04/2019
8.	*Pseudomonas* sp. Strain DSMZ 13134	–	Approved	01/02/2014	31/01/2024
9.	*Pythium oligandrum* M1	Fungi	Approved	01/05/2009	30/04/2019
10.	*Streptomyces* K61 (formerly *S. griseoviridis*)	Fungi	Approved	01/05/2009	30/04/2019
11.	*Streptomyces lydicus* WYEC 108	Fungi and bacteria	Approved	01/01/2015	31/12/2024
12.	*Trichoderma asperellum* (formerly *T. harzianum*) strains ICC012, T25 and TV1	Fungi	Approved	01/05/2009	30/04/2019
13.	*Trichoderma asperellum* (strain T34)	Fungi	Approved	01/06/2013	31/05/2023
14.	*Trichoderma atroviride* (formerly *T. harzianum*) strains IMI 206040 and T11	Fungi	Approved	01/05/2009	30/04/2019
15.	*Trichoderma atroviride* strain I1237	Fungi	Approved	01/06/2013	31/05/2023
16.	*Trichoderma gamsii* (formerly *T. viride*) strain ICC080	Fungi	Approved	01/05/2009	30/04/2019
17.	*Trichoderma harzianum* strains T-22 and ITEM 908	Fungi	Approved	01/05/2009	30/04/2019
18.	*Trichoderma polysporum* strain IMI 206039	Fungi	Approved	01/05/2009	30/04/2019
19.	*Verticillium albo-atrum* (formerly *Verticillium dahliae*) strain WCS850	Fungi	Approved	01/05/2009	30/04/2019
20.	Zucchini yellow mosaic virus, weak strain	Fungi	Approved	01/06/2013	31/05/2023

Table 1.6 Beneficial fungi based biocontrol of plant pathogen

S. no.	Biocontrol agent	Target pathogen	Host plant
1.	AM: *Glomus mosseae*	*Ralstonia solanacearum*	Tobacco
2.	AM: G. *intraradices*	*Nacobbus aberrans*	Tomato
3.	AM: *Glomus fasciculatum*	*Alternaria alternata*	Tomato
	Avirulent/Hypovirulent strains: *Cryphonectria parasitica*	*Cryphonectria parasitica*	Chestnut
4.	ECM: *Thelephora terrestris* and *Pisolithus tinctorius*	*Phytophthora cinnamomi*	Shortleaf pine
5.	ECM: *Laccaria bicolor*	*F. moniliforme*	Scots pine
6.	ECM: *Suillus luteus*	*F. Oxysporum*	Stone pine scots pine
7.	Endophyte: *Piriformospora indica*	*R. solani*	Rice
8.	Endophyte: *Cryptosporiopsis quercina*	*Pyricularia oryzae*	Rice
9.	Endophyte: *Penicillium* sp.	*Pseudomonas syringae* pv. Tomato	Arabidopsis
10.	*Trichoderma asperellum*	*Fusarium oxysporum* f. sp. lycopersici	Tomato
11.	*T. koningii*, *T. viride*, *T. harzianum*	*Sclerotium rolfsii*	Groundnut
12.	*T. harzianum*	*Alternaria brassicae* and *A. brassicicola*	Mustard
13.	*T. harzianum*	*Meloidogyne javanica*	Tomato
14.	*T. harzianum*	*Botrytis cinerea*	Bean
15.	*T. hamatum*	Phytophthora	Rhododendron
16.	Yeast: *Cryptococcus laurentii* and *Sporobolomyces roseus*	*B. cinerea*	Apple
17.	Yeast: *Pichia anomala*	*B. cinerea*	Apple
18.	*Verticillium nigrescens*	*V. dahliae*	Cotton

1.6 Conclusion and Future Prospects

To feed the world population which grows at a rapid rate there is always need for synthetic pesticides. However, by using biological control agents we can minimize the use of synthetic pesticides, especially in developed countries. Biological system approach may provide a better alternative to suppress or inhibit the activity of disease causing agents. Several examples of successful and even outstanding results of biological control agent methods with plant pathogenic agents in agricultural fields are known. Also, sometimes suitable agents may not be available for all species for which biocontrol would be required. Therefore, opportunities for application of biocontrol techniques may be limited. The possible use of genetic engineering techniques to manipulate the virulence or host specificity of pathogens and thus produce right agents for biocontrol purposes may solve the problem. Biological control agents could be used as integrated management programs where there is

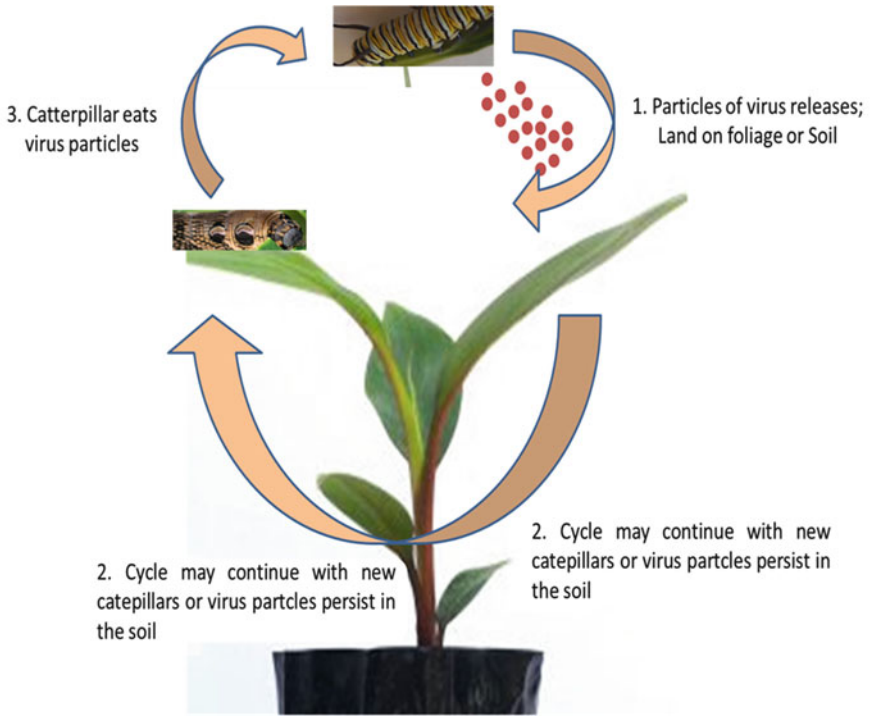

Fig. 1.3 Generalized life cycle of insect virus

necessity of adding biocontrol agents and the success of disease management is not totally dependent on the biological activity.

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Overview of Nutrient and Disease Management in Banana

2

Bhawna Dipta, Sonal Bhardwaj, and Manoj Kaushal

Abstract

Bananas are one of the most important crops for consumption as dessert and a staple food. In world trade for export, it ranks fourth among all agricultural commodities. The intensive cultivation of this crop warrants high yield and quality but requires extensive use of chemical fertilizers and pesticides that not only pollutes the environment by residue accumulation but is also against the interests of sustainable agricultural practices. Integrated nutrient management is an economically sound preventive management option which could restore soil fertility and the productivity of banana. Other feasible management strategies include the use of resistant cultivars and the introduction of microorganisms or their mixtures in the rhizosphere to protect them against diseases, thereby leading to improved establishment as well as overall performance. In this chapter, we provide an overview of the strategies for the management practices to control the soil-borne pathogens in banana besides maintaining soil fertility.

Keywords

Antibiosis · Banana · Cytokinin · Nitrogen · Organic manure · Siderophore

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2.1 Introduction

Banana (*Musa* spp.) is a monocotyledonous perennial giant herb belonging to the family Musaceae and order Zingiberales. It is one of the fourth key food resources in the world after rice, wheat, and maize (Ngamau et al. 2014). Banana is a native of the tropical regions of Southeast Asia (Fuller and Madella 2009) and mostly grown in tropical and subtropical locales of the world (Dodo 2014). Banana is a great source of dietary fiber, vitamin C, vitamin B_6, and provides sufficient levels of minerals like potassium, phosphorus, calcium, manganese, copper, and magnesium. Processed items such as bread, chips, wafers, puree, jam, powder, pulp, beer, and wine can be produced using a banana. Besides, the tender stem of the banana bearing inflorescence is utilized as a vegetable. The banana plant also has medicative applications as it helps in combating arteriosclerosis, bronchitis, diabetes, hysteria, epilepsy, leprosy, ulcers, hemorrhages, acute dysentery as well as cure skin afflictions. Banana is primarily grown by small-scale farmers both as a household food security and as a source of income throughout the year, thus, playing a vital role in poverty alleviation of the regions involved in its production. Unlike other fruit crops, banana cannot get water and nutrients from the deeper soil profile as it bears adventitious and horizontal roots proliferating topsoil. This undeveloped root system inhibits the consumption of essential mineral nutrients and limits the large-scale production of bananas under adverse tropical soil conditions. Thus, banana trees require great quantities of nutrients for their development and fruit production which is chiefly exploited from a very limited soil depth.

These nutrients may be supplied by the soil or by fertilization to obtain optimum yield on a sustainable basis (Rajput et al. 2015). In conventional farming, the intensive use of chemical fertilizers and pesticides has proved to be a tremendous threat not only to food safety but also to the ecosystem's health and its sustainability (Carvalho 2017). Moreover, the outbreak of several diseases due to intensive cultivation has substantially inflicted yield losses of staggering dimension both in quantity and quality aspects to the farming community. Consequent problems such as soil acidification, soil alkalinity, as well as soil, water, and atmospheric contamination have not only caused continuing soil deterioration but also resulted in declined plant and microbe biodiversity. Even worse, pesticides and residues that are frequently found in foods lead to different toxic mechanisms such as the poisoning of gastrointestinal, renal, and nervous systems and pulmonary fibrosis (Eddleston and Bateman 2012). The present chapter focuses on the integrated nutrient management (INM) practices, which could restore soil fertility and the productivity of banana. INM is a strategic, eco-friendly, and economical preventive management option that includes the use of organic wastes, biofertilizers, and inorganic fertilizers which improves crop yields and preserves sustainable and long-term soil fertility while minimizing nutrient losses and improving the nutrient-use efficiency of crops. The chapter also discusses plant growth promoting attributes of beneficial microorganisms with special attention to sustainable disease management of major banana diseases by biocontrol agents.

2.2 Integrated Nutrient Management

Although chemical fertilizers contribute a lot in fulfilling the nutrient requirement of banana, their regular, excessive, and unbalanced use may lead to deterioration of the physical, chemical, and biological properties of the soil, causing nutrient imbalances and environmental pollution. INM envisages the use of chemical fertilizers with organic sources like farmyard manure, poultry manure, neem cake, oil cake, vermicompost, etc., along with biofertilizers in judicious combinations for agricultural productivity and farm profitability (Selim 2020). INM is known to have a promising effect in arresting the decline in productivity through the correction of marginal nutrient deficiencies and their positive influence on the physical, chemical, and biological soil properties is depicted in Fig. 2.1.

2.2.1 Basic Components of INM

2.2.1.1 Organic Sources

Organic food production is a holistic system that enhances soil fertility and biological diversity as well as improves the quality of fruit. Organic manures influence soil productivity through their effect on the physical, chemical, and biological properties of soil. Different kinds of organic materials such as farmyard manure, urban and rural compost, green manure, press mud have large nutrient potential to increase the productivity of many crops. Other potential organic sources of nutrients include industrial byproducts, municipal solid wastes, sewage sludge,

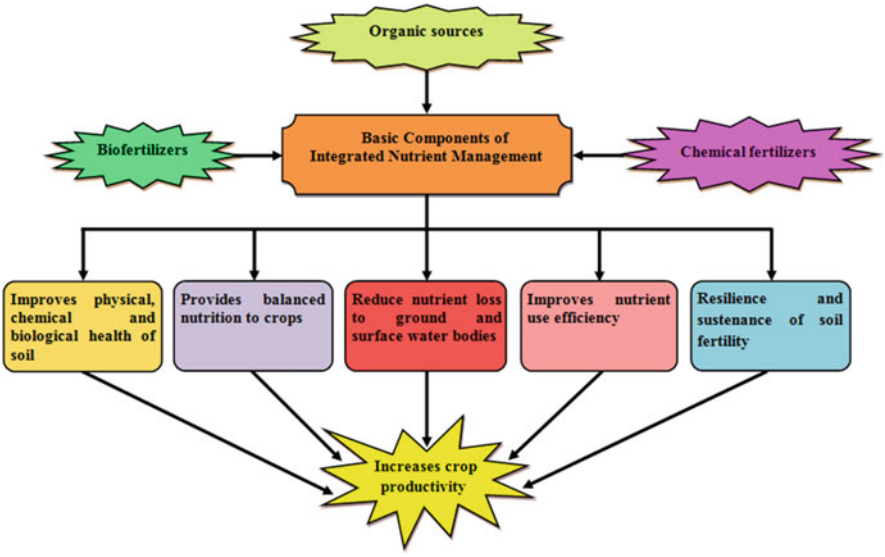

Fig. 2.1 Role of integrated nutrient management in plant growth promotion

and food industry wastes. A sizeable quantity of crop residues left in the field also acts as a great source of nutrient supply. Organic sources such as farmyard manure, agro-waste, press mud, and agro-industrial waste could be properly recycled into value-added products such as compost and applied alone or together with mineral sources for plant nutrition.

2.2.1.2 Biofertilizers

Biofertilizers are the preparations containing living/latent cells of efficient strains of agriculturally beneficial microorganisms that help in increasing the availability and uptake of nutrients by plants when inoculated in soil or seeds (Richardson et al. 2009; Giri et al. 2019). Moreover, the use of biofertilizers is essential not only to reduce the quantum of inorganic nutrients or organic manures to be applied but also to increase the beneficial soil flora and fauna. Inoculation of the crop with nitrogen-fixing microorganisms before sowing improves nodulation and nitrogen fixation, which in turn is translated into enhanced growth and grain yield. The majority of the nitrogen-fixing species belong to α-proteobacteria containing six genera, namely *Rhizobium*, *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* (=*Ensifer*), and *Azorhizobium* (Sawada et al. 2003). The β-proteobacteria such as *Burkholderia* and *Ralstonia* also has been reported to fix nitrogen (Willems 2006). *Rhizobium meliloti* RMP3 and RMP5 significantly enhanced the percentage of seed germination, seedling biomass, nodule number, and nodule fresh weight in groundnut (Arora et al. 2001). In another study, Verma et al. (2013) reported significantly increased nodulation, dry weight of root and shoot, grain and straw yield, uptake of nitrogen and phosphorus by chickpea when treated with *Mesorhizobium* sp. BHURC03 and *Pseudomonas aeruginosa* BHUPSB02. Similarly, *Rhizobium laguerreae* strain PEPV40 significantly increased leaf number, size, and weight, as well as chlorophyll and nitrogen contents in spinach (Jimenez-Gomez et al. 2018).

The utilization of phosphate solubilizing microorganisms (PSM) reduces the use of expensive phosphatic fertilizers and enhances the availability of aggregated phosphates in an environment-friendly and sustainable manner. Viruel et al. (2014) reported that *Pseudomonas tolaasii* IEXb significantly stimulated seedling emergence, shoot length, grain yield, grain weight, total dry biomass, and P content in maize. In another study, increased total P content and total P uptake in cucumber were observed on treatment with *Trichoderma asperellum* T34 (Garcia-Lopez et al. 2015). The co-inoculation of P-solubilizers with other beneficial bacteria and/or mycorrhizal fungi also enhances the efficiency of P-solubilization. Franco-Correa et al. (2010) reported that treatment of clover with *Streptomyces* MCR9 and *Glomus* sp. significantly increased shoot and root biomass and mycorrhizal root length. Inoculation of *Pantoea agglomerans* and *Burkholderia anthina* enhanced shoot and root length, shoot and root dry matter, and P uptake in mung bean (Walpola and Yoon 2013).

2.2.1.3 Chemical Fertilizers

Banana is a gross feeder and requires large amounts of nitrogen and potassium followed by phosphorus, calcium, and magnesium for its growth, yield, and biomass

production (Purabi 2017). To fulfill its nutritional attributes, it is essential to apply these elements in the soil, which mostly comes from inorganic chemical sources. Nitrogen, an indispensable component of amino acids and nucleic acids, occupies a prominent place in the plant metabolism system. Plants usually depend upon combined or fixed forms of nitrogen, such as ammonia and nitrate because they are unable to use atmospheric nitrogen. Nitrogen is mainly responsible for better vegetative growth in banana. Although nitrogen is distributed evenly throughout the plant, its highest proportion is found in the suckers and leaves (Babu et al. 2004).

Potassium is the essential plant nutrient in banana production due to its higher accumulation in the fruit and plant tissue. The adequate supply of potassium fertilizers not only increases the growth and yield but also improves the quality of fruits, physiology of the plant and induces resistance against biotic and abiotic stresses (Wang et al. 2013). Potassium also catalyzes important reactions such as respiration, photosynthesis, translocation of photosynthates, chlorophyll formation, and water regulation in banana (Kumar et al. 2020). Banana demands a very high quantity of potassium for its growth and development up to the flowering stage (Kumar and Kumar 2008). The phosphorus requirement of banana is very much less compared to nitrogen and potassium. Phosphorus has a promotive effect on the young root system and stimulates growth and has depressing effects on the number, weight, and size of fingers when applied in excess.

2.2.2 Effect of INM Practices on Banana Production

2.2.2.1 Leaf Nutrient Status

Jeeva et al. (1988) reported that *Azospirillum* inoculation + 100% N application enhanced height, the mid girth of pseudostem, leaf production, leaf area, and bunch weight of banana cv. Poovan and also enhanced the N, P, Ca, and Mg contents of leaves as compared to uninoculated control plants which received 100% nitrogen alone. Maximum plant height, pseudostem girth, the total number of leaves, and total leaf area were recorded with poultry manure @ 15 kg/plant + 80% recommended NPK (200:50:400 g NPK/plant) followed by rice husk ash @ 15 kg/plant + 80 percent recommended NPK and press mud @ 15 kg/plant + 80% recommended NPK in ratoon of Poovan banana (Jeyabaskaran et al. 2001). Ziauddin (2009) recorded higher N, P, and K concentrations in index leaf in banana cv. Ardhapuri when treated with 100% recommended dose of fertilizer combined with farmyard manure or combined with vermicompost. Selvamani and Manivannan (2009) reported that combined application of organic manures (FYM, vermicompost, and neem cake), biofertilizers (VAM, *Azospirillum*, PSB, and *Trichoderma harzianum*) with inorganic fertilizers enhanced the leaf nutrient contents in banana leaf in different stages of growth.

Highest pseudostem height, pseudostem girth, the total number of leaves, days taken to shooting, and less number of days for harvesting were registered on an application of 20 kg FYM + 1 kg neem cake + 200:40:200 g NPK/plant (Badgujar et al. 2010). Application of 80% RDF (inorganic form: nitrogen @ 43.4 g,

phosphorus @ 40.0 g, and potassium @ 33.3 g in 12 equal splits) + 20% RDF (organic form: vermicompost @ 4.285 kg/plant) along with biofertilizers (*Azospirillum* @ 50 g, PSB @ 50 g, and KMB-*Frateuria aurantia* @ 25 g/plant) showed highest plant height at 3 MAP (months after planting) (129.67 cm) and 5 MAP (184.29 cm), highest pseudostem girth at 3 MAP (35.61 cm) and 5 MAP (49.74 cm), higher nitrogen concentration in index leaf at 3 MAP (2.93%) and 5 MAP (3.28%), higher phosphorus content in index leaf at 3 MAP (0.26%) and 5 MAP (0.24%), and higher potassium content in index leaf at 3 MAP (3.25%) and at 5 MAP (3.54%) (Hussain et al. 2015).

2.2.2.2 Quality Parameters

El-Moniem and Radwan (2003) reported that treatment with biofertilizers + 75% NPK resulted in higher total soluble solids (TSS), acidity, and starch followed by biofertilizers + 50% NPK and 100% NPK alone in banana. FYM (10 kg) + neem cake (1.25 kg) + vermicompost (5 kg) + wood ash (1.75 kg) per plant + triple green manuring with sunn hemp + double intercropping of cowpea + vesicular arbuscular mycorrhizae (25 g) + *Azospirillum* (50 g) + phosphate solubilizing bacteria (50 g) and *Trichoderma harzianum* (50 g) per plant recorded maximum TSS, acidity, ascorbic acid, non-reducing and total sugars besides enhancing the shelf life of banana and reducing physiological loss in weight in banana cv. Grand Naine (AAA) (Vanilarasu and Balakrishnamurthy 2014).

Lenka et al. (2016) reported that 100% RDF + PSB + *Azospirillum* increased pulp weight (103.81 g), peel weight (32.44 g), TSS (22.2 brix), reducing sugar (8.12), and non-reducing sugar (3.75) of banana cv. Grand Naine. Ganapathi and Dharmatti (2018) reported maximum TSS (23.52 brix), total sugars (20.30%), reducing sugars (20.30%), non-reducing sugars (17.87%), pulp-to-peel ratio (3.81), shelf life (6.33 days), and titratable acidity (0.25) when treated with vermicompost @ 24.20 t/ha + urea @ 535.73 kg/ha + sunn hemp @ 8.88 t/ha + *Azospirillum* @ 30.86 kg/ha and PSB @ 30.86 kg/ha in banana cv. Grand Naine.

2.2.2.3 Yield Attributes

Soil and foliar application of nitrogen in combination with *Azotobacter* have resulted in increased plant height, plant girth, the number of hands/bunch, and the number of fingers/hands in banana cv. Robusta (Kumar and Shanmugavelu 1988). Chezhiyan et al. (1999) reported an increased bunch weight of 15.3 kg in hill banana var. Virupakshi when applied with biofertilizers (*Azospirillum*, phosphobacteria, and VAM), organic manure (FYM), and 75% NPK. Sabarad (2004) found that inoculation of VAM + *Trichoderma harzianum* + 180:108:225 g NPK/plant produced better growth in banana. Gogoi et al. (2004) observed that combined application of *Azospirillum*, PSB, and ½ RD of N, RD of P and K increased the number of hands/bunch, gingers/hand, bunch weight, yield, harvest index of banana, and soil NPK availability. Bhalerao et al. (2009) reported that application of a recommended dose of NPK (200:40:200 g per plant) with 10 kg FYM per plant and biofertilizer (*Azospirillum* and PSB) @ 25 g per plant resulted in maximum plant height (216.0 cm), pseudostem girth (70.92 cm), minimum days required to flower

(258.5 days), and crop duration (356.9 days) in banana cv. Grand Naine. Gaikwad et al. (2010) found that application of 100% RDF + *Azospirillum* (50 g/plant) + PSB (50 g/plant) + VAM (250 g/plant) + *Trichoderma harzianum* (50 g/plant) recorded a maximum number of hands per bunch (10.7), number of fingers per bunch (154), number of functional leaves (12.7), maximum bunch weight (18.0 kg/plant), plant height (196 cm), stem girth (70.9 cm), and yield (79.8 t/ha) with monetary returns of Rs. 2,79,300/ha. The treatment with 100% recommended doses of NPK in combination with farmyard manure and biofertilizers significantly influenced plant growth and yield of banana (Hazarika and Ansari 2010).

Enhanced yield attributes, viz. the number of fingers/hands, finger length, finger volume, circumference, and weight of finger in Grand Naine banana were recorded on treatment with 100% RDF + VAM + *Azospirillum* + PSB + *Trichoderma harzianum* (Hazarika et al. 2011). Butani et al. (2012) studied the effect of chemical fertilizer and vermicompost on yield, nutrient content, and its uptake in banana (*Musa paradisiaca* L.) cv. Grand Naine. The highest yield, nutrient content, and uptake were recorded with the application of the 300:90:200 g of NPK and 8 kg of vermicompost per plant. Patel et al. (2012) reported that 300 g N + *Azotobacter* registered the highest yield of banana (*Musa paradisiaca* L.) cv. Grand Naine. 50% RDF + FYM + *Azotobacter* (50 g/plant) + PSB (50 g/plant) + *Glomus fasciculatum* (250 g/plant) registered maximum plant height (190.84 cm) and plant girth (81.34 cm) in banana cv. Ardhapuri (*Musa* AAA) (Patil and Shinde 2013). Chhuria et al. (2016) observed maximum bunch weight, number of hands/bunch, and number of fingers/bunch in banana cv. Grand Naine when treated with 100% RDF (300:100:300 g NPK) + 125 g of *Azotobacter*, *Azospirillum*, and PSB. Pattar et al. (2018) reported better length of the bunch, number of hands per bunch, the weight of bunch, number of fingers per hand and bunch, the weight of the finger, length and girth of the finger, yield per plant, and the total yield on treatment with 100% RDF (200:100:300 g N:P_2O_5:K_2O + 20 kg FYM per plant) + PSB (20 g) + *Azospirillum* (20 g) dose in banana cv. Rajapuri.

2.3 Plant Growth Promoting Rhizobacteria

The rhizosphere is the area of soil surrounding the roots where microbes flourish due to the release of an enormous amount of sugars, amino acids, organic acids, vitamins, enzymes, and organic or inorganic ions through root exudates. Rhizosphere harbors an extremely complex microbial community including saprophytes, epiphytes, endophytes, pathogens, and beneficial microorganisms. The beneficial plant–microbe interactions in the rhizosphere termed as plant growth-promoting rhizobacteria (PGPR) are the primary determinants of plant health and soil fertility (Jeffries et al. 2003; Rosier et al. 2018). PGPR are efficient microbial competitors in the root zone which enhances the plant growth by (1) increasing the availability of nitrogen, phosphorus, and other essential nutrients to plant (Cakmakci et al. 2006; Wang et al. 2020), (2) synthesizing phytohormones for plant growth promotion (Chen et al. 2013; Cakmakci et al. 2020), and (3) controlling diseases and pests by

the production of antimicrobial metabolites (Farag et al. 2016; Awad et al. 2017; Jin et al. 2020).

2.3.1 Nitrogen Fixation

Nitrogen is an essential element for synthesizing nucleic acids, proteins, and other organic nitrogenous compounds. Biological nitrogen fixation (BNF) plays a vital role as a substitution to commercially available nitrogen fertilizer in crop production and reduction of the environmental problems besides enriching the soil with nitrogen for the subsequent crops, thereby restoring the degraded ecosystems. The atmospheric dinitrogen is converted into ammonia by nitrogen-fixing microorganisms using a nitrogenase enzyme complex, which consists of two-component metalloenzyme, viz. dinitrogenase reductase, the iron protein and dinitrogenase, the molybdenum-iron protein (Kim and Rees 1994). Biologically fixed nitrogen is used directly by the plant as it is less susceptible to volatilization, denitrification, and leaching and concomitant benefits accruing in terms of effects on the global nitrogen cycle, global warming, ground and surface water contamination. Nitrogen-fixing microorganisms generally include members of the family Rhizobiaceae which forms a symbiosis with leguminous plants (Boakye et al. 2016; Lindstrom and Mousavi 2020), actinomycete *Frankia* which fixes nitrogen in non-leguminous trees (Wall 2000), and non-symbiotic, free-living, nitrogen-fixing forms such as *Azospirillum*, *Acetobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Azoarcus*, *Azotobacter*, *Nostoc*, and *Anabaena* (Steenhoudt and Vanderleyden 2000; Choudhary and Bimal 2010).

2.3.2 Phosphorus Solubilization

Phosphorus (P) is an essential macronutrient for biological growth and development after nitrogen. Although P content in soil on an average is 0.05%, only 0.1% of the total P present in the soil is available to the plants because of its chemical fixation and low solubility. PSM plays a key role in solubilizing inorganic P and makes it available to the plants (Dipta et al. 2019). The production of organic acids is the principal mechanism for microbial solubilization of inorganic phosphates (Alam et al. 2002; Tandon et al. 2020). Gluconic acid is reported to be the major organic acid involved in P-solubilization (Vassilev et al. 1996; Castagno et al. 2011). Secretion of phosphatase, phytase, and phosphonatase plays an important role in the mineralization of organic P substrates (La Nauze et al. 1970; Qiao et al. 2019). Among the diverse and naturally abundant microorganisms dwelling the rhizosphere, *Aspergillus flavus, A. candidus, A. niger, A. terreus, A. wentii, Fusarium oxysporum, Penicillium* sp., *Trichoderma isridae*, *Trichoderma* sp. (Akintokun et al. 2007), *Pseudomonas cedrina*, *Rahnella aquatilis*, *Rhizobium nepotum,* and *R. tibeticum* (Rfaki et al. 2015), *Bacillus pumilus* (Dipta et al. 2017), *Azospirillum lipoferum* and *A. brasilense* (Mohamed et al. 2017) have been reported to meet the P

demands of plants. *Anabaena* sp., *Calothrix braunii*, *Nostoc* sp., *Scytonema* sp., and *Tolypothrix ceylonica* have also been reported as efficient P-solubilizing cyanobacteria (Gupta et al. 1998).

2.3.3 Phytohormone Production

Production of various phytohormones such as auxins, cytokinins, and gibberellins by beneficial microorganisms influences physiological and developmental processes in plants. Indole-3-acetic acid (IAA) is an important signal molecule that may exert pronounced effects on plant growth and establishment including cell elongation, phototropism, geotropism, apical dominance, lateral root initiation, ethylene production, and fruit development (Woodward and Bartel 2005). Tryptophan is the main precursor for modulating the level of IAA biosynthesis (Zaidi et al. 2009). IAA is also reported to increase root surface area and root length, thus providing the plant better access to soil nutrients. Moreover, IAA loosens plant cell walls resulting in root exudation that provides additional nutrients to support bacterial growth (Glick 2012). Production of microbial IAA has been reported to promote the growth attributes of various crops (Aziz et al. 2012; Mohite 2013; Raut et al. 2017).

Cytokinin, an adenine derived phytohormone, regulates cell division, seed germination, bud formation, the release of buds from apical dominance, root development, and delay of senescence. Cytokinin also mediates the responses to biotic and abiotic stress (Werner and Schmulling 2009). Xu et al. (2012) reported that engineered *Sinorhizobium* strains synthesized more cytokinin that improved the tolerance of alfalfa to severe drought stress without affecting nitrogen fixation. In another study, cytokinin-producing rhizobacteria have been reported to alleviate drought stress in an arid environment (Liu et al. 2013). Gibberellins, a class of tetracyclic diterpenoid compounds, are essential for the regulation of diverse developmental processes in plants such as seed germination, stem elongation, leaf formation, flower, and fruit development. Kang et al. (2014) reported that gibberellin-producing PGPR *Leifsonia soli* SE134 stimulated shoot growth in mutant rice plants deficient in gibberellin synthesis.

2.3.4 Siderophore Production

Nearly all microorganisms depend on the uptake of iron (III). Under aerobic conditions this essential element is present in the form of insoluble oxide hydrates ($Fe_2O_3.nH_2O$); therefore, its concentration is far too low to sustain the microorganisms. One way to overcome this problem is the production of effective iron complexing compounds named siderophores. Siderophores are low molecular weight, ferric ion-specific chelating agents produced by bacteria, actinomycetes, fungi, and certain algae growing under low iron stress. The term siderophore was coined by Lankford in 1973. The main roles of these compounds are to selectively bind iron (Fe^{3+}) and actively transport the iron-siderophore complex into the cytoplasm making it available to the microbial cell (Verma et al. 2012). After

complexation, iron (III) is available in a water-soluble form as ferric-siderophore and can be taken up by microorganisms (Braun and Braun 2002). Siderophore producing microorganisms have been reported to promote the growth of various crops such as potato (Bakker et al. 1986), mung bean (Mahmoud and Abd-Alla 2001), maize (Sharma and Johri 2003), cowpea (Dimkpa et al. 2008), pepper (Yu et al. 2011), and cucumber (Qi and Zhao 2012).

2.3.5 Hydrogen Cyanide Production

Hydrogen cyanide (HCN), a secondary metabolite commonly produced by rhizosphere microorganisms may inhibit or enhance plant establishment. The precursor of microbial cyanide is glycine. Alstrom and Burns (1989) tested the efficacy of two cyanogenic strains, viz. *Pseudomonas fluorescens* strain S241 and *P. fluorescens* strain S97 on bean and lettuce seedlings before planting in soil. S241 reduced the growth, whereas S97 increased growth initially. Inhibition by S241 was related to consistently higher levels of rhizosphere cyanide in comparison with S97 treated plants and control soils. HCN production by rhizobacteria has been postulated to play a chief role in the biological control of pathogens. HCN secretion by *Pseudomonas fluorescens* strain CHA0 stimulated root hair formation and suppressed black root rot in the tobacco (Voisard et al. 1989). Multifarious plant growth-promoting traits of rhizobacteria associated with banana are shown in Table 2.1.

2.3.6 Biocontrol

Increased use of chemical inputs causes the development of pathogen resistance to the applied agents and their non-target environmental impacts. Biocontrol agents are, thus, being considered as a supplemental way of reducing the use of chemicals in agriculture. A biological control refers to the use of introduced/native microbial antagonists to suppress or inhibit the activity of one or more pathogens. Several bacterial strains such as *Actinoplanes missouriensis* (El-Tarabily 2003), *Pseudomonas* sp., *Pantoea dispersa*, *Enterobacter amnigenus* (Gohel et al. 2004), *Bacillus subtilis* (Chang et al. 2010), *Rhizobium japonicum* (Al-Ani et al. 2012), and *Bacillus pumilus* (Kaushal et al. 2017) have been reported to act as biocontrol agents against various phytopathogens. Among fungi, *Trichoderma* has been recognized as a major biocontrol agent against various phytopathogenic fungi by several workers (de Marco et al. 2000; El-Katatny et al. 2001; Herath et al. 2015; Khatri et al. 2017).

Bananas are susceptible to a wide range of diseases. Some of these diseases are highly destructive and very contagious, and once introduced they are persistent and difficult to eradicate. The specific banana cultivars, prevailing environmental conditions, and the specific disease or pest affect the severity and occurrence of disease outbreaks and plant damage. The major fungal and bacterial diseases include panama disease, yellow sigatoka, black sigatoka, crown rot, anthracnose, cigar end tip rot, stem end rot, moko disease, and tip-over. Banana bunchy top virus (BBTV), banana bract mosaic virus (BBrMV), banana mild mosaic virus (BanMMV), banana

Table 2.1 Plant growth-promoting traits of rhizobacteria associated with banana

Microorganisms	Plant growth-promoting traits	References
Enterobacter sp. C3C9, *Klebsiella* sp. VI, and *Citrobacter* sp. III	Nitrogen fixer	Martinez et al. (2003)
Pseudomonas aeruginosa FP10	P-solubilizer, IAA, and siderophore producer	Ayyadurai et al. (2006)
Flavimonas oryzihabitans K50V2s (43)	P-solubilizer, siderophore producer, and nitrogen fixer	Ngamau et al. (2012)
Bacillus megaterium, Lactobacillus casei, and *Bacillus subtilis*	IAA producer	Mohite (2013)
Stenotrophomonas maltophilia BE25	IAA producer	Ambawade and Pathade (2013)
Bacillus amyloliquefaciens strain NJN-6	IAA and GA_3 producer	Yuan et al. (2013)
Pseudomonas aeruginosa	Siderophore and HCN producer	Shaikh et al. (2014)
Ralstonia sp.	P-solubilizer, IAA, siderophore, HCN, and ammonia producer	Jimtha et al. (2014)
Bacillus subtilis EB-126 and *Bacillus* sp. EB-47	P-solubilizer, IAA producer, and nitrogen fixer	Andrade et al. (2014)
Bacillus sp. EB. 78	P-solubilizer	Matos et al. (2017)
Pseudomonas putida strain PF1P	IAA and GA_3 producer	Heng and Zainual (2017)
Bacillus siamensis BE 76	IAA producer	Ambawade and Pathade (2018)

virus X (BVX), and banana streak virus (BSV) are the main banana viral diseases. A list of major banana diseases along with their causal organisms and symptoms is shown in Table 2.2. Different biocontrol mechanisms, alone or in combination, might be used to suppress plant diseases directly or indirectly as given below.

2.3.6.1 Antibiosis

Antibiosis involves the production of low-molecular-weight antimicrobial compounds by the biocontrol agents that suppress or reduce the growth and/or proliferation of the phytopathogens (Fravel 1988; Mazzola et al. 1995). The production of multiple antibiotics probably suppresses diverse microbial competitors and enhances biocontrol potential. An antibiotic, pyoluteorin isolated from the culture of *Pseudomonas fluorescens* was inhibitory to *Pythium ultimum* (Howell and Stipanovic 1980). Gurusiddaiah et al. (1986) isolated and characterized antibiotic from cultures of *Pseudomonas fluorescens* 2–79 (NRRL B-15132) that showed excellent activity against *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani*, and *Pythium aristosporum*. Howie and Suslow (1991) examined the role of an antibiotic, oomycin A, isolated from *Pseudomonas fluorescens* strain Hv37aR2 in the suppression of *Pythium ultimum* infection in cotton. They recorded a 70% reduction in root infection and an average of 50% increase in seed emergence in cotton.

Table 2.2 List of major banana diseases, their causal organisms, and symptoms

Disease	Causal organisms	Symptoms
Panama disease	*Fusarium oxysporum f. sp. cubense*	Gradual yellowing of lower leaves including leaf blades. The yellowing progresses to the leaf midriff eventually collapsing the petiole and longitudinal splitting of the outer leaf sheaths in the pseudostem causing death of banana plants
Yellow sigatoka	*Mycosphaerella musicola*	Tiny yellow spots/light green streaks appear on the upper surface of leaves. Streaks widen, and the center develops a rusty coloration. Later on, the center of the lesion dries up and develops a black ring with a yellow halo
Black sigatoka	*Mycosphaerella fijiensis*	The appearance of brown rusty streaks especially on the lower surface of the leaf. The lesions becomes round to elliptical and darken giving characteristic black streaking to the leaves
Crown rot	*Colletotrichum musae*, *Verticillium theobromae*, *Musicillium theobromae*, *Lasiodoplodia theobromae*, *Fusarium semitectum*, *F. verticillioides*, *F. oxysporum*, *F. graminearum*, *F. solani, F. sporotrichoides*, *F. pallidoroseum*, *Nigrospora sphaerica*, *Ceratocystis paradoxa*, *Acremonium* sp., *Aspergillus* sp., *Cladosporium* sp., and *Penicillium* sp.	Blackening of the crown tissue spreads to the pulp resulting in the separation of fingers from the hand
Anthracnose	*Colletotrichum musae*	The fungus attacks the flower, skin, and distal ends of heads. The diseased fruit turns blackish and shrivels
Cigar end tip rot	*Trachysphaera fructigena*, *Verticillium theobromae,* and *Gloeosporium musarum*	Gray to black rot spreads from the perianth to the tip of the fruits
Rhizome rot/tip-over	*Erwinia carotovora*	Rotting of rhizome with brown discoloration from the peripheral region to the core of the rhizome. Later, the tissue becomes massive soft, watery, and dark brown to black
Moko disease	*Ralstonia solanacearum*	The appearance of yellowish discoloration on the inner leaf lamina. Young suckers are blackened showing stunted growth and leaves turn yellow and necrotic
Bunchy top	Banana bunchy top virus (BBTV)	Infected suckers bear chlorotic leaves. Dark green streaks appear on the midrib of the petiole. The diseased plants remain stunted and produce a poor bunch

(continued)

Table 2.2 (continued)

Disease	Causal organisms	Symptoms
Streak	Banana streak virus (BSV)	Leaves develop yellow streaking that becomes progressively necrotic and gives a black-streaked appearance in older leaves
Bract mosaic	Banana bract mosaic virus (BBrMV)	Yellowish green bands or mottling appear on young leaves resulting in abnormal thickening of veins
Mosaic	Cucumber mosaic virus (CMV)	Light green or yellowish streaks on young leaves. Bands give a mottled and distorted appearance

2,4-diacetylphloroglucinol (DAPG) production by *Pseudomonas fluorescens* strain CHA0 resulted in the suppression of black root rot of tobacco and take-all of wheat caused by *Thielaviopsis basicola* and *Gaeumannomyces graminis* var. *tritici*, respectively (Keel et al. 1992). Karunanithi et al. (2000) reported inhibition of root rot of sesamum caused by *Macrophomina phaseolina* due to the production of an antibiotic compound, pyrrolnitrin by *Pseudomonas fluorescens*. The growth of *Fusarium oxysporum* f. sp. *cubense*, *Cylindrocladium floridanum* ATCC 42971, *C. scoparium* ATCC 46300, *C. spathiphylli* ATCC 44730, and *C. spathiphylli* Gua5 causing wilt and root necrosis in banana was inhibited by the production of 2,4-diacetylphloroglucinol (DAPG) from *Pseudomonas aeruginosa* FP10 (Ayyadurai et al. 2006).

2.3.6.2 Parasitism

Parasitism involves the direct utilization of one organism as food by another. Mycoparasites are fungi that are parasitic on other fungi and are known to play an important role in disease control (El-Katatny et al. 2001). Mycoparasitism is mediated by physical penetration of the mycoparasite into the host hyphae via the development of peculiar organs such as haustoria and secretion of various enzymes or secondary metabolites leading to degradation of fungal structures followed by nutrient/metabolite uptake from the host fungus (Daguerre et al. 2014).

2.3.6.3 Competition

This process is considered to be an indirect interaction whereby biocontrol agents or phytopathogens are excluded by depletion of a food base or by the physical occupation of the site. Rapid colonization and exhaustion of limited available substrates are the common processes used by nonpathogenic plant-associated microbes to protect the plant so that none is available for pathogens to grow. The biocontrol agents are more competent in uptake or utilizing a substance than pathogens. A competitive root tip colonization procedure was applied to a random Tn*5luxAB* mutant bank of the efficient colonizer *Pseudomonas fluorescens* WCS365. Mutant PCL1285 showed competitive root-tip-colonizing abilities equal to those of wild-type WCS365. However, mutant PCL1286 showed a strongly enhanced competitive root-tip-colonizing phenotype on tomato and grass compared

to its parental strain (de Weert et al. 2004). The production of siderophore discussed earlier is an example of competition, where the nutrient being competed for is available Fe^{3+}.

2.3.6.4 Cell-Wall Degrading Enzymes

Diverse microorganisms secrete extracellular hydrolytic enzymes such as chitinases, cellulases, β-1,3-glucanases, and proteases that can interfere with pathogen growth and/or activities, playing a role in the suppression of phytopathogen (Mishra et al. 2020). Production of hydrolytic enzymes by mycoparasitic fungi such as *Trichoderma* allows them to parasitize the hyphae of phytopathogenic fungi using prehensile coils and hooks that penetrate the cell walls of respective host and consume nutrients for their development. The mycelium of *Rhizoctonia solani* was degraded by chitinases and β-1,3-glucanases produced by *Stachybotrys elegans* (Tweddell et al. 1994). An extracellular protease from *Stenotrophomonas maltophilia* strain W81 inhibited *Pythium* mediated damping-off in sugar beet (Dunne et al. 1997).

El-Katatny et al. (2001) reported inhibition of phytopathogenic basidiomycete *Sclerotium rolfsii* by chitinases and β-1,3-glucanases produced by *Trichoderma harzianum* Rifai T24. Similarly, a significant positive correlation was observed between percentage growth inhibition of *Aspergillus niger* and lytic enzymes (chitinase, β-1,3-glucanase, and protease) in the culture medium of *Trichoderma viride* 60 (Gajera and Vakharia 2012). In another study, Ashwini and Srividya (2014) reported that mycolytic enzymes, viz. chitinase, glucanase, and cellulase from *Bacillus subtilis* effectively inhibited mycelia of *Colletotrichum gloeosporioides* OGC1. *Aspergillus griseoaurantiacus* KX010988 produced chitinase with a molecular mass of 130 kDa. It was found to be optimally active at pH 4.5 and temperature 40 °C. The chitinase showed antifungal activity against the pathogenic fungus *Fusarium solani* (Shehata et al. 2018). *Streptomyces luridiscabiei* U05 produced chitinase which inhibited the growth of *Alternaria alternata*, *Fusarium oxysporum*, *F. solani*, *F. culmorum*, *Botrytis cinerea*, and *Penicillium verrucosum* (Brzezinska et al. 2019).

2.3.6.5 Induction of Systemic Resistance

Two different types of systemic resistance can be conferred to host plants by microorganisms named induced systemic resistance (ISR) and systemic acquired resistance (SAR). Soil-borne microorganisms that competitively colonize plant roots and stimulate plant growth mediate ISR, whereas SAR is induced by pathogens (Romera et al. 2019). ISR is mediated by jasmonic acid (JA) and/or ethylene, whereas SAR is mediated by salicylic acid (SA) which is responsible for the expression of pathogenesis-related (PR) proteins. These defense pathways involve the evolution of specific pattern-recognition receptors (PRRs) for recognition of microbe-based signals referred to as pathogen or microbe-associated molecular patterns (PAMPs or MAMPs) or plant-based signals generated upon invasion, i.e., damage-associated molecular patterns (DAMPs) (Boller and Felix 2009). Biological control of major diseases of banana is depicted in Table 2.3.

Table 2.3 Management of major diseases of banana by biocontrol agents

Biocontrol agents	Diseases	Target pathogens	References
Pseudomonas putida strain 93.1	Root rot	*Cylindrocladium* sp.	Sutra et al. (2000)
Streptomyces violaceusniger G10	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Getha and Vikineswary (2002)
Streptomyces sp. strain S96	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Cao et al. (2005)
Pseudomonas aeruginosa FP10	Wilt and root necrosis	*Fusarium oxysporum* f. sp. *cubense*, *Cylindrocladium floridanum* ATCC 42971, *C. scoparium* ATCC 46300, *C. spathiphylli* ATCC 44730, and *C. spathiphylli* Gua5	Ayyadurai et al. (2006)
Cordana sp. and *Nodulisporium* sp.	Anthracnose	*Colletotrichum musae*	Nuangmek et al. (2008)
Pseudomonas fluorescens strain CHA0	Bunchy top	Bunchy top virus	Kavino et al. (2008)
Trichoderma viride, *T. harzianum*, and *T. koningii*	Post-harvest crown rot	*Lasiodiplodia theobromae* and *Colletotrichum musae*	Sangeetha et al. (2009)
Pantoea agglomerans and *Flavobacterium* sp.	Crown rot	*Colletotrichum musae* and *Lasiodiplodia theobromae*	Gunasinghe and Karunaratne (2009)
Bacillus subtilis	Leaf spot and post-harvest anthracnose	*Pseudocercospora musae* and *Colletotrichum musae*	Fu et al. (2010)
Trichoderma harzianum DGA01 and *Bacillus amyloliquefaciens* DG14	Black sigatoka and leaf spot	*Mycosphaerella fijiensis* and *Cordana musae*	Alvindia (2012)
Bacillus amyloliquefaciens strain NJN-6	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Yuan et al. (2013)
Pseudomonas fluorescens FP7	Anthracnose	*Colletotrichum musae*	Peeran et al. (2014)
Burkholderia spinosa	Anthracnose	*Colletotrichum musae*	Silva and De Costa (2014)
Pseudomonas fluorescens	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Selvaraj et al. (2014)
Trichoderma atroviride	Black sigatoka	*Mycosphaerella fijiensis*	Cavero et al. (2015)
Serratia marcescens CFFSUR-B2	Black sigatoka	*Mycosphaerella fijiensis*	Gutierrez-Roman et al. (2015)
Pantoea agglomerans and *Enterobacter* sp.	Anthracnose	*Colletotrichum musae*	Khleekorn et al. (2015)

(continued)

Table 2.3 (continued)

Biocontrol agents	Diseases	Target pathogens	References
Trichoderma asperellum	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Chaves et al. (2016)
Bacillus amyloliquefaciens strain W19	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Wang et al. (2016)
Trichoderma virens BRIP60169, *T. harzianum* BRIP60170, and *T. harzianum* BRIP60384	Yellow sigatoka and leaf spot	*Mycosphaerella musicola*, *Deightoniella torulosa and Cordana musae*	Samuelian (2016)
Candida tropicalis YZ1, *C. tropicalis* YZ27, and *Saccharomyces cerevisiae* YZ7	Anthracnose	*Colletotrichum musae*	Zhimo et al. (2016)
Bacillus subtilis strains (PP and CL3)	Rhizome rot	*Pectobacterium carotovorum* subsp. *carotovorum*	Rajamanickam et al. (2018)
Pseudomonas aeruginosa DRB1 and *Trichoderma harzianum* CBF2	Wilt	*Fusarium oxysporum* f. sp. *cubense*	Wong et al. (2019)

2.4 Conclusion

Being an exhaustive crop, banana requires large quantities of nutrients from the soil. INM ensures the efficient and judicious use of all the major sources of plant nutrients in an integrated manner, which helps not only in bridging the existing wide gap between nutrient removal and addition, but also ensures a balanced nutrient supply, thereby enhancing nutrient response efficiency and crop productivity of desired quality. A wide range of pathogens infects the banana plant. Microbial-mediated biological control is a powerful and alternative tool against phytopathogens. These biological agents involve quite diverse metabolites and complex signaling pathways, which may act alone or synergistically to prevent, mitigate, or control plant diseases.

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Introduction of Potato Cyst Nematodes, Life Cycle and Their Management Through Biobased Amendments

3

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Abstract

Potato (*Solanum tuberosum* L.) is the most important non-cereal food crop worldwide and is popularly called as king of vegetables because of its nutritional attributes. The potato cyst nematodes (PCN) (*Globodera* spp.) are major pests of potato crops worldwide which comprise two species *Globodera rostochiensis* (Woll) and *G. pallida* (Stone) and eight pathotypes (Ro_1 to Ro_5 of *G. rostochiensis* and Pa_1 to Pa_3 of *G. pallida*). They cause significant yield reductions and severely impact the movement of potatoes around the globe through quarantine restrictions. Emergence of juveniles from the cysts is stimulated by host root diffusates, second stage juveniles (J_2) of PCN invade the root tip in the zone of elongation and migrate intra-cellularly to the cortex surrounding the vascular tissue. Mature cysts get detached from the roots, and can remain viable in the soil for several years. In general, in the advance stage of PCN infestation, the symptoms may appear as signs of mineral deficiency, yellowing of plants in patches, wilting of plants during sunny hours as well as stunted plants with poor root system. There are various options for controlling and limiting the damage they cause, including the use of nematicides, following cultural practices like crop rotation with non-host crops, intercropping with antagonistic crops, growing of resistant cultivars and summer ploughing. However, due to the formation of cysts, it will survive in the soil even in the absence of a host, making many of the cultural management strategies unattractive. Chemical control of PCN involves the use of very harmful pesticides but due to the increasing concern about environmental issues, it cannot be followed practically. Therefore, combining of different management approaches is most desirable and effective method of control.

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Keywords

Globodera spp. · Cultural control · Chemical control · Physical control · Biological control · Integrated management

3.1 Introduction

Among the parasitic nematodes, the plant parasitic nematodes are very small and cannot be seen by naked eyes. Generally, it is very difficult to recognize the nematode damage as it is often looks similar to nutrient deficiency. In addition, nematodes damage also leads to secondary infection by other organisms. During the year 1881, Julius Kuhn first recorded the cyst nematode infection in potato from Germany. *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone) are popularly known as Golden cyst nematodes which hinder the sustainable potato production worldwide. Among top ten plant parasitic nematodes it stands second based on economic importance (Grenier and Benjamin 2017). Even in the absence of host long-term survival of PCN in the soil presents them challenging to the scientists and policy makers. Wherever the PCN occur, it become a quarantine issue for the domestic and international commerce in potatoes. Recently new species, *Globodera ellingtonae* is reported in potatoes from America (Grenier and Benjamin 2017).

3.2 Origin and Distribution of Potato

Worldwide Potato (*Solanum tuberosum*) is considered to be the most important food crop and it belongs to the *Solanaceae* family. At least 8000 years ago potato occurred in the valleys of the Andes in South America. During 1531 due to Spanish invasion in Peru it became the well adapted important food crop there later on it is appeared in Europe during the sixteenth century (Turner and Evans 1998). Potato introduced into England from Andes during 1590. As per records, potato introduced into the Canary Islands from Peru in 1622. From the original two introductions, it spread too many parts of the world (Fig. 3.1). From the Spanish introduction, it was diffused throughout the continental Europe and parts of Asia (Turner and Evans 1998) (Table 3.1). Nearly 1680s, it was introduced into India which is now grown under different agro climatic conditions. While, Mr. John Sullivan, the founder of present day Udhagamandalam, initially introduced potato to Tamil Nadu in 1822 where the PCN was first reported during 1961 (Jones 1961).

3.3 Origin and Distribution of Potato Cyst Nematodes

The Andean Mountain of South America, the original home for potato is also the place of origin for PCN. PCN introduced into Europe in the 1850's along with the soil remaining on potato tubers brought for late blight resistance breeding and soon

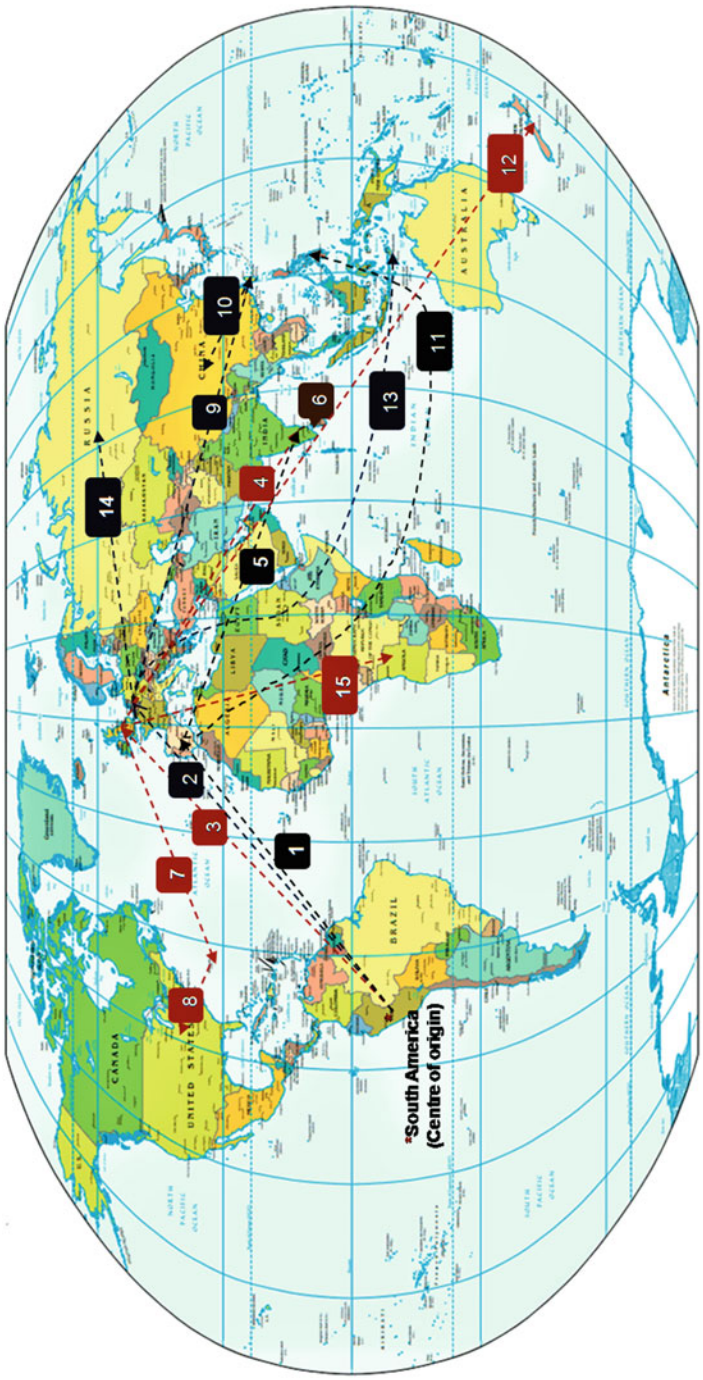

Fig. 3.1 Symptoms of field infected with potato cyst nematode

Table 3.1 Distribution of potato from its centre of origin

S. No.	Introduced		Year
	From	To	
1.	South America	Spain	1570
2.	South America	Holland	>1573
3.	South America	UK	1590
4.	UK	India	<1610
5.	Portugal	India	<1610
6.	India	Sri Lanka	<1610
7.	UK	Bermuda	1613
8.	Bermuda	USA	1621
9.	Holland	Taiwan	<1650
10.	Taiwan	China	<1650
11.	Spain	Philippines	<1700
12.	UK	Holland	1773
13.	Holland	Java	1794
14.	Holland	Russia	<1800
15.	UK	Africa	1830

after it spread throughout the world through the introduction of varieties from Europe. Hence Europe has been considered as the secondary centre for PCN distribution. According to Franco et al. (1998) the precise pathways of introduction from South America to Europe of PCN must remain a matter of assumption. Evans and Stone (1977) described that PCN probably spread from Europe to other counties with exported seed tubers of breeding materials.

There is some assumption that PCN were introduced into the Asian countries during the Second World War while the transportation of human resources, food and military equipment to many parts of the Asia. But one exception was that from Peru to Japan, PCN may be transferred through contaminated sacks of Guano, the dried remains of birds' semisolid urine, makes excellent fertilizer.

3.3.1 Species

Heterodera rostochiensis was first described by Wollenweber (1923) who differentiated it from the beet cyst nematode, *H. schachtii* and proposed as new species. Later, the heterogeneity within *H. rostochiensis* led to the description of new species, *H. pallida* with white or cream coloured females by Stone (1973). Subsequently, *H. rostochiensis* and *H. pallida* were assigned to the new genus *Globodera* which lacked a terminal cone, whereas the genus *Heterodera* contains the lemon shaped cyst nematodes (Mulvey and Stone 1976; EPPO 2020).

G. rostochiensis was first discovered in the USA in 1941, in India during the 1960s and in Mexico during the 1970s (Grenier and Benjamin 2017). Presently PCN have been reported from 83 countries with *G. rostochiensis* (CABI/EPPO 2020a) and 64 countries with *G. pallida* (CABI/EPPO 2020b) in six continents, viz., Africa,

Fig. 3.2 Occurrence of *Globodera* spp. in Asian countries

North America, South America, Asia, Europe and Oceania. In Europe, *G. rostochiensis* is present in 39 countries and *G. pallida* in 38 countries. In North America, *G. rostochiensis* is present in six countries and *G. pallida* in 5 countries. In South America, both the species are present in eight countries. In Asia, *G. rostochiensis* is present in 16 countries and *G. pallida* in 6 countries (Fig. 3.2). In Africa, *G. rostochiensis* is present in 11 countries and *G. pallida* in 5 countries. In Oceania, *G. rostochiensis* is present in three countries and *G. pallida* in one country. Populations of *G. rostochiensis* (Ro1 race) in the British have the virulence against H1 (ex-*andigena*) gene which was similar to South American population of *G. rostochiensis* (Ro1 race). Whereas, which show restricted genetic introduction of *G. rostochiensis* into Europe or other races may be failed to establish due to climatic condition in Europe (Turner and Evans 1998; Grenier and Benjamin 2017). Due to scarcity of *G. pallida* resistance varieties make the control of this species much more difficult.

3.3.2 Pathotypes

Continuous breeding and selection of resistant potatoes in Britain, Netherlands and Germany brought to light the occurrence of variation within species, which were designated separately. International scheme proposed by Kort et al. (1977) designates five pathotypes of *G. rostochiensis* from Ro1 to Ro5 and three pathotypes of *G. pallida* from Pa1 to Pa3 (Table 3.2).

In India, the differential host reactions of PCN populations from The Nilgiris and Kodaikanal hills revealed that the pathotypes Ro1 of *G. rostochiensis* and Pa2 of *G. pallida* are the most prevalent forms (Prasad 1996). The other prevalent pathotypes are Ro2 and Ro5 of the former and Pa1 and Pa3 of the latter (Table 3.3).

Table 3.2 Classification of pathotypes of PCN species

	Pathotypes							
	Globodera rostochiensis					*Globodera pallida*		
Differential hosts	Ro_1	Ro_2	Ro_3	Ro_4	Ro_5	Pa_1	Pa_2	Pa_3
S. tuberosum spp. *tuberosum*	+	+	+	+	+	+	+	+
S. tuberosum spp. *andigena* (H1)	–	+	+	–	+	–	–	+
S. kurtzianum KTT/60.21.19	–	–	+	+	+	+	+	+
S. vernei GLKS 58.1642.4	–	–	–	+	+	+	+	+
S. *vernei* $(VT^n)^2$ 62.33.3	–	–	–	–	+	–	+	–

'+' indicates susceptibility; '–' indicates resistance (Kort et al. 1977)

Table 3.3 Pathotypes of *Globodera* spp. at different localities of Nilgiri hills in India

Locality	*G. pallida*	*G. rostochiensis*
Adigaratty	Pa1, Pa2, Pa3	Ro1, Ro2
Fernhill	Pa1, Pa2, Pa3	Ro1, Ro2, Ro5
Kallatty	Pa1, Pa2, Pa3	Ro1
Nanjanad	–	Ro1, Ro2, Ro5
Vijayanagaram	Pa2	Ro1, Ro2, Ro5

Prasad (1996).

3.3.3 Spread of Disease

The disease normally spreads by the movement of infested soil containing cysts and larvae, through the following agencies.

1. Movement of seed potatoes from infested fields to the clean fields (cysts, about 0.8 mm in diameter size can be easily escaped in the tuber eyes or in soil that may adhere to tubers at the harvesting time).
2. Wind, irrigation and rain water (by wind-blown of contaminated soil and water runoff in the clean field).
3. Raising of seedling from infested area and planting to clean area (cyst may transfer through seedlings in new field).
4. Movement of compost from infested area (movement of compost from PCN infested area to clean field may increase the chance of infection).
5. Use of agricultural implements first in the infested area and then in clean plots (PCN transported on tractor tyres, spade, etc.).
6. Through shoes of the workers and feet of cattle (cysts transfer through boots and shoes of works and feet of farm animal, dogs, etc.)
7. Through the use of old gunny bags in which the potatoes from infested plots were packed/stored previously (re-use of old gunny bags in which already stored potatoes from the infected field).

3.3.4 Host Range

Potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena*) are the agronomic crops attacked by both species on PCN. The known host range of PCN includes few species of *Datura, Hyoscyamus, Lycopersicon, Physalis, Physoclaina, Salpiglossis* and *Saracha* all in the Solanaceae family. *Oxalis tuberosa* Molina, a native Andean tuber crop of economic importance, is also considered to be a host of PCN (Sullivan 2006). Sullivan et al. (2007) reported that *Datura stramonium, Nicandra physalodes* and *Solanum nigrum* allowed nematode penetration in the roots but no further development of *G. rostochiensis* pathotype Ro1. Similarly, *Solanum sisymbriifolium* reported as a potential trap crop for both the species of PCN (Scholte and Vos 2000).

3.4 Symptoms

The disease caused by this nematode is often referred to as 'potato sickness'. The presence of the golden nematode in soil is often unnoticed in lightly infested crop which does not show any above ground symptoms at all. This is because most of the plants can tolerate nematode invasion by developing more lateral roots as wound response. However, as the degree of invasion increases, the plants become unable to defend against PCN and finally express a range of symptoms.

When the infestation is sufficiently heavy and localized, poor growth of plants appear in small patches (Fig. 3.3) which may be occurred like wilted plant during hot parts of the day. This is often the first evidence above ground of the presence of the golden nematode.

More evenly distributed infestations may cause a sudden failure of crops in whole fields. Repeated cultivation of potatoes encourages the rapid multiplication and build-up of the parasite. Heavily attacked plants remain severely stunted with dull and unhealthy looking foliage. As the season advances, the lower leaves turn yellow

Fig. 3.3 Symptoms of field infected with potato cyst nematode

and brown and wither, leaving only the young leaves at the top, the entire plant now presenting a somewhat 'tufted head' appearance. The browning and withering of the foliage gradually extend and ultimately causes the premature death of the plant. The root system is poorly developed, the yield and size of the tubers are reduced considerably depending upon the degree of infestation. Badly infested plants give little or no harvest. Close examination of the roots of infected plants reveals the presence of white or yellow female nematodes sticking to the roots. Symptoms may vary from year to year, depending on growing conditions and fluctuations in populations of nematode (Prasad 2006).

3.5 Biology

Potato root diffusates (PRD) consist some chemical substances which stimulated the hatching of cysts called hatching factor. There are at least 25 hatching factors responsible for hatching of both the species of PCN. After 3 weeks of plant emergence only activity of PRD is increased from the root tips. Some hatching stimulants like α-solanine, α-chaconine and solanoeclepin-A occur naturally in potatoes (Blaaw et al. 2001). Hatching mainly depends on host root diffusates, prevailing weather and physical conditions of the cyst.

The second stage juvenile (J_2) coming out of the cysts moves actively in soil and invade the roots by rupturing with its stylet. It enters through the epidermal cell walls and eventually settles with its head towards the stele and feeds on cells in pericycle, cortex or endodermis by forming a feeding tube. This induces enlargement of root cells and breakdown of their walls to form a large 'syncytium' or 'transfer cell' with dense granular cytoplasm that provides nourishment for nematode development. The nematode molts and remains in the syncytium until its development is complete (Evans and Stone 1977).

The sex of the nematode is determined during J_3 stage, the females become sedentary, swollen and remain attached to the roots and posterior part of the body comes out by rupturing the root cells. Males retain their thread shape and come out of the roots to locate and mate the females. The immature females of *G. rostochiensis* are golden yellow in colour while that of *G. pallida* are white or cream in colour (Fig. 3.4). The white female remains white or cream coloured before finally turning brown, whereas the yellow female passes prolonged golden-yellow phase before it turns to brown (Evans and Trudgill 1978).

After the female dies, the body wall becomes thick and forms a hard brown cyst which is resistant to unfavourable weather. Each cyst contains 200–500 eggs which displaced in soil during harvest of tubers. The cysts can easily survive in the absence of a suitable host for next 20–30 years (Turner and Evans 1998). PCN complete life cycle within 35–40 days therefore it complete normally one generation in one crop season. However, there are facts that *G. rostochiensis* has two generation because of its shorter dormancy (45–60 days) and long crop duration (120 days) whereas *G. pallida* has a longer dormancy of 60–75 days and one generation. After completing the life cycle, cysts enter in the extreme form of dormancy, known as 'diapause'

Fig. 3.4 Infection of *Globodera* species

in this period they cannot be stimulated to hatch. After completing diapauses only 60–80% of juveniles can be stimulated to hatch in the presence of host root diffusates but it never reaches 100% and this is a part of the survival strategy. About 30–33% spontaneous hatching occurs annually in the absence of host but it can be influenced by the environmental conditions (Oostenbrink 1950). In the temperate zones in the absence of the host crop, soil infestation with viable PCN may persist for 20–30 years. The high reproductive capacity of this nematode (up to 70 times) and their slow rate of decline (40% per year) make them a persistent and serious pest of potatoes (Evans and Trudgill 1978).

3.6 Yield Losses

The tolerance limit of PCN is 1.3–2.1 eggs/g soil (Greco 1993) while the economic threshold level is around 20 eggs/g of soil (Evans and Stone 1977). Earlier world-wide it caused estimated yield loss of 30% (Oerke et al. 1994), whereas Urwin et al. (2001) reported estimated losses of more than 12%. Under Indian conditions, the tuber yield loss estimates vary from 5 to 80% depending on the initial inoculum level (Prasad 1996).

3.7 Management

PCN once established in the fields become very difficult to be eradicated therefore it remains as a serious endemic pest of potato worldwide. Since the single method for PCN control is not fully effective for the suppression therefore incorporation of blend of various management options like host resistance, chemical, biological and cultural methods is being advocated to bring down the PCN population to levels that permit profitable cultivation of potato.

3.7.1 Cultural Control

3.7.1.1 Crop Rotation

Urwin et al. (2001) reported that the use of crop rotations keep the PCN population densities below the damaging level. The best sequence affecting PCN density, potato yield and profitability was determined as maize and lima beans preceding the potato crop in Peru (Canto 1995), Ecuador (Ravelo 1984) and Cochabamba (Proinpa 1996). In western Europe a gap between potato crops of susceptible varieties of up to 7 years is necessary (Oostenbrink 1950; Jones 1970). Crop rotation with non-solanaceous crops is widely recommended for management of PCN because of their narrow host range. Menon and Thangaraju (1973) observed the effect of crop rotation of 4 years involving potato, French beans, peas and peas recorded 98.7–99.9% reduction of PCN in the fourth year and increased the yield more than 90% when potato was grown at the end of rotation period. Incorporation of resistant varieties alone in a 4 year crop rotation programme resulted 67–78% yield increase. Growing of non-host crop between host crops will reduce the population density of PCN (Whitehead 1995). Prasad (1993) also reported that crop rotation with the PCN non-host crops radish, cabbage, cauliflower, turnip, garlic, carrot, green manure crop like lupin, etc. for 3–4 years brings down the cyst population by 50%. All the non-solanaceous crops tested reduced the PCN multiplication ratio. Among different non-solanaceous crops, radish recorded 19.6–21.0% reduction in number of cysts and 12.2–16.2% reduction in number of eggs per cyst. Which was followed by garlic, it recorded 15.9–17.7% and 10.3–11.6% reduction in number of cysts and eggs respectively (Aarti et al. 2017).

3.7.1.2 Intercropping

Manorama et al. (2005) recorded higher potato equivalent yield and reduced cyst population when potato is intercropped with French Beans (75:50). Intercropping of potato with mustard in 1:1 plant ratio combined with carbofuran application reduced PCN infestation and enhanced potato yield (Devrajan and Balasubramanian 2008). Non-solanaceous crops, viz., marigold and radish were evaluated as a intercrop, potato intercropped with radish in the ratio of 2:1 was found to be effective in decreasing the PCN population (Rf: 0.99) (Aarti et al. 2017).

3.7.1.3 Trap Cropping

Growing potatoes to stimulate the hatching of PCN and destroying of potato plant after the incursion of nematodes in the potato roots can lessen soil infestations (Webley and Jones 1981). In France, *G. pallida* populations reduce by 80% per annum by trap cropping and 98.5% with two trap crops and application of ethoprophos. Growing potato as a PCN trap crop must be destroyed before the females are fertilized. The exact time of trap crop destruction is much more important as delay in destruction will lead the development of a female. Tolerant cv. Cara grown in full ridges for 6 weeks in heavily infested soil decreased *G. pallida* by 75% (Whitehead 1977; Whitehead et al. 1994). In India, trap cropping with susceptible potato cultivar attracted more juveniles than the resistant potato cultivar and reduced

nematode population by 53% (Aarti et al. 2017). The non-tuber bearing solanaceae wild plant *Solanum sisymbriifolium* (Lam.) is a promising source of resistance to PCN and it reduced the PCN populations by 50–80% (Scholte and Vos 2000).

3.7.1.4 Host Plant Resistance

Wolters et al. (1996) identified resistance in 18 out of 22 *Solanum* accessions, with the highest levels in *S. gourlayi* BGRC7180 and *S. neorossi* BGRC7211 as well as in the *S. sanctae-rosae, S. sparsipilum* and *S. sucrense.* In Germany, Rouselle-Bourgeois and Mugniery (1995) found resistance to *G. rostochiensis* R_1A in *S. andigena*, *S. gourlayi, S. spegazzini* and *S. vernei* and resistance to *G. pallida* P_4A/P_5A in *S. gourlayi, S. spegazzini, S. sparsipilum* and *S. vernei.* Initially, all resistance was based on the H_1 allele derived from *S. tuberosum* ssp. *andigena* CPC 1673, which was effective only against pathotypes R_1A and R_1B of *G. rostochiensis.* Now these pathotypes widely expand and become virulent so H_1 allele is not effective against all populations of *G. rostochiensis* (Phillips et al. 1998). Mulder (1994) reported that resistant cultivars derived from *S. tuberosum subsp. andigena* had high level of tolerance compared to *S. vernei*. Hockland et al. (2012) reported that UK and Europe have the resistant potato varieties to *G. rostochiensis* (Ro1) but there is no cultivars resistance to all the pathotypes of *G. pallida*. In India, for locating resistance and for incorporation in commercial potato varieties, a large collection of germplasm was screened against PCN by Kishore et al. (1969). Dalamu et al. (2012) documented the potato germplasm resistant to both the species of PCN located in *tuberosum* and *andigena* accessions. In India, *S. vernei* derived resistant cultivars Kufri Swarna (Khan et al. 1985), Kufri Neelima (Joseph et al. 2012) and Kufri Sahyadri (Joseph et al. 2019) were developed to reduce PCN multiplication. However, in this area conflict between breeder and nematode continues because the development of virulence in both the species of *Globodera*.

3.7.2 Physical Control

Soil solarization is most suitable for small areas having long hot summers as in temperate areas only few centimetre layer of soil get lethal temperature (Whitehead and Turner 1998). *G. rostochiensis* eggs (97%) were unable to hatch in the top 10 cm layer of the soil during hot summer (LaMondia and Brodie 1984). Soil solarization for 62 days reduced 95% *G. rostochiensis* population (Mani et al. 1993).

3.7.3 Chemical Control

Nematicides are an effective and trustworthy method to bring down the nematode population quickly. The efficacy of soil fumigation depends heavily on soil condition and temperature. Soil can be fumigated successfully above 5 °C (methyl bromide), 7 °C (1,3-D) or 10 °C (MITC fumigants) (Whitehead and Turner 1998). In tomato and potato, methyl bromide @488–1464 kg/ha controlled the PCN

population when applied under gas-tight polythene sheet. However, it has been banned in some countries as it is harmful to the ozone layer. In UK, soil fumigant Dazomet found better in controlling the nematodes effectively than equivalent dose of Telone (Whitehead et al. 1973). Whitehead et al. (1994) reported that ethoprophos @11.2 kg/ha partially controlled *G. pallida* in the silty loam soil.

In India, trials have been attempted with different nematicides like DD, DBCP, Nemafos, V.C.13 and Dasanit 10G. DD applied @1000 l/ha in two split doses in 15 days interval resulted 98–100% control. Dasanit 10G was recommended for three crop seasons @300 kg/ha in the main season followed by 150 kg/ha in each of the subsequent two seasons (Gill 1974). Application of Furadon 3G @2 kg a.i./ha at the time of planting is being recommended for PCN after the standardization as a part of package of practices for potato in the Nilgiris (Prasad 2006). However, these chemicals have been banned in the recent past. Fumigant molecule Dazomet (Basamid 90G) @ 40–50 g/m^2 also found to be effective in bringing down the PCN population but after application the soil needs to be covered with polythene sheet (Aarti et al. 2016).

Use of calcium hypochlorite solution containing 9% available chlorine as a seed treatment was found to be effective in reducing PCN population (Manoharan et al. 1978). Cyst adhering potato tubers can be destroyed by immersion in sodium hypochlorite solution for 2 h and then rinsing in water (Wood and Foot 1977). Soaking of PCN infested un-sprouted seed potato tubers in 2.0% NaOCl solution (containing 4% available chlorine) resulted 100% cyst disintegration after 30 min and there was no harmful effect on tuber sprouting after 2 months of storage (Aarti et al. 2020). However, repeated use of nematicides is not only expensive but also hazardous to environment. Hence this has to be supplemented with other non-chemical approaches to contain the nematode population at low levels.

3.7.4 Bio-control Agents and Organic Amendments

Several workers have studied biological control of *Globodera* spp. in vitro and in vivo but no field trial data are available. However, in the recent past some products have come to market that have nematicidal effects. Most other potential bio-control agents are still being tested to overcome problems with application methods. Application of biological control agents, viz., *P. fluorescens* and *P. lilacinus* (Seenivasan et al. 2007) and organic amendments like neem cake (5 t/ha) combined with *Trichoderma viride* (5 kg/ha) confirmed the decreasing in PCN population (Umamaheswari et al. 2012). Biofumigation with incorporation of radish leaves @ 1 kg/m^2 and covering with polyethylene sheet recorded maximum yield (25.97 t/ha) and 1.21 PCN reproduction factor (Rf) (Umamaheswari et al. 2015).

3.7.5 Integrated Nematode Management

PCN can be managed well when combined the effective control measures, reduction of *G. pallida* infestation could be achieved with 5 years of crop rotations with non-host crops, effective soil fumigation and use of an effective trap crop (Whitehead and Turner 1998). To decrease large populations rapidly, a fumigant nematicide or a trap crop should be followed by the growing of a potato crop protected by a granular nematicide (Phillips et al. 1998). Whitehead et al. (1991) reported that granular nematicides with susceptible potato cultivars and with crop rotations effective for the management of *G. rostochiensis*. Intercropping of potato with mustard in 1:1 plant ratios applied with carbofuran 3G @ 1 kg a.i./ha reduced PCN infestation and enhanced potato yield (Devrajan and Balasubramanian 2008). Devrajan et al. (2004) suggested an integrated approach for PCN management wherein application of *Pseudomonas fluorescens* (2.5 kg/ha) + neem cake (1 t/ha) + mustard intercrop (between potato rows) + carbofuran 3G (1 kg a.i./ha) increased the tuber yield and decreased the PCN population. Manorama et al. (2016) reported effective nematode reduction of 47% in 2 years by rotating PCN susceptible and resistant variety along with application of carbofuran @ 2.0 kg a.i./ha. For eradication of PCN, soil solarisation (4 weeks) followed by appliance of neem cake (5 t/ha) in combination with *Trichoderma viride* (5 kg/ha) recorded decrease in PCN population (Aarti et al. 2017).

Apart from IPM some of the control measures are recommended at international level for potato growers by OEPP/EPPO (OEPP/EPPO 2014). In Netherland the possible control measures, i.e. growing of resistant potato varieties, growing of potato as a trap crop for 40 days, growing of *Solanum sisymbriifolium* as a catch crop and soil fumigation are followed. After the confirmation by photo sanitary inspectors only the official ban is lifted. In the Slovenia, growing of resistant varieties, crop rotation for minimum 4 years and removal of volunteer potatoes has been recommended. In England and Wales, use of PCN resistant potato cultivars, crop rotation, chemical control, trap cropping (with *Solanum sisymbriifolium*), use of green manures and fumigants are followed. In Belgium, the official control programme includes the use of resistant potato cultivars for the PCN pathotypes, application of nematicides, viz., metam sodium, metam potassium, ethoprophos, fosthiazate and oxamyl before planting susceptible cultivar, crop rotation for ware (1 crop every 3 years) and seed (1 crop every 4 years) potato production. In Denmark, resistant varieties must be grown in two consecutive years. All machinery must be cleaned before use in fields, harvested tubers of ware potatoes in infested fields must not go on at the same time as in seed potato fields, soil and other waste must be handled carefully to avoid further spread. The usefulness of the control programme is checked by soil testing after 3 years of application. In Germany, use of highly resistant varieties, 6 year rotation as no nematicides is available in Germany. In France, for seed potatoes testing is always done before planting if PCN detected than not allowed to grow potatoes for 6 years, all volunteer potatoes must be destroyed. Growers may grow plants such as grass, maize, cereals to avoid the risk of exporting soil in new field.

3.8 Future Strategies for PCN Management

Breeding resistant varieties from diverse sources of resistance in wild tuber bearing *Solanum* spp. needs to be explored for resistance to wide spectrum of PCN pathotypes. This also necessitates identification of molecular markers for identification of resistance gene in varieties for the species of PCN. By co-ordination between nematologists and molecular biologists, the possibilities should be explored to inhibit the activity of genes responsible for production of hatching factors in root diffusates and also identify, characterize and inhibit the genes which involved in parasitization of PCN in host plant which may be done through RNAi. As biological control agents hold a promise in control of potato cyst nematode, identification of native antagonistic bacteria and fungi from potato rhizosphere and characterization of their bioactive compounds may serve as novel nematicides against potato cyst nematode. Researchers around the world quickly adopting the approach CRISPR Clustered Regularly Interspaced Short Repeats Palindromic Repeats) to edit the DNA sequences of interested organism. Possibilities of using aeroponic root leachates for inducing the hatching of potato cyst in the absence of host may become novel strategy for the management which leads to premature death of juveniles.

3.9 Conclusion

PCN are tiny yet strong pests of potato and create a serious threat to potato cultivation and global potato trade because of their quarantine significance. Though eradication of PCN is very difficult if once established but, more recently Western Australia has been declared free of potato cyst nematodes, after a battle of about 24 years, opening up big opportunities for its $45 million potato industry. This indicates the possibility of the eradication of this nematode from India as well as in other countries with the strong background of science based biosecurity policies, strict regulatory and sanitation measures and management strategies.

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Integrated Nutrient and Disease Management Practices in Root and Tuber Crops

4

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Abstract

Root and tuber crops are the second group of cultivated species after cereals as global sources of carbohydrates. These crops have become the major issue of interest in the last few years since their production requires very low inputs and hence they contribute significantly to world food and nutritional security with immense industrial uses. The flexible adaptability of these crops to marginal soils and contribution to food security in households have made them an important component in improving the welfare of poor farmers. However, the repeated use of fertilizers, fungal chemicals, and antibiotics for prolonged times has not only led to resistance development in concerned crops but also enhanced toxicity in the environment. Also, diseases due to bacterial and fungal pathogens are the second major cause of concern for reducing the productivity that causes fiscal losses to the growers. Sustainable methods to enhance the income of the farmers growing these tropical food crops employ the judicious use of natural resources like soil microbes, crop residues, and applied resources such as chemical fertilizers, organic manures, and bio-fertilizers. Further, the use of bio-agents/biological control offers the best possible option to increase crop yields by managing the pathogens in an environment-friendly manner. This chapter presents, collates, and discusses the application of sustainable management practices for the

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improvement of soil health and use of potential biocontrol agents which paves way for enhanced productivity to the development of these crops.

Keywords

Tubers · Biocontrol mechanisms · Bio-agents · Nutrient management · Soil fertility · Sustainability

4.1 Introduction

Root and tuber crops constitute the third important group of food crops, after cereals and grain legumes as global sources of carbohydrates. They are consumed as either staple or subsidiary food by about one-fifth of the world population and have utmost importance for the world food security. These crops are well known for their high calorific value and possess the ability to resist adverse soil and climatic conditions (Saravaiya and Patel 2005). The chief roots and tubers, i.e. cassava, potato, and sweet potato rank among the top ten food crops produced in the developing countries. Due to their valuable table yields in conditions where other crops fail, these crops serve for a long time as the source of food and nutrition for many of the world's poorest and immense malnourished households. Hence, these crops are expected to contribute significantly in increasing the income and nutritional well-being of the people in the next few decades. Despite as food, they are also used as livestock feed and raw material for agro-based industries. In the next few decades, cultivation of root and tuber crops is expected to increase further as there is a declining trend observed in the production of cereals and pulses in developing countries due to the effect of climate change.

However, repeated, heavy, and unbalanced applications of chemical fertilizers not only cause soil erosion but also lower the crop yield and in turn disturb the environment, subvert ecology, and degrade soil productivity. The integrated nutrient management (INM) approach consists of the replacement of heavy doses of chemical fertilizers with effective and balanced quantities of organic manures and inorganic fertilizers along with specific microorganisms. This methodology is becoming a quite promising practice for eco-friendly and stable production of crops besides maintaining higher productivity (Selim 2020). Furthermore, low productivity due to soil-borne pathogens is the major constraints that reduce the quality, quantity, and market value of these crops besides causing yield losses in field and storage conditions. Implementation of biological control methods as the earliest effort in the plant pathogen interactive environment offers an attractive way to replace chemical fertilizers, pesticides, and other supplements, manage pathogen and pest control in crops, and increase crop production in an environment-friendly manner (Panth et al. 2020). Due to the concerns on soil and human health, escalating awareness on the concept of recycling of available wastes for better nutrient management, insufficient manual labor involved in undertaking farming, the thrust

nowadays is to develop sustainable, eco-friendly, and cost-effective methodologies taking into account the constraints as mentioned above.

This chapter discusses the present status of nutrient management practices in root and tuber crops through the judicious use of organic manure, chemical fertilizers, and bio-fertilizers for the improvement of soil health to ensure a step forward towards productive and profitable crops. Since most of the studies on biocontrol of diseases are focused only on potato crop and very little interests have been given to other tuber crops like cassava and sweet potato, therefore, a basic understanding of postharvest biocontrol systems, upgradation of microbial antagonists exhibiting a broad spectrum of antifungal potential on different produce and environmental impacts need to be reconnoitered. Keeping this in consideration, this chapter also highlights the advancements made in recent years on the spectrum of bacteria and fungi used as antagonists for control of major diseases of root and tuber crops, their mechanisms of action, and different modes to augment biocontrol efficiency of the antagonists.

4.2 Importance of Root and Tuber Crops in the Accomplishment of Sustainable Development Goals

Root and tuber crops have boosted sustainable food production due to many captivative reasons. Firstly, these crops are a convenient source of staple food to tackle food and nutrition security as food yield per unit area of land is more in comparison to other crops. Secondly, the short cropping cycles (3–4 months) of potato and sweet potato make them well suited to the double-cropping seasons. Further, yam and cassava have longer cropping cycle that plays an important role in the annual cycle of food availability due to their broader agroecological adaptation, diverse maturity period, and in-ground storage capability that permits flexibility in the harvesting period for sustained food availability. These crops are also efficient converters of natural resources into a more usable product, caloric energy in the growing season, and almost double that of wheat and rice. Being cheap but nutritionally rich staple food meets the dietary demands due to the abundance of protein, vitamin C, vitamin A, zinc, and iron in them. These crops have high demand both in local and national markets. Last but not the least, these are far less susceptible to large-scale market shocks and price speculation during the international market crisis that is experienced by more widely traded staple crop, such as grains. All these elements make their contribution to a more stable food system besides being a predictable source of income. The five major groups of root and tuber crops grown across the world include crops, viz. cassava (*Manihot esculenta*), yams (*Dioscorea* spp.), sweet potato (*Ipomoea batatas*), potato (*Solanum tuberosum*), and edible aroids known variously as taro (*Colocasia esculenta*) and tannia (*Xanthosoma*), but often denoted as cocoyams. The secondary staples include cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*), whereas elephant foot yam

(*Amorphophallus paeoniifolius*), greater yam (*Dioscorea alata*), and taro (*Colocasia esculenta*) are being used as vegetable crops (Mohanty et al. 2010; Sahoo et al. 2012; Nedunchezhiyan et al. 2013). However, root and tuber crops have major confronts associated with quality seed production, new variety adoption, losses due to insects and diseases, low productivity in nutrient-poor soils, tolerance to stress associated with heat and drought, consumer preferences, are bulky, have high water content and a relatively short shelf-life that creates a problem in storage of harvested products. Improved productivity of these crops is a prerequisite since the pressure on agricultural land has increased.

Crop productivity can be increased by using fertilizers as one of the key inputs. Nowadays, modern varieties of crops require a relatively high quantity of fertilizers for obtaining higher yields as compared to the traditional cultivars. In the 1960s high yielding varieties were introduced with an excessive amount of chemical fertilizers requirements to increase production. This was done to make the country self-sufficient in food requirements but has impaired the prevailing soil conditions. The continuous and imbalanced application of chemical fertilizers has been confirmed by many researchers not only to deteriorate soil health but also led to an ecological imbalance resulting in decreased nutrient uptake efficacy in plants (Saravaiya et al. 2010). The soils obtaining plant nutrition barely via chemical fertilizers are showing abating yields due to deteriorated physical conditions besides suffering from micronutrient deficiency with the excessive use of chemical fertilizers. Groundwater contaminations, environmental pollution, destruction of the ozone layer through N_2O production are other causes of excessive use of nitrogenous fertilizers. On the other hand, the reconsideration of substitutes has become the need of the hour due to low organic matter content of most of the soils. The growing rate of world population soared hidden hunger and demands a sustainable agricultural approach for improved crop yield with a high nutritional value (Roriz et al. 2020).

4.3 Soil Fertility Management

Soil fertility is the outcome of a combination between soil properties and crop management on plant growth and tuber yield (Patzel et al. 2000). Soil fertility and crop production can be increased through the framework of Integrated Soil Fertility Management (ISFM). This framework works in conjunction with the combined application of organic and mineral nutrient sources (Chivenge et al. 2011; Kearney et al. 2012; Vanlauwe et al. 2010, 2015). Due to the limitation in available resources, multiple nutritional deficiencies crop up in roots and tubers because of insufficient availability of soil nutrients (NPK). The INM strategy for root and tuber crops involves three major components, i.e. the conjoint application of chemical fertilizers, organic manures, and bio-fertilizers. This strategy of integrated soil fertility management incorporating practices of both organic and inorganic plant nutrients leads to averting soil degradation, knowledge to adapt these to local conditions, the

achievement of higher crop productivity, and thus helping meet the requirements of future food supply (Bonierbale et al. 2006). The effect of positive interaction between organic and inorganic mineral inputs on crop productivity and sustainable soil health is fully acknowledged in the integrated nutrient management paradigm (Vanlauwe et al. 2002). In an integrated nutrient management strategy, the soils are recognized as the storehouse of utmost plant nutrients that are indispensable for plant growth. How these key nutrients are managed has a major impact on plant growth, soil fertility, and hence sustainability (Janssen 1993). Other factors that upsurge the importance of using locally available organic sources of plant nutrients to maintain soil productivity are the escalating costs of fertilizer and the economic conditions of farmers. Research evidence have also signified maximum corm yields along with the highest starch and protein content under integrated use of organic manures along with chemical fertilizers (Kumar et al. 2015).

Unlike chemical fertilizers, organic manures are available locally and used by farmers to provide nutrients for the crop plants. Organic manure is one of the most important inputs for increasing the productivity of the crop. The crop production potential is directly associated with the organic matter content of soils. Important organic manures include farmyard manure, bio-compost, poultry manure, neem cake, and vermicompost, etc. however, these are not accessible in such an adequate quantity that they can escalate food production. An alternative to this is exploiting organic waste usage and using it as integrated manure by coalescing with bio-fertilizers and chemicals. Singh and Kalloo (2000) have also recommended the use of bio-fertilizers in combination with chemical fertilizers and organic sources in an integrated nutrient approach.

The organic matter present in the soil is the chief source of energy and food for most of the soil organisms. The organic matter influences soil structure and texture besides having direct and indirect influence on the microbial population and activity. The organic matter is a store house of innumerable vital nutrients provides a congenial environment for the growth and multiplication of diverse microbial communities present in the soil. Hota et al. (2014) reported that fungal (AM) inoculation of *Colocasia* along with optimum doses of NPK and organic manure improved various microbial colonies in the soil; while conjoint application of FYM along with NPK and $MgSO_4$ fostered microbial counts in the soil. Conjoint application of mineral and organic fertilizers has been reported by several co-workers to boost yields in yam crop as compared to non-fertilized controls (Ennin et al. 2013; Susan John et al. 2016). The effects of mineral fertilizers, however, are unexpected. Hgaza et al. (2012) observed an increase in tuber yield of *Dioscorea alata* (yam) due to the addition of mineral NPK fertilizers to low fertile savanna soils. They concluded that NPK addition accelerated the mineralization rate of soil organic matter after observing a triggered increase in nitrogen uptake by crop from the soil without causing any change in root morphology and growth (Hgaza et al. 2011). However, the negative effects of NPK additions on the soils having very low organic matter contents have been reported so these results further need to be investigated.

Rana et al. (2020) have also stressed that the employment of proper irrigation and improved nutrient management practices increases the yield in potato cultivation.

McGarry et al. (1996) reported an increase in tuber rotting of potato crops during storage due to the addition of mineral inputs that negatively affected the organoleptic properties of tubers. Since the use of either organic or mineral has become obligatory to increase the production of yam, the need of the hour requires further analysis on the consequences of fertilizer application on tuber quality (Vernier et al. 2000). The nutrient utilization competencies of motile nutrients N and K can vary according to species. Though fertilizer application is acknowledged to enhance yields in crops, however, this practice has led to a reduction in the N and K nutrient use efficiencies of both these motile nutrients and hence reduced agronomic productivity. This emphasizes the need for future studies on fertilizer responses for these species. Further extensive fertilizer applications in different soils could cause losses of added nutrients, thus instigating fiscal losses owing to the high rates of fertilizers besides causing problems of water and soil contamination. Also, throughout 3–-6 months pre and postharvest losses of these crops are extreme that may range from 30 to 60%. The losses depend on the diverse species cultivated and the storing conditions (Proctor et al. 1981). The triggering factors leading to loss include (1) weight loss due to aridness, (2) loss of carbohydrate and water due to respiration, (3) sprouting on breakage of dormancy, (4) losses due to rodents and insects, and (5) losses due to fungal, bacterial, and viral diseases. Farmers can foster strategies to cope up with the reduction of soil fertility by selecting and cultivating less demanding cultivars, introducing rotations to benefit from the residual effect of fertilizers added to previous crops and lessen pests and diseases pressure, and the cultivation of tuber crops in sites where water, organic matter, and nutrients tend to accumulate such as lowlands.

The soil biological community is distinct. The soil offers an extremely diverse and heterogeneous habitat for microorganisms. A single ounce of soil is comprised of over 10,000 species of bacteria and thousands of species of fungi. Soil biodiversity is an important factor to maintain the activity of the soil biota in the complex soil habitat and for supporting the critical soil functions such as nutrient cycling. The microbes in any soil system are present in a state of dormancy, waiting for conditions that are favorable for their growth. Soil acts as a virtual desert for microbes outside the rhizosphere (microecological zone in direct proximity of plant roots). As a result, alternate periods of high and low activity of microbes are common. The microbes experience a boom in development and activity after offered with finite, high-quality organic matter in the form of root exudates, crop residues, organic amendments, or dying roots. Further, the microorganisms experience a bust due to starvation and breakage of cells apart. Much of the cellular material after breakage is recycled, but a larger part of the subsequent dead microbial biomass becomes associated with mineral surfaces via polysaccharides plus fungal hypha. In this association, further decomposition does not occur, and the building up of steady long-lived SOM starts. It is this SOM that besides acting as a nutrient reservoir for soil biology also helps to maintain good soil structure, water-holding capacity, and cation exchange capacity (Nin et al. 2015) of soil that in turn governs the soil fertility and supports plant

growth by providing water and nutrients. High-quality organic materials like legume cover crops, fresh cereals, poultry manures, or slurry manures having a nitrogen concentration above 2% and a C:N ratio <25 are the direct approaches to stimulate microbial activity and growth rates. Microbial biomass can be increased by around 36% in just a year or two with the addition of these organic materials (Kallenbach and Grandy 2011).

The physical and chemical properties of soil have a great influence on crop productivity and organic matter decomposition by soil microorganisms. Bio-fertilizers play a supplementary role in crop productivity and are not alternatives to chemical fertilizers. Application of bio-fertilizer aids in increasing soil microflora and fauna ultimately upsurging the rate of decomposition, crop productivity, and soil sustainability. During the decomposition of organic matter, organic acids are released that help to dissolve soil's available nutrients and make them available to be used by the crops. They supply nitrogen to certain crops under specific soil conditions. The primary types of bio-fertilizers used in India include symbiotic and non-symbiotic nitrogen-fixing cyanobacteria and phosphate-solubilizers. Another predominant nutrient is phosphorus that is required in the early stages of plant growth for optimum production. The indiscriminate use of phosphatic fertilizers has an adverse effect on the nutritive properties of crops besides posing a chronic threat to the soil health in the sustainability of crop production worldwide. The efficient use of phosphate solubilizing microorganisms is the holistic approach that opens up a new horizon in supplementing phosphorus to the plants resulting in higher plant productivity besides reducing the quantity of P-fertilizer application to the soil (Dipta et al. 2019). The development of such multifunctional bio-fertilizers with the potential to reduce almost 50–75% of chemical fertilizer application to augment and maintain soil fertility has become a significant concern. Dipta et al. (2017) recorded that the application of bio-fertilizers along with different P-sources (tricalcium phosphate, rock phosphate, and bone meal) improved soil nutrients, i.e. maximum available NPK over initial soil nutrient content. Table 4.1 depicts the efficacy of integrated nutrient management practices on growth parameters of root and tuber crops.

4.4 Biocontrol

The menaces of soil-borne disease epidemics in production yields, escalating prices of chemical fungicides, variations in climate, novel disease outbreaks, and evident rising distress due to environmental and soil health necessitates the use of integrated disease management approaches for sustainable crop production. Soil-borne diseases are problematic that distress the development, vigor, tuber quality, and even act as restraining factors on the harvestable produce of tubers. When the management of most of the diseases becomes tricky, the consequences lead to the use of soil fumigants, which are unfavorable for the growth of beneficial soil-borne organisms. Therefore, sustainable and biologically constructed disease management

Table 4.1 Effect of integrated nutrient management on growth parameters of root and tuber crops

Crop	Application	Growth parameters	References
Elephant foot yam	Pre-planting corm inoculated with *Azotobacter* culture	Maximum corm yield and highest cost benefit ratio of 1:3.37	Mukhopadhyay and Sen (1999)
Sweet potato	Inoculation of *Azotobacter* and AM alone and in combination with different levels of N and P	Maximum tuber yield, highest net return, and highest B/C ratio (2.69)	Pushpakumari and Geethakumari (1999)
Sweet potato	Application of 1/3rd (33 kg N ha^{-1}) of the 100 kg N ha^{-1} and three strains of *Azospirillum brasilense,* viz. spp. 7, UPMB 12, and UPMB 14	Higher root yield, vigorous vegetative growth, and higher N content in the roots and leaves	Saad et al. (1999)
Sweet potato	Urea coated with cow dung or inoculated with bio-fertilizer (*Azospirillum*)	Higher dry matter production of tubers	Nair et al. (2001)
Sweet potato	Application of 50% recommended doses of inorganic N (FN), organics like farm yard manure (FYM N), poultry manure (PLM N), pig manure (PGM N), and bio-fertilizer (*Azospirillum*)	Improved bulk density, organic carbon, and available NPK content of the soil	Nedunchezhiyan and Reddy (2004)
Elephant foot yam	Application of 75% RDF (inorganic) + 25% RDF (organic) along with *Trichoderma* (5 kg ha^{-1}) + *Pseudomonas* (5 kg ha^{-1})	Highest height of the shoot, pseudostem girth, and canopy spread	Sengupta et al. (2008)
Tubers	Cattle manure levels (0, 10, 20, 30, 40, and 50 t ha^{-1}) applied in a main plot, the sub plot assigned with bio-fertilizer concentrations (0, 15, 30, and 45%)	Bio-fertilizer concentrations provided the greatest productivities of total tubers	Ademar et al. (2010)
Sweet potato	Application of FYM, green leaf manure, vermicompost as organic manures and *Azotobacter*, phosphorus solubilizing bacteria, *Trichoderma* as bio-agents along with conventional production system	Improved soil microbial biomass and carbon content	Nedunchezhiyan et al. (2010)
Elephant foot yam	Application of 75% RDF (through inorganic source) + 25% RDF (through organic source) + arbuscular mycorrhizal fungi and	Maximum corm yield	Patel et al. (2010)

(continued)

Table 4.1 (continued)

Crop	Application	Growth parameters	References
	Azospirillum both at 5 kg ha^{-1}		
Elephant foot yam cv. Gajendra	100% RDF (through inorganic sources) applied along with *Azospirillum* and phosphorous solubilizing bacteria	Highest corm yield	Saravaiya et al. (2010)
Amorphophallus paeoniifolius (Dennst.) Nicolson cv. Gajendra	75% of RDF with inorganic sources and 25% organic manure (FYM) application along with AMF and *Azospirillum* (5 kg ha^{-1} each)	Highest corm yield	Murthy et al. (2011)
Sweet potato cv. Sree Bhadra	Application of FYM, poultry manure, vermicompost, mustard cake, *Azospirillum*, and phosphobacterium at different levels and combinations along with a recommended dose of manures and fertilizers	Maximum dry weight (26.33%), single tuber weight (214 g), number of tubers per plant (3.45), tuber yield per plant (450.16 g), and tuber yield per hectare	Rahul et al. (2011)
Greater yam	Application of 75% RDF (through IOS) + 25% RDN (through OS: FYM) + *Azotobacter* @ 5 kg ha^{-1} + PSB 5 kg ha^{-1}	Maximum tuber yield	Saravaiya et al. (2011)
Cassava	Application of Sunn hemp @ 50 kg ha^{-1} + RD K + 50% RD NP + *Azospirillum* @ 5 kg ha^{-1} + phosphorus solubilizing bacteria (PSB) @ 5 kg ha^{-1}	Highest tuber yield	Ashok et al. (2013)
Greater yam	Application of vermicompost @ 4.72 t ha^{-1} + castor cake @ 1.35 t $ha^{-1}$and bio-compost @ 5.07 t ha^{-1} + neem cake @ 0.51 t ha^{-1}	Higher tuber yield	Kaswala et al. (2013)
Elephant foot yam	Vermicompost @ 5 t ha^{-1}, ash @ 5 t $ha^{-1}$with *Azospirillum* and PSB each @ 5 kg ha^{-1}	Higher crop growth, corm yield, and increased organic carbon of soil	Kolambe et al. (2013)
Elephant foot yam	50% of the recommended dose of NPK applied along with bio-fertilizers (*Azospirillum* and phosphobacteria) and vermicompost	Maximum corm yield	Krishnakumar et al. (2013)

(continued)

Table 4.1 (continued)

Crop	Application	Growth parameters	References
Sweet potato	Vesicular arbuscular mycorrhiza (VAM) applied along with lime + FYM + NPK	Higher yield (10%) over FYM + NPK only	Laxminarayana (2013)
Cassava	3/4 RD of FYM+ NK + *Gliricidia* green leaf manure @ 25 t ha^{-1} applied alongwith 3% panchagavya	Highest tuber yield, the maximum net return, B:C ratio, and nutrient status of soil	Mhaskar et al. (2013)
Elephant foot yam var. Gajendra	75% RDF with inorganic source and 25% RDF with organic source applied along with arbuscular mycorrhizal fungi and *Azospirllum* both @ 5 kg ha^{-1}	Highest corm yield	Venkatesan et al. (2013a)
Elephant foot yam	Application of FYM @ 10 t ha^{-1} with *Azospirillum* and phosphobacteria both @ 5 kg ha^{-1} along with ash @ 5 t ha^{-1}	Highest values of all vegetative and yield traits i.e. plant height, pseudostem girth, canopy spread, and corm yield	Venkatesan et al. (2013b)
Elephant foot yam cv. Gajendra	75% recommended dose of NPK as an inorganic source applied along with 25% recommended dose of NPK through FYM in combination with *Azospirillum* and arbuscular mycorrhizal fungi both @ 5 kg ha^{-1}	Maximum corm yield, highest starch (14.09%), and highest protein content	Kumar et al. (2015)
Elephant foot yam	Bio-fertilizers application i.e. combination of AZ, PSB, and KMB each at 5 L ha^{-1}	Maximum plant height, canopy spread, and number of leaflets per plant	Navya et al. (2017)
Potato	50% recommended dose of N applied through inorganic fertilizer and remaining 50% recommended dose of N through organic manures (25% FYM plus 25% vermicompost)	Higher growth characters, tubers quality, and tuber yield	Taha et al. (2017)
Potato	Application of FYM @ 13.5 t ha^{-1} and blended NPS @ 245.1 kg ha^{-1}	Highest marketable and total tuber yields of 43.52 and 47.04 t ha^{-1}, respectively	Alemayehu et al. (2020)
Sweet potato	*Azotobacter* sp. IBCB 10 and *Azotobacter vinelandii* IBCB15 applied along with 50% doses of nitrogen fertilizer	Increased crop yield by 57% along with higher dry matter	Castellanosone et al. (2020)

strategies need to be developed in root and tuber production. The use of microorganisms and/or their metabolites to defend plants against pathogenic threats is known as biological control (Tomar et al. 2013). Various researchers have extensively studied the efficacy of biological control agents to combat pathogens in tuber/seed crops to reduce environmental pollution, ecological disturbance due to the addition of pesticides being used in fumigation and pre-sowing treatments (Lin et al. 2018). The suppression of diseases by potential microbial agents via different mechanisms takes place mainly in the rhizosphere (Van Loon 2007; Badri et al. 2009; Reinhold-Hurek and Hurek 2011). In 1904, Dr. Lorenz Hiltner introduced the concept of rhizosphere and described it as the soil compartment under the influence of roots (Smalla et al. 2006; Hartmann et al. 2008). He also proposed that non-pathogenic microorganisms colonize the vigorous roots.

Bio-agents refer to the naturally occurring living organisms found in the rhizosphere, phylloplane that aid not only in the management of the diseases but also increase the yield of the crop (Lal et al. 2016). Then there is rhizoplane directly outside the root matter that acts as an interface amid root and soil and always constitutes a plethora of microorganisms (Foster 1986). These microorganisms associated with plant roots can have a constructive, deleterious, or neutral effect on the plant (Raaijmakers et al. 2009). Nowadays, biological control methods epitomize a noteworthy complement to other methods of disease control that are based on genetic approaches and chemical treatments. This is a serious concern since certain diseases affect the parts underground that are normally beyond the influence of applied germicidal treatments. However, due to diverse soil biotic and abiotic factors, the ability of disease control management by these protecting agents generally depends upon the conditions of their colonization and sufficient biomass. Many published accounts have shown a lack of consistency on the reliability of these biocontrol practices (Compant et al. 2005; Latour et al. 2009). Thus, Abd-Elgawad and Askary (2020) have also suggested the need for characterization of various important aspects of innumerable organisms residing in the rhizosphere of plants to grasp the contribution of these microbes in the biocontrol process. Presently, biocontrol encompassing the use of microorganisms has gained extreme popularity and is being used to counteract innumerable fungal and bacterial diseases on the seed of potatoes, yams, aroids, and sugar beet (Lebot 2009). Potential bacteria with antifungal properties have become an interesting subject of study all over the world for potato pathologists and producers.

Biological control of potato late blight is an effective alternative to synthetic fungicides that have proven toxic to environmental health (Cao and Forrer 2001). In addition to combating late blight disease in potatoes, these microbes also produce certain plant growth regulators (phytohormones), improve phosphorus nutrition in plants, and fix atmospheric nitrogen (Zakharchenko et al. 2011). Since biocontrol agents colonize their plant host and increase their potential activity with time, they have the advantage over synthetic fertilizers application. Numerous microorganisms with significant levels of success rate have been tested for their potential to prevent major infection of late blight in potatoes. Research with *Bacillus cereus* showed that colonization of bacteria on the surface of potato increased to until 61 days even after

planting (Wharton et al. 2012). *Erwinia carotovora* colonizes the potato roots and tubers and is known to cause preemergence seed-piece decay, blackleg, soft stem rot, and soft rot of tubers. As the pathogen population exceeds 10^6 colony-forming units per gram of soil under favorable environmental conditions; at the same time, fluorescent pseudomonads increase their population via colonizing the rhizosphere and hence efficiently hinder the growth of pathogens (Azad et al. 1985). Sunaina et al. (1997) have also reported the ability of fluorescent pseudomonads which when applied to potato seeds reduced the population of *E. carotovora* in the subsequent roots by about 95–100% and in tubers by 27%–100% as compared to untreated plants. Table 4.2 describes the list of biocontrol agents that have been used to control various bacterial and fungal diseases of root and tuber crops.

4.4.1 Biocontrol Mechanisms

Bacteria follow the natural mechanisms of biocontrol to combat plant pathogens. The different strategies to suppress plant pathogens include competition for nutrients and/or space, antibiosis, siderophore production-mediated suppression of disease, parasitism, cell-wall lytic enzymes and induced systemic resistance of host plant (Sharma et al. 2009). An efficient biological control is generally achieved by the combination of more than one mechanism and in no case, a single mechanism has been found yet to be satisfactory. Living microorganisms or their metabolites act as efficient biocontrol agents against plant pathogens via the production of antibiotics and biosurfactants. These microbes possess the ability to deteriorate the viability of pathogens and hence prevent the development of disease (Daayf et al. 2003). The numerous possible mechanisms, operating in an interaction system, to suppress pathogen infection are shown in Fig. 4.1.

Production of low molecular weight antifungal compounds by several microorganisms to antagonize the pathogens directly has been reported. Inhibition of growth of fungal pathogen occurs via secretion of antifungal volatile compounds (VOCs) (Mari et al. 2012) that are a mixture of low molecular weight lipophilic compounds. Secretion of lipoproteins from *Bacillus subtilis* consisting of a lipophilic fatty acid chain and a hydrophilic peptide ring is an excellent example of biocontrol properties shown by these bacteria (Chen et al. 2008). Another example is of a bacterial metabolite named Serenade secreted by *Bacillus subtilis* strain. This metabolite stops the plant pathogen spores from germination through three groups of lipoproteins by disrupting the mycelia and germ tube growth of the pathogen. Further, these bacterial metabolites also prevent the pathogen to attach itself to the surface of the leaf (Stephan et al. 2005).

Biosurfactants produced extracellularly or as part of the cell membrane by several microorganisms are highly specific, naturally occurring, amphiphilic compounds that help to reduce surface and interfacial tension. Their ability to inhibit fungal pathogens and biodegradation make them suitable candidates as biocontrol agents for controlling major diseases of late blight (Tomar et al. 2013). There are certain other molecules that indirectly affect the pathogens before infection done by the

Table 4.2 List of biocontrol agents against bacterial and fungal diseases of tuber crops

Crop	Biocontrol agent	Disease	Target pathogens	References
Potato	Nonpathogenic *Ralstonia solanacearum*	Bacterial wilt/brown rot	*Ralstonia solanacearum*	Kempe and Sequiera (1983)
Sweet potato	Nonpathogenic *Fusarium oxysporum*	Fusarium wilt	*Fusarium oxysporum*	Ogawa and Komada (1985)
Potato	*Bacillus polymyxa*	Bacterial wilt/brown rot	*Ralstonia solanacearum*	Aspiras and de la Cruz (1986)
Cassava	Fluorescent pseudomonads	Root rot	*Erwinia carotovora*	Hernandez et al. (1988)
Potato	Fluorescent pseudomonads	Ring rot	*Clavibacter michiganensis* spp. *sepedonicus*	de la Cruz et al. (1992)
Potato	*Verticillium bigutattum*	Rhizoctonia black scurf and stem canker	*Rhizoctonia solani*	Van den Boogert and Velvis (1992)
Potato	*Pectobacterium* spp.	Blackleg and soft rot	*Dickeya* spp./ *Pectobacterium* spp.	Costa and Loper (1994)
Potato	*Talaromyces flavum*	Verticillium wilt	*Verticillium dahliae*	Nagtzaam and Bollen (1997)
Potato	Fluorescent pseudomonads	Blackleg and soft rot	*Dickeya* spp./ *Pectobacterium* spp.	Cronin et al. (1997), Kastelein et al. (1999)
Potato	*Enterobacter cloacae* and fluorescent pseudomonads	Fusarium dry rot	*Fusarium* spp., mainly *Fusarium roseum var. sambucinum* and *Fusarium oxysporum*	Schisler et al. (2000)
Potato	*Streptomyces* bacteriophage	Scab	*Streptomyces* spp. mainly *Streptomyces scabiei*	McKenna et al. (2001)
Potato	*Bacillus* sp.	Fusarium rot	*Fusarium roseum* var. *Sambucinum*	Sadfi et al. (2002)
Potato	Nonpathogenic *Streptomyces*	Scab	*Streptomyces* spp. mainly *Streptomyces scabiei*	Neeno-Eckwall et al. (2001), Hiltunen et al. (2009)
Potato	*Bacillus subtilis* B5	Late blight of potato	*Phytophthora infestans*	Ajay and Sunaina (2005)
Potato	*Bacillus subtilis* and *Trichoderma virens*	Stem canker	*Rhizoctonia solani*	Brewer and Larkin (2005)
Cassava and yam	*Trichoderma harzianum* and	Rot	*Botryodiplodia theobromae*, *Fusarium*	Manjula et al. (2005)

(continued)

Table 4.2 (continued)

Crop	Biocontrol agent	Disease	Target pathogens	References
	Penicillium oxalicum		*solani,* and *Sclerotium rolfsii*	
Yam	*Bacillus subtilis*	Rot	*Botryodiplodia theobromae*, *Aspergillus niger*, *Penicillium oxalicum,* and *Rhizoctonia* spp.	Okigbo (2005)
Sweet potato	*Penicillium* spp.	Rot	*Streptomyces ipomoeae, Ceratocystis fimbriata, Macrophomina phaseolina*	Ooshiro et al. (2007)
Potato	*Bacillus subtilis, Paenibacillus macerans,* and fluorescent pseudomonads	Bacterial wilt/brown rot	*Ralstonia solanacearum*	Naser et al. (2008)
Potato	*Pseudomonas putida*	Late blight/ mildew	*Phytophthora infestans*	Andreote et al. (2009)
Potato	*Enterobacter cloacae*	Fusarium dry rot	*Fusarium sambucinum*	Al-Mughrabi (2010)
Potato	*Pseudomonas koreensis*	Late blight/ mildew	*Phytophthora infestans*	Hultberg et al. (2010)
Water yam	*Trichoderma harzianum*, *Pseudomonas syringae,* and *Pseudomonas*	Rot	*Botryodiplodia theobromae* and *Fusarium solani*	Okigbo and Emeka (2010)
Potato	*Pseudomonas* spp.	Black scurf	*Rhizoctonia solani* Khun AG-3	Tariq et al. (2010)
Potato	*Trichoderma koningii* and *Bacillus megaterium*	Nematode infection and fusarium wilt	*Meloidogyne javanica* and *Meloidogyne incognita* of root-knot nematodes and the wilt fungus *Fusarium oxysporum*	El-Shennawy et al. (2012)
Potato	*Bacillus subtilis* and *Trichoderma viride*	Late blight of potato	*Phytophthora infestans*	Lal et al. (2014)
Yam	*Trichoderma harzianum*	Rot	*Penicillium purpurogenum*	Gwa and Abdulkadir (2017)
Yam	*Trichoderma harzianum*	Rot	*Colletotrichum* spp.	Gwa and Ekefan (2017)
Potato	*Bacillus amyloliquefaciens* Ba01	Potato scab	*Streptomyces scabies*	Lin et al. (2018)

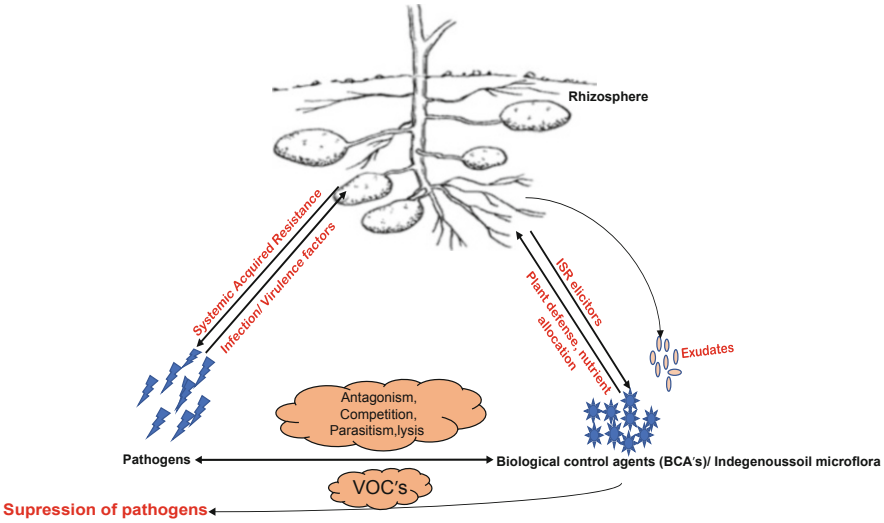

Fig. 4.1 An overview of a defense mechanism by biocontrol agents

pathogen through activation of the host plant's innate immune defense system (Cao and Forrer 2001). The major phenomenon of defense systems in host-pathogens include induced systemic resistance (ISR)/systemic acquired resistance (SAR). Heller and Gessler (1986) and Doke et al. (1987) were the pioneers to validate this event for protection against late blight in tomatoes and potatoes, respectively. In response to pathogenic stress, plants produce certain phytoalexins and/or other pathogenesis-related proteins as excellent defense mechanisms. A linear water-insoluble β-1,3-glucan is Curdlan produced via the fermentation of *Agrobacterium* sp. activates the plant's defense system prior to pathogen attack (Li et al. 2014). Direct antagonists/inducers are either beneficial/effective when the plant is already infected by a pathogen or they are defensive in case infection has occurred in the plant beforehand (Stephan et al. 2005).

4.4.1.1 Commensalism

It is a symbiotic interaction among two living entities, where one organism gets the benefit and the other one is neither harmed nor benefited. In respect to certain host plants, most of the associated microbes are assumed to be commensals as their presence either alone or in combination seldom explicits positive or negative consequences to the host plant. Their presence might extant multiple challenges to an infection causing pathogen. Whereas, an absence of a measurable decrease in pathogen infection or disease severity is an indicative of commensal interactions.

4.4.1.2 Protocooperation

The organisms involved in this type of mutualism do not depend solely on each other for their survival and in this case, disease suppression varies on the prevailing environmental conditions.

4.4.1.3 Competition for Nutrients and Space

Remarkable decrease in growth, activity and/or fecundity of the interacting organisms occurs at time when competition between pathogens and nonpathogens within and between species occurs. Competition for space includes the competition for infective sites and occurs when the specific recognition sites of host-pathogen are occupied by antagonists. When these places become unavailable for occupying by the pathogens, recognition, and hence infection fails to appear (Janisiewicz et al. 2000). For successful implementation of this phenomenon and hence biocontrol, the microbial antagonists must be able to survive under unfavorable conditions and grow more rapidly than the pathogen (Droby et al. 1992). Evaluation of microbial agents for wound competence under environmental conditions is another important character to commence them as commercial potential.

4.4.1.4 Siderophore Production

Furious competition is fomented when a shortage of bioavailable iron in soil environments and on plant surfaces occurs (Loper and Henkels 1997). Plant growth-promoting bacteria produce certain low molecular weight compounds known as siderophores (Das et al. 2007). These compounds competitively acquire ferric ions under iron-limiting conditions (Whipps 2001). Siderophores are chelating compounds that form a tight and stable complex by binding with ferric ions and transport it into the cell (Saraf et al. 2014). Certain species of bacteria, fungi, and plants are known to produce siderophores as an efficient strategy to overcome iron deficiency (Shanmugaiah et al. 2015). Bhardwaj et al. (2017) also reported isolates from rhizosphere of cauliflower to show antagonism against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Pythium* spp. known to cause root rot, stem rot, and damping off diseases, respectively, in the concerned crop.

Several plant growth-promoting strains are recognized to allure iron from heterologous siderophores produced by cohabiting microorganisms (Whipps 2001; Lodewyckx et al. 2002). The fundamental of diverse bacterial siderophores is to deprive pathogenic fungi of this essential element with distinct capacities to sequester iron, (O'Sullivan and O'Gara 1992; Loper and Henkels 1999). Bhardwaj et al. (2018) reported maximum siderophore production by an isolate SB_{11}, followed by the reference strain (MK_5). Further, based on multiple plant growth-promoting activities, the application of PGPR isolates SB_{11} and the reference isolate (MK_5), with 75% recommended doses of NP fertilizers, increased the number of non-wrapper leaves, curd diameter, gross weight of curd, and net curd weight as compared to control at all the three locations.

4.4.1.5 Parasitism

A symbiosis where two phylogenetically unrelated organisms coexist over a prolonged period is referred to as parasitism. This type of symbiosis involves one organism, being physically smaller of the two (parasite) that gains benefit from the other (host) being harmed to certain considerable extent. The activities of hyperparasites and avirulent pathogens result in the achievement of biocontrol via stimulation of host defense systems.

4.4.1.6 Predation

A biological communication encompassing the hunting and killing of one organism by another for consumption and sustenance is known as predation. The animals that feed at higher trophic levels in the macroscopic world are typically referred to as predators but this term has also been applied to the actions of microbes such as protists, and mesofauna, e.g. fungal feeding nematodes and microarthropods, that devour pathogen biomass for their sustenance.

4.4.1.7 Production of Cell-Wall Lytic Enzymes

Several microbial antagonists are known to produce lytic enzymes such as glucanase, proteinases, and chitinases that degrade the cell-wall of pathogenic fungi (Lorito et al. 1993; Castoria et al. 2001; Chernin and Chet 2002). Swain and Ray (2008) studied the interaction between *Fusarium oxysporum*, the postharvest rotting pathogens of yam (*Dioscorea* spp.) tubers and *Bacillus subtilis* isolated from cow dung microflora via scanning electron microscopy and reported the lysis of fungus cell wall by *B. subtilis* owing to the production of extracellular chitinase.

4.4.1.8 Antibiosis/Allelochemicals

Antibiotics are those microbial toxins that must be produced at low and sufficient concentrations (doses) near the pathogen to poison or kill other microorganisms. Allelochemical substances have been reported to be produced by biological control agents (BCA) (He et al. 2006) and the inhibition of plant pathogens by antibiotics or chemicals produced by BCA is an allelopathic process. The complete knowledge of this process is mandatory so as to guarantee high crop yield in any ecosystem (Dania et al. 2015). Vey et al. (2001) reported *Trichoderma* strains effectively inhibit the growth of plant pathogens via the production of volatile and non-volatile toxic metabolites such as alamethacin, harzianic acid, viridian, tricholin, and peptaibols. *Bacillus subtilis* has also been reported to produce allelochemicals such as bacillomycin, surfactin (Tsuge et al. 1995), subtilin, bacitracin, and subtenolin (Manjula et al. 2005). In another study, *Pseudomonas aeruginosa* PNA1 was shown to strongly reduce the root rot disease tissue culture-derived cocoyam plantlets (Tambong and Hofte 2001). The biocontrol formulations of antagonistic microbes can achieve successful commercialization depending upon the determined and consistent balance of target disease control. Some of the antagonistic microbe-based biocontrol product formulations available in the market are given in Table 4.3 (Dukare et al. 2018).

Table 4.3 Microbe-based commercialized biocontrol products for control of spoilage/diseases in potato

Biocontrol product	Active antagonists	Producing firm	Spoilage pathogens
Biosave	*Pseudomonas syringae* (bacterial based)	Jet harvest solutions USA	*Penicillium, Botrytis,* and *Mucor*
Shemer	*Metschnikowia fructicola* (yeast based)	Bayer/Koppert, the Netherlands	*Botrytis, Penicillium, Rhizopus,* and *Aspergillus*

4.5 Conclusion

As the root and tuber crops are formed inside the soil thus the preservation of preeminent soil physical conditions is a crucial step. In the course of time, as the developments foster in the INM strategy, much focus will rest on the exploration of sustainability of the currently developed low input management practices in the long run for various root and tuber crops with the intention of creating awareness among the farmers for sustainable crop production through nutrient management strategies. Despite our understanding of the biological control mechanisms via which the antagonists offer disease resistance to root and tuber crops, the elementary information on the ecology of microbes and survival mechanisms of biocontrol agents on crop surfaces is less recognized and its understanding is necessary for the successful implementation of biocontrol technology. Further, a better apprehension of the intensity of infection levels occurring in the field and the mode of action of these biocontrol agents are critical factors that need to be addressed. Commercial-scale formulation of microbial antagonists can also tackle to foster a robust ecosystem and could gain impetus eventually. All these approaches as a constituent of an integrated nutrient and disease management represent a great potential for wider public acceptance in the near future.

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Microbial Biopesticides Use in Insect-Pest Management: An Overview

5

Preeti Sharma and Neeta Gaur

Abstract

Insect, pathogens, weeds, and invertebrates as pests cause significant crop losses worldwide and act as a barrier in achieving the aim of global food security and reduction in poverty, as in terms of food security the annual crop losses due to pests correspond to a huge amount of food supply which can otherwise feed millions of people. The use of synthetic pesticides for crop protection plays a major role in insect-pest management but simultaneously poses various challenges; hence, for sustainable agriculture, we need to chalk out alternative methodologies to meet the need of crop protection. Integrated Pest Management (IPM) helps in Sustainable Intensification by producing more output from the same area of land while reducing the negative environmental impacts and at the same time increasing contributions to natural capital and the flow of environmental services by using various methods and techniques. Among which, one of the major methods is Microbial Control, in which pathogens are exploited for biological control of insect pests through introductory or inundate applications. Microbial pathogens of insects are intensively investigated to develop environment friendly pest management strategies in agriculture. Entomopathogenic viruses, bacteria, and fungi, as biopesticides are currently used as an alternative to traditional insecticides which overcome the harmful effect of the chemicals on non-target organism. This chapter reviews the insecticidal properties of microbes, their potential utility, recent advancement, and case studies in insect-pest management.

Keywords

Microbes · Integrated pest management · Sustainable agriculture · Biopesticide

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5.1 Introduction

Over the past few decades besides providing for the livelihood of farmers and laborer, the agricultural sector also addresses food security for the nation. Due to various factors the consumption pattern of food in the country has been changing and food security demand has been raised. According to FAO, food security is a situation where all people have, at all times, physical and economic access to sufficient, safe, and nutritious food that meets the dietary needs and food preferences for a healthy and active life (Thakore 2006). As per 2014 estimates, despite high levels of agricultural production in India, 15% of the population continues to be undernourished (Kristiofferesen et al. 2008). To feed the ever-growing global population, we need to produce more food. India enacted the National Food Security Act in 2013, the main aim of this act is to provide food and nutritional security to people by ensuring access to adequate amount of quality food from less per capita arable land and available water (Roh et al. 2007). As of 2015, 68% of the population of India, i.e. 81 crore persons (of which 77% are in rural areas and 23% in urban areas) are covered under the Act. But the crop damage by pests including insects, fungi, weeds, viruses, nematodes, animals, and birds has a serious impact on farming and agricultural practices for a long time which leads to reduced agricultural production. In India alone, 30% of the crop yield potential is lost as a result of insect pests, diseases, and weeds, corresponding to 30 million tons of food grain (Vendan 2016). It has been estimated that about 67,000 pest species damage agricultural crops. Therefore, significant efforts are required for pest management to protect the crops (Kumar and Singh 2014). Although chemical pesticides use in the intensive agriculture to control pests, have certainly contributed towards improving agricultural production, in terms of both yield and quality. But this pest management strategy adversely affects the whole environment including beneficial organisms as well as leaves harmful residues in food, feed, and fodder and also causes environmental pollutions. Extensive use of synthetic pesticides for insect-pests management in high yielding varieties provides protection to crops; but it also led to the various problems such as pesticide residues in food and environment, subsequent impact on the food chain, groundwater contamination, and pest resistance (Khachatourians 2009; Damalas 2009). To overcome the hazards associated with chemical pesticides, the use of biopesticides (pesticides derived from such natural materials as animals, plants, microorganisms and certain minerals) came into existence (Frampton et al. 2012; Mishra et al. 2015). In this regard, the conventional pesticide industry and market have undergone major changes over recent decades (Carvalho 2017), which have entailed greater efficiency of pesticide use than in the past through major improvements to pest management technology and practices in the context of Integrated Pest Management (IPM) programs. Over the past 150 years, a great deal of knowledge has been gathered on the use of microbial biopesticides including bacterial, fungal, viral, protozoan, or nematode-based preparations as pest control agents (Kabaluk and Gazdik 2005; Koul and Cuperus 2007; Kaushal 2018). Microbial control agents can be an alternative to chemical pesticides when used as part of an ecologically based integrated pest management (EBIPM) or area-wide pest

management strategy (AWPM) (Kabaluk and Gazdik 2007; Pelaez and Mizukawa 2017). These days microbial biopesticides are gaining more interest due to many reasons, including the problem associated with conventional synthetic pesticides as resistance, residual problem, and toxicity to environment. In comparison to conventional insecticides they are host specific and safe to the environment but improvement in the production and upgradation in formulation technology of microbial biopesticides is needed for large level adoption (Koul and Cuperus 2007; Koul et al. 2008 and Gautam et al. 2018). In this view, this chapter reviews microbial biopesticides forms, utility, their production and development, opportunities and challenges associated with them.

5.2 Status of Biopesticide

5.2.1 Indian Status

In India commercial production of biocontrol agents was started by Biocontrol Research Laboratories (BCRL), a division of Pest Control (India) Limited, under contract with Plant Protection Research Institute (PPRI). The rise of biopesticides in India is being encouraged by the government as part of the integrated pest management (IPM) program. In the last few years, microbes exhibiting good biocontrol potential which have been discovered by many workers and researchers (Rabindra 2001; Ignacimuthu et al. 2001; Koul et al. 2003; Ranga Rao et al. 2007). Most of the microbial products belong to the group antagonistic fungi (especially *Trichoderma* spp.), bacteria (especially Bt and *P. fluorescens*), and viruses consist of NPV and granuloviruses (GV) and contribute major part for biopesticides market (Kunimi 2007; Kabaluk and Svircev 2010). At present, organic pesticides contribute 4.2% of the entire pesticide market in India and also CAGR is likely to increase by 20.2% from 2010 to 2020. In India, organic pesticides have been estimated to have market value of about 23.92 million USD (Arora 2015) and the market is consistently growing. In India, different types of bioinsecticides are registered under Insecticides Act, 1968 (Table 5.1) and there are about 150 biopesticide producing companies in India (Rabindra 2005). State of Maharashtra is the biggest consumer of biopesticides in India and *Trichoderma viride, Pseudomonas fluorescens*, and *Bacillus thuringiensis* are the best-selling biopesticides in India. India's Department of Biotechnology provides cooperation in research and production of biopesticide; ICAR and DBT support 31 and 22 producer units, respectively. Biocontrol labs have been set up in different states of the country to promote biopesticides (Gautam et al. 2018).

5.2.2 Global Status

Crop pests cause about 40% reduction in the world's crop yield, for the management of these pests 5.6 billion pounds of pesticide are used worldwide and among which most of the chemical pesticides are responsible for the unbalancing of our environment (Alavanja 2009). Whereas biopesticides application showed lesser or no

Table 5.1 Microbial pesticides registered in India as of July 2018

S. no	Microbial biopesticides
Bacteria	
1	*Bacillus thuringiensis* var. israelensis
2	*Bacillus thuringiensis* var. kurstaki
3	*Pseudomonas fluorescens*
4	*Bacillus subtilis*
	Bacillus sphaericus
Fungi	
5	*Ampelomyces quisqualis* Ces.
6	*Beauveria bassiana*
7	*Metarhizium anisopliae*
8	*Paecilomyces lilacinus*
9	*Trichoderma harzianum*
10	*Trichoderma viride*
11	*Verticillium chlamydosporium* Godd.
12	*Verticillium lecanii*
13	*Paecilomyces lilacinus*
14	*Hirsutella thompsonii*
Virus	
15	NPV of *Helicoverpa armigera*
16	NPV of *Spodoptera litura*
	NPV of *Pseudomonas fluorescens*

Source: Central Insecticide Board (2018).

toxicity to crops and environment. Application and development trend of biopesticides has been reviewed by Leng et al. (2011). But they are not found dominating globally as synthetic pesticides do. Worldwide, approximately 1400 biopesticide products are being sold (Marrone 2007) as liquid concentrates, wettable powders, and ready-to-use dusts and granules. Among them, bacterial products are more frequently used especially those from Bt-based products (Lisansky 1997). Currently it is the main bacterium being used in agricultural pest control (Ali et al. 2008). According to the facts, in biopesticide industry 60% of the world biopesticide market (Fig. 5.1) are occupied by about 200 Bt-based products (Kabaluk and Svireev 2010; CABI 2010; Rabindra 2005), and almost 50% of this are consumed by USA and Canada in America (Guerra et al. 2001). According to recent study during 2012–2018 during, the largest market shares in biopesticide belonged to Latin America (includes Mexico) with 27.9%, followed by Asia with 18.6%, Europe (18.1%), North America (USA and Canada) 18%, and 18.3% for rest of the world (Damico 2017) (Fig. 5.2).

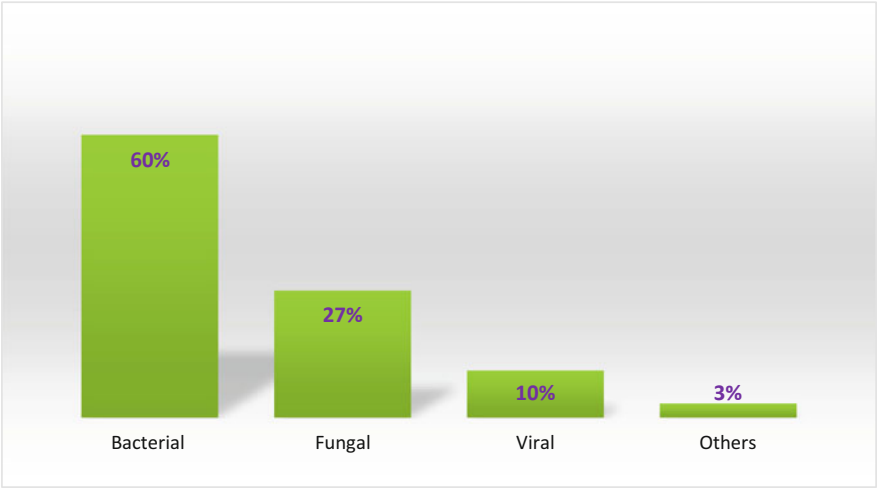

Fig. 5.1 Global biopesticide market based on types of microbes used (Source: Kabaluk 2010)

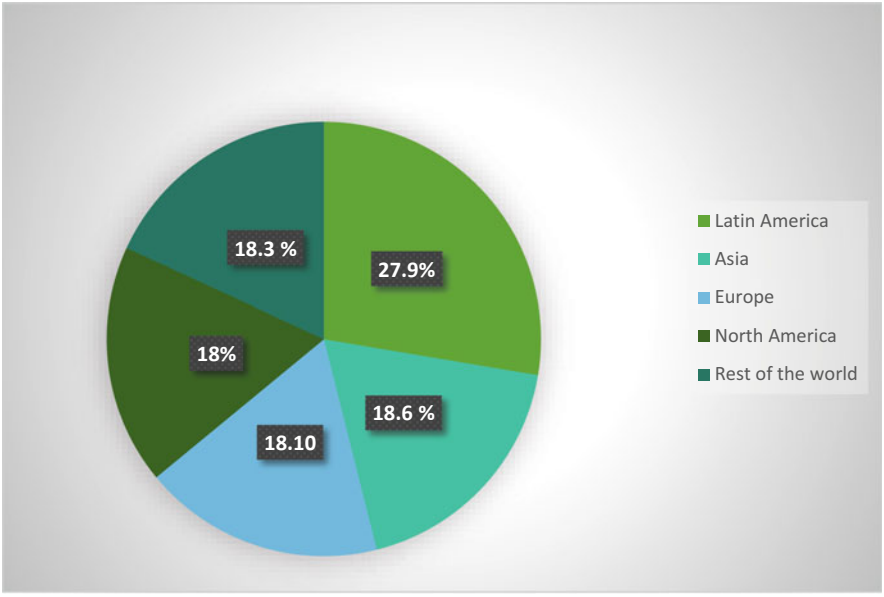

Fig. 5.2 Global market shares in biopesticides (Source: Damico 2017)

5.3 Biopesticide

As defined by USEPA, biopesticides are pesticides derived from natural materials such as animals, plants, bacteria, minerals and also include living organisms that destroy agricultural pests. The EPA separates biopesticides into three major classes based on the type of active ingredient used as biochemical, plant-incorporated protectants, and microbial pesticides (USEPA 2008). At a global level, there is an inconsistency in understanding the term biopesticide which was defined by USEPA and that is why International Biocontrol Manufacturer's Association (IBMA) and the International Organization for Biological Control (IOBC 2008) promote to use the term biocontrol agents (BCAs) instead of biopesticide (Guillon 2003). IBMA classifies biocontrol agents into four groups: (1) macrobials, (2) microbials, (3) natural products, and (4) semio-chemicals (insect behavior-modifying agents). Biochemical pesticides are chemicals either extracted from natural sources or synthesized to have the same structure and function as the naturally occurring chemicals. Biochemical pesticides are distinguished from conventional pesticides by their structure and mode of action (O'Brien et al. 2009). The most important biocontrol agents are microbials (41%) followed by macrobials (33%) and finally other natural products (26%) (Guillon 2003). Biopesticides are reaching importance all over the world nowadays because of their nontoxic eco-friendly mode of actions that are helpful for the management of various insect pests (Mazid and Kalita 2011). In general, biopesticides can be classified into three major categories (Fig. 5.3): (1) plant-incorporated protectants, (2) microbial pesticides, and (3) biochemical pesticides. When they are used for the management of insect pests, the efficacy of biopesticides can be equal to that of conventional pesticides, particularly for crops like fruits, vegetables, nuts, and flowers and they also do not cause any residue problem (Koul 2011). By combining synthetic pesticide performance and environmental safety, biopesticides execute efficaciously with the tractability of minimum application limitations and with superior resistance management potential (Kumar 2012; Senthil-Nathan 2013). Biopesticides are gaining attention and interest among those concerned people who are developing environmentally friendly and safe integrated crop management (ICM) compatible approaches and tactics for pest management (Pandey et al. 2010). In particular, farmers' adoption of biopesticides may follow the recent trend of "organically produced food" and the more effective introduction of "biologically based products" with a wide spectrum of biological activities against key target organisms, as well as the developing recognition that these agents can be utilized to replace synthetic chemical pesticides (Copping and Menn 2000; Chandrasekaran et al. 2012; Senthil-Nathan 2013).

5.3.1 Microbial Biopesticides

Microbial pesticides act as a natural biopesticide in crop protection extend a unique chance to research in developing countries. The utilization of biopesticide programs would be required to prevent insect-pest resistance to synthetic chemical pesticides

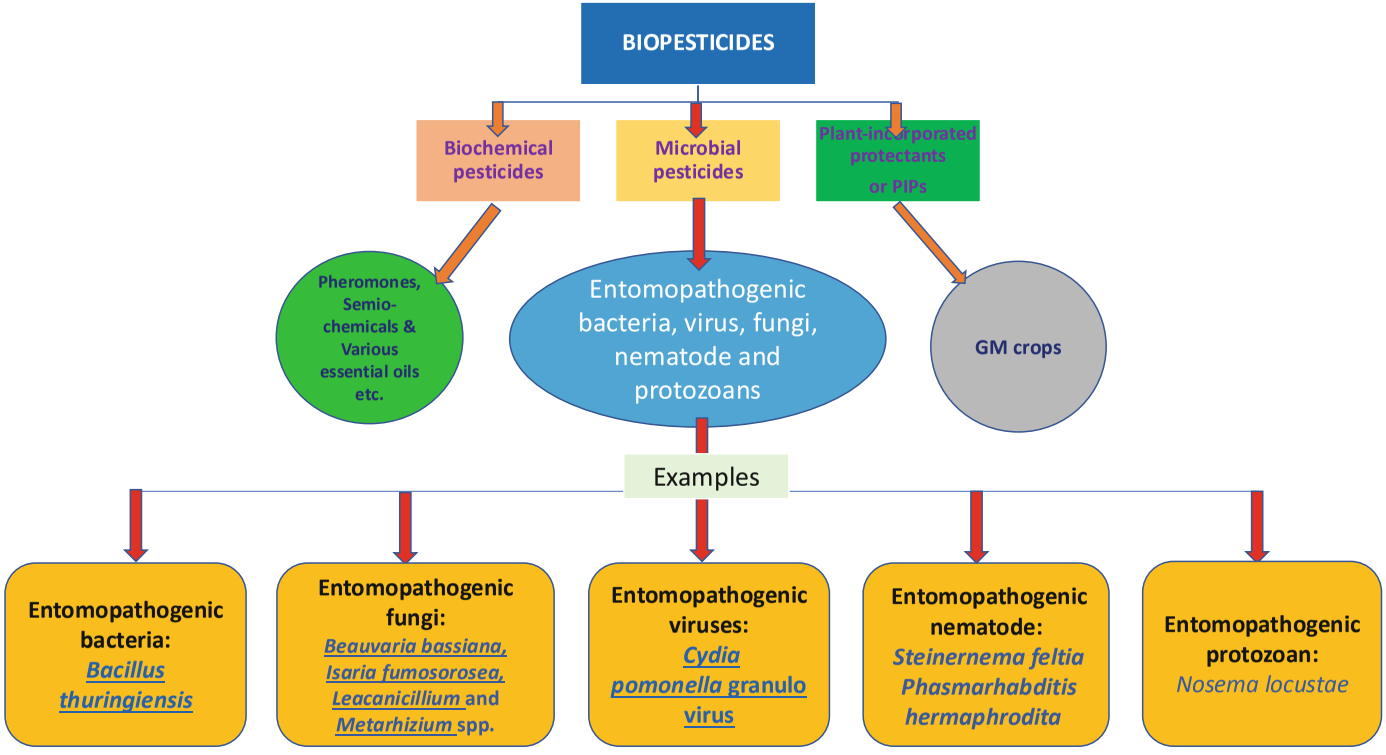

Fig. 5.3 Classification of biopesticides

(Copping and Menn 2000; Senthil-Nathan 2006; Senthil-Nathan et al. 2006, 2009). Microbial pesticides are a form of biological control agents, which contain microorganism (bacterium, fungus, virus, protozoan or alga, rickettsia, mycoplasma, and nematodes) as the active ingredient (MacGregor 2006). They offer the advantages of higher selectivity and less toxicity in comparison to conventional chemical pesticides (Khachatourians 2009). They produce toxic metabolites specific to the pest and also prevent establishment of other microorganisms through competition or can suppress the pest through various other modes of action (Dowds and Peters 2002; Harris 2009). The most commonly used microbial biopesticides are biofungicides (*Trichoderma*, *Pseudomonas*, *Bacillus*), bioherbicides (*Phytophthora*), and bioinsecticides (*Bt*) (Harris 2009). Microbial pesticides come from naturally occurring or genetically altered bacteria, fungi, algae, viruses, or protozoans (Gupta and Dikshit 2010; Clemson 2007). In biopesticide market, bacterial biopesticides claim about 74%; fungal biopesticides about 10%; viral biopesticides, 5%; predator biopesticides, 8%; and "other" biopesticides, 3% (Thakore 2006). By 2008, there were approximately 73 microbial active ingredients that were registered by the USEPA. The registered microbial biopesticides included 35 bacterial products, 15 fungi, 6 nonviable (genetically engineered) microbial pesticides, 8 plant-incorporated protectants, 1 protozoan, 1 yeast, and 6 viruses (Steinwand 2008). Microbial biopesticides may be delivered to crops in many forms as live or dead organisms, and spores or in the form of microbe-based pesticides that are being used presently (CPL 2010; Koul 2011). Various forms of biopesticides like *Bacillus thuringiensis* (Bt), a large array of fungi, viruses, protozoa, and some beneficial nematodes have been formulated for insect-pest management of various insect pests and for greenhouse, turf, field crop, orchard, and garden use (Butt et al. 2001a, b; Grewal et al. 2005; EPA 2006).

5.3.1.1 Bacteria

Bacterial biopesticides are the most common form of microbial biopesticides and also can be used to control the growth of plant pathogenic bacteria and fungi. Bacteria are prokaryotic, unicellular organism. Most of the insect pathogenic bacteria belong to the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, and Micrococcaceae. Among them member of family Bacillaceae has received maximum attention as microbial control agents (Koul and Cuperus 2007). Various bacterial species and subspecies, especially *Bacillus*, *Pseudomonas*, etc., have been established as biopesticides and are primarily used to control insect pests and plant diseases (Koul 2011). Several subspecies of *Bacillus thuringiensis* Berliner as *B. thuringiensis* ssp. *kurstaki* and *aizawai* found effective against insect pests, in which the highest activity found against lepidopteran larval species; *B. thuringiensis israelensis* and other insect pests as mosquito larvae, black fly (simuliid), fungus gnats; *B. thuringiensis tenebrionis*, coleopteran adults and larvae; Colorado potato beetle (*Leptinotarsa decemlineata*); and *B. thuringiensis japonensis* strain Buibui, against soil-inhabiting beetles (Copping and Menn 2000; Senthil-Nathan 2015). They are generally species specific for different insect orders. Its principal characteristic is the synthesis of crystalline inclusions containing proteins

known as δ endotoxins or Cry proteins, which have insecticidal properties (Aronson and Shai 2001). They start to act against pest when come into contact. These toxins ingested by the larvae lead to gut paralysis after that, the infected larvae stop feeding and finally they die from the combined effects of starvation and midgut epithelium impairment (Betz et al. 2000; Darboux et al. 2001). Due to their high specificity and environmental safety *B. thuringiensis* and Cry proteins are efficient, safe, and sustainable alternatives to chemical pesticides for the control of insect pests (Thakore 2006). Use of microbial pesticide showed significant decrease of synthetic chemical insecticide usage in studies (James 2009).

5.3.1.2 Fungi

Entomopathogenic fungi act as a major biological control agents for insect-pest populations (Charles et al. 1996). Insect pathogenic fungal species are found from different classes with a wide range of adaptations (Park et al. 2009; Khandelwal et al. 2012). The mode of action is varied and depends on both the pesticidal fungus and the target pest. One advantage of fungal biopesticides in comparison with other microbial biopesticides is that they do not need to be eaten to be effective. Infection starts when spores come in contact with integument surface, where the formation of the germinative tube initiates, the fungi starting to excrete enzymes which degrade the insect's cuticle and help in the process of penetration by mechanical pressure (Koul 2011). Once fungi enter inside the insect, it develops as hyphal bodies that disseminate through the hemocoel and invade diverse muscle tissues, fat bodies, Malpighian tubules, mitochondria, and hemocytes, leading to death of the insect within 3–14 days after infection. Once the insect dies and many of the nutrients are exhausted, fungi start micelles growth and invade all the organs of the host. Finally, hyphae penetrate the cuticle from the interior of the insect and emerge at the surface, where they initiate spore formation under appropriate environmental conditions (Park et al. 2009; Koul 2011). However, they are living organisms that often require a narrow range of conditions including moist soil and cool temperatures to proliferate. The speed with which death occurs is determined in part by the environmental conditions. Under optimal conditions, target pests may be killed in 3–7 days but when conditions are not ideal death may be caused in 3–4 weeks (Berry et al. 1991; Senthil-Nathan 2015). The main route of entrance of the entomopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trachea (Lasa et al. 2007).

Metarhizium anisopliae Sorokin var. *anisopliae* has the potential to be used as a biocontrol agent. It propagates worldwide in the soil, demonstrating a wide range of insect host species. This species comprises a huge number of different strains and isolates of various geographical origins (Roberts and St Leger 2004). In moist soil *Metarhizium* develop filamentous growth and infectious spores, called conidia, which infect soil-dwelling insects upon contact (Mnyone et al. 2010; Koul 2011). These entomopathogenic fungi have been registered as microbial agents and are also under commercial development for the biological control of several pests (Butt et al. 2001a, b).

Fungal biopesticides include *Trichoderma harzianum*, which is an antagonist of *Rhizoctonia*, *Pythium*, *Fusarium*, and other soil-borne pathogens *Trichoderma* are acclaimed as an effective, eco-friendly, and cheap, nullifying the ill effects of chemicals, and very effective for the management of various foliar- and soil-borne plant pathogens like *Ceratobasidium*, *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Sclerotium*, *Pythium*, and *Phytophthora* spp. (Bailey and Gilligan 2004; Harman 2005; Corato 2020; Rosskopf et al. 2020). *Trichoderma* is a fungal antagonist that grows into the main tissue of a disease-causing fungus and secretes enzymes that degrade the cell walls of the other fungus and then consumes the contents of the cells of the target fungus and multiplies its own spores (Dominguesa et al. 2000; Anand and Reddy 2009). *B. bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin are naturally occurring entomopathogenic fungi that infect sucking pests which include *Nezara viridula* (L) and *Creontiades* sp. (Khandelwal et al. 2012).

5.3.1.3 Virus

Viral biopesticides play a significant role in antagonizing pathogens especially bacteria in the form of bacteriophages. These viruses are widely used for the control of various insect pests of vegetable and field crops globally, and also effective against plant-chewing insects. For the order Lepidoptera it is found much effective against gypsy moths, pine sawflies, Douglas fir tussock moths, and pine caterpillars. Codling moth is controlled by *Cydia pomonella* GVs on fruit trees (Lacey et al. 2008) and potato tuberworm by *Phthorimaea operculella* GVs in stored tubers (Arthurs et al. 2008). Virus-based products are also available for cabbage moths, corn earworms, cotton leafworms and bollworms, beet armyworms, celery loopers, and tobacco budworms (Cory and Myers 2003; England et al. 2004; Raymond et al. 2005; Hewson et al. 2011). Viruses have been isolated from more than 1000 species of infected insects from at least 13 different insect orders (Roh et al. 2007). Entomogenous viruses fall into two categories, viz. inclusion viruses (IV) producing inclusion bodies in the host cells and non-inclusion viruses (NIV) which do not produce inclusion bodies. The IV are further sub divided into polyhedron viruses (PV) or polyhedroses, which produce polyhedral bodies and granulosis virus which produce granular bodies. Polyhedroses could inhabit the nucleus and are called nuclear polyhedrosis viruses (NPV) or the cytoplasm which are called cytoplasmic polyhedrosis virus (CPV) (Crickmore 2005; Koul 2011). Most of the insect-infecting viruses have been isolated from Lepidoptera (560) followed by Hymenoptera (100), Coleoptera, Diptera, and Orthoptera (40) (Khachatourians 2009; Senthil-Nathan 2015). Some of them have been commercialized for use as biopesticides. The viruses used for insect control are the DNA-containing baculoviruses (BVs), Nucleopolyhedrosis viruses (NPVs), granuloviruses (GVs), acoviruses, iridoviruses, parvoviruses, polydna-viruses, and poxviruses and the RNA-containing reo-viruses, cytoplasmic polyhedrosis viruses, nodaviruses, picrona-like viruses, and tetraviruses. However, the main categories used in pest management have been NPVs and GVs. Among the insect viruses found in nature, those belonging to the baculovirus family (Baculoviridae) were considered for the

development of most commercial viral biopesticides (Bravo et al. 2007; DeMaagd et al. 2001). Baculoviruses are found as safe for vertebrates and to date, no negative impacts on plants, mammals, birds, fish, or non-target insects have been reported (Whalon and Wingerd 2003). Major advantages of Baculoviruses are that they can cause sudden and severe outbreaks for complete control of the pest and they can replace and serve as an alternative to the chemical pesticides (O'Brien et al. 2009; Koul 2011). The mechanism of viral pathogenesis is through replication of the virus in the nuclei or in the cytoplasm of target cells. When Baculovirus ingested by the larvae it initiates infection. After ingestion, they enter the insect's body through the midgut and from there they spread throughout the body. Once in the larval gut, the virus's protein overcoat quickly disintegrates, and the viral DNA proceeds to infect digestive cells. Within a few days, the host larvae are unable to digest food, so weaken and die (Thakore 2006). As of 2010, over 24 baculovirus species have been reported to be registered for use in insect-pest management throughout the world (Kabaluk and Svircev 2010; Moscardi et al. 2011).

5.3.1.4 Nematodes

Another group of microbial biopesticides is the entomopathogenic nematodes, which control weevils, gnats, white grubs, and various species of the Sesiidae family (Grewal et al. 2005). Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. They are useful for the suppression of soil-borne pests and stem borers. It can kill them within 48 h through the expulsion of pathogenic bacteria (Copping and Menn 2000). Nowadays many Nematodes under two families Heterorhabditidae and Steinernematidae have been effectively used as biological insecticides in pest management programs. Insect juveniles (IJs) are free-living organisms, which can enter into the host body through mouth, anus, spiracles, or cuticle, after entering into the host body it releases their bacterial symbionts in to the hemocoel of hosts, killing the host within 24–48 h (Dowds and Peters 2002; Koul 2011). The nematodes can complete up to three generations within the host, the parasitic cycle is initiated by the third-stage IJs and after completion of life cycle IJs leave the cadaver to find the new hosts. Entomopathogenic nematodes found effective against insect-pest families found in stored goods like Pyralidae (Shannag and Capinera 2000) and Curculionidae (Shapiro and McCoy 2000). A field concentration of >2.5 billion nematodes/ha against some of the major insect pests of row crops, but concentrations few times higher (7–15 billion/ha) are demanded to accomplish the control of pest population (Loya and Hower Jr 2002). Artificial selection is useful in increasing entomopathogenic infectivity and nematicide resistance. The recent discovery that maize roots damaged by the western corn rootworm emit a key attractant for insect-killing nematodes has opened the way to explore whether a selection strategy can improve the control of root pests (Hiltpold et al. 2010). Entomopathogenic nematodes (EPN) can be mass-produced in vivo and in vitro through solid media or liquid fermentation. However, there is need of extensive studies and research to optimize application parameters and develop efficient strains to achieve significant control of pests through nematodes (Senthil-Nathan 2015). Entomopathogenic

nematodes are considered nontoxic to humans, relatively specific to their target pests, and can be applied with the help of standard pesticide equipment (Shapiro-Ilan et al. 2006).

5.3.1.5 Protozoans

Entomopathogenic protozoans, commonly referred as microsporidians, infect and also induce chronic and debilitating effects on a wide range of insect pests and reduce the target pest populations (Grewal et al. 2005). Protozoa are taxonomically subdivided into several phyla, some of which contain entomogenous species. Almost 1000 protozoan species, mainly microsporidia, attack invertebrates, including numerous insect species like grasshoppers and *Heliothis* moths. Microsporidia, such as *Nosema* spp., are generally host specific and slow acting, most frequently producing chronic infections. *N. bombycis*, the first reported microsporidium for silkworm pebrine disease, which persisted in Europe, North America, and Asia during the mid-nineteenth century. Pébrine is still an epidemic disease and cause heavy economic losses in silk-producing countries such as China (Cai et al. 2012; Koul 2011). Insect pathogenic protozoan species are *Nosema* spp. and *Vairimorpha necatrix*. The biological activities of most entomopathogenic protozoa are complex. Microsporidia species are among the most commonly observed, and their main benefits are persistence, recycling in host populations and their debilitating effect on reproduction and overall fitness of target insects. (Solter and Becnel 2000). Protozoans produce spores, which are the infectious phase in several susceptible insects as lepidopteran, orthopteran, and hoppers (Lewis 2002; Senthil-Nathan 2015). *Nosema* spp. spores are assimilated by the host and develop in the midgut. The spore formed by the protozoan is the infectious stage and has to be ingested by the insect host for pathogenic effect. Spores invade host target cells cause massive infection, demolishing organs and tissues. The infection results in reduced feeding, vigor, fecundity, and longevity of the insect host as inundatively applied microbial control agents. The only protozoan registered for use as a biopesticide is the microsporidian, *Nosema locustae*, which infects nymphal stages of grasshoppers and kills them within 3–6 weeks post-infection (Koul 2011). However, not all infected grasshoppers are killed by this protozoan infection because of difficulty in assessing of a highly mobile insect (Kaya and Gaugler 1993). *Nosema pyrausta* is another beneficial microsporidian that reduces fecundity and longevity of the adults and also causes mortality of the larvae of European corn borer (Koppenhofer and Kaya 2002). A study of *Nosema pyrausta*, a microsporidium infects the European corn borer, *Ostrinia nubilalis*, showed both horizontal and vertical transmissions maintain *N. pyrausta* in natural populations of European corn borer. *N. pyrausta* suppresses populations of European corn borer by reducing oviposition, percentage hatch, and survival of infected neonate larvae (Koul and Dhaliwal 2002).

5.4 Effects of Microbial Biopesticides

The main advantages of using microbial insecticides for pest management are their environmental safety, specificity, and biodegradability. Microbial biopesticide based on viruses and bacteria are mainly host specific while others, such as fungi and nematodes, may affect a fairly wide range of insects and related arthropods. Commercially available microbial pathogens are target specific and safe to the environment and ecosystem. The microbial pesticides do not leave any harmful residues in the environment, and do not enter the food chain. It has been documented that Bt has rapid breakdown and low toxicity towards aquatic systems, mammals, and other non-target organisms (Koul 2011). Bt-sprays are safe to non-target organisms such as soil microorganisms (protozoa and fungi), Collembola, Mollusca, Crustacea, Arachnida, aquatic insects, predators, parasitoids, honeybees, earthworms, salamanders, bird, and mammals (Boomathi et al. 2005; Senthil-Nathan 2015). Entomopathogenic fungi are also safe for the ecosystem as they do not affect the specific host, its infected cadavers that drop on the soil, sporulate under congenial microclimatic conditions, and overwinter in the soil. Particular environmental conditions are necessary for their infection and their attack starts again as the pest population prevail. Baculovirus, among the other insect viruses, are regarded as safe and selective. They have been used worldwide against many insect pests (mainly Lepidoptera). But the problems associated with the limited use of baculoviruses as narrow host range, slow killing speed, technical and economic difficulties for in vitro commercial production, timing of application based on host population monitoring, and variability in their efficacy in the field under diverse climatic conditions (Vimala Devi and Hari 2009). Epizootics of baculovirus diseases are frequent in Lepidoptera and sawflies with very high larval mortality, resulting in a substantial reduction in insect population. These days research has been focused on the identification of virulent microbial isolates for effective management of the target pests and their safety to the natural enemies, persistence in the environment, phytotoxicity in addition to generating information on the bio-efficacy (Jeyarani et al. 2008). Ranga Rao et al. (2008) recorded low reduction (3%) of *H. armigera* parasitoid, *Campoletis chlorideae* Uchida, and other natural enemies in the HaNPV sprayed plots as compared to 60% reduction in the endosulfan treated plots in chickpea. HaNPV (@ 250 LE ha^{-1}) application on chickpea resulted in a reduction of aerial and soil-inhabiting natural enemies by 15 and 22%, respectively, over the control plots, while the reduction in the Endosulfan sprayed plots was 52.4 and 63.1%, respectively.

5.5 Constraints Related to Microbial Biopesticides Production and Use

Biopesticides although offer a promising approach in integrated pest management for sustainable agriculture, but still their adoption is not up to the mark because of various constraints. It is necessary to maintain the production of quality

biopesticides, since they are important in rendering sustainability to farming systems. Insufficient knowledge, lack of adequate machinery, inappropriate handling and improper distribution, importation laws for live inoculants, and several other issues can lead to lack of quality products and loss of market (Mishra et al. 2015). Some of the important constraints associated with microbial biopesticides are as follows:

5.5.1 Lack of Faith and Awareness Among People

These days Agriculture market is moving towards an increase in demand for environment friendly, chemical residue-free organic products. But adoption of eco-friendly biopesticides is less due to the lack of awareness about benefits of microbial biopesticides, and their uneven efficiency. The lack of awareness, knowledge, and confidence in farmers is one of the main reasons for lagging of usage of these eco-friendly pest control alternatives. Condition is worst in developing countries where most of the farmers are even not familiar with the term "biopesticide" and lack efficiency and skills to practice and use them (Alam 2000). Certain extension activities such as organizing teaching programs, workshops, and entrepreneurs dealing with the idea of promoting sustainable agriculture and efforts of various government agencies to popularize the use of biopesticides. The National Farmers Policy (2007) in India has strongly recommended the promotion of biopesticides for increasing agricultural production and sustaining the health of farmers and environment (Arora et al. 2010). Hence, it is essential that training and teaching should be given to the farmers in regard to the use of Microbial biopesticides (Amin 2013).

5.5.2 Inconsistent Field Performance

Extremely unreliable supply and very inconsistent performance of microbial biopesticides are the main reasons that many farmers stopped using biopesticides (Alam 2000). Factors responsible for the poor performance are the rapid decline in the size of populations of active cells. Abiotic soil factors (e.g., textural type, pH, temperature, and moisture) exert their (direct) effect on inoculant population dynamics by imposing stresses of various natures on the living microbial cells introduced in the fields. Furthermore, efficient introduction into soil during the growing season is a major technical constraint. It is extremely important that a minimum effective threshold population of the introduced biopesticide is maintained in the soil so as to combat the pests and pathogens (Arora et al. 2010). Sophisticated quality control measures, monitoring facilities, reliability, specificity, and replicability in its activity should be implemented. Technical and chemical compatibility along with innovative application methods is also necessary.

5.5.3 Poor Quality and Shelf Life of Microbial Biopesticides

Poor quality and performance are the main reasons those hinder the microbial biopesticides takeover on the market. It has been reported that the biopesticides being sold in the market are contaminated and have a low count of microorganisms with low shelf life result in inconsistent performance (Alam 2000; Arora et al. 2010). Bacterial survival in the desired formulation is affected by several variables: the culture medium used for bacterial cultivation, the physiological state of the bacteria when harvested from the medium, the use of protective materials, the type of drying technology used, the presence or absence of contaminants, and the rate of dehydration. It is also important that precautions should be taken to avoid adulteration during packaging, storage, and application of biopesticides. For the solution of this problem some techniques as air-dried and lyophilized preparations of biopesticides can be a better solution (Nakkeeran et al. 2005). Formulations with extended shelf life include granules, pellets, and dry powder based biopesticides. Granules can protect the active agent from desiccation and also provide basic food for the agent. Powder formulation is easy to apply by suspending it in water with wide area of application (Amin 2013).

5.5.4 Imbalance Between Production and Agribusiness

In industrial point of view raw material and instrumentation facility initially required for the biopesticide production are costly. The established companies relinquished their wish to do business in microbial pesticides and finally left the field due to huge losses in the agribusiness, these are main reasons for less production. Enormous caution at the stage of manufacture/culture, transportation/distribution, and application is needed (in packaging, storage, and use of suitable carrier materials) (Arora et al. 2001, 2010). However, consistency and long-term returns can reduce the cost and enhance the profits. A number of features of the agricultural economy make it difficult for companies to invest in developing new biopesticide products and at the same time, make it hard for farmers to decide about adopting the new technology (Chandler et al. 2011). Biopesticide industry is now being forecasted by leading global management consulting and market research firms which is helpful for market competency (Leng et al. 2011). The profit in biopesticide business could be made only by using novel techniques and tools and multifaceted bioformulations based on microbial consortia with diverse activities can be useful in bringing down the costs (Arora et al. 2013).

5.5.5 Regulatory Framework and Registration

Regulatory framework related to registration of biopesticide is the main hurdle in the development which is expensive as well as time consuming (Ehlers 2006). The governments can frame regulations at the global level by organizing meetings,

workshops, and conferences regarding uplifting the status of biopesticides/bioformulation. At present, different countries have different rules and regulations due to which problems related to registration, use, import, and export do occur. Government can set up globally accepted uniform acts or laws, so that there is a common policy regarding the registration and regulation of biopesticides (Kumar 2012).

5.5.6 Health and Environmental Issues

Microbial biopesticides may possess some health risks if not used according to the instructions mentioned on the product. Bacterial biopesticides containing Bt as active ingredient which is not reported to show any major adverse effects on human health, but in some cases, occupational exposure confirmed health risks (Doekes et al. 2004). But in case of *Trichoderma*, *M. anisopliae*, and *B. bassiana* allergy to farmworkers has been reported (Darbro and Thomas 2009). Recently studies show that *M. anisopliae* treated mice found as a robust fungal allergen (Ward et al. 2011). So that governments should also set up defined standards and permissible limits in regard to using biopesticides so that it diminishes the health risks. Thus, it is necessary that before developing a biopesticide strain monitoring should be extensively done (Copping and Menn 2000; Kumar 2012).

5.5.7 Competition with Chemical Pesticides

Practically, biopesticides are not as effective as chemicals. In case of chemical pesticides, lesser quantity is sufficient to kill a vast quantity of pests which is the main reason why farmers choose chemical pesticides over biopesticides. Studies shows that the production of major crops around the globe depends on chemical insecticides in large extant (Aktar et al. 2009). Synergistic action of microbial biopesticides and chemical pesticides may be helpful (Irigaray et al. 2003). Research on combining microbial biopesticides with synthetic pesticides has showed improvement in control of some pest species including pesticide-resistant varieties (Cuthbertson et al. 2005). Microbial biopesticides production and development can only be enhanced by the removal of above-mentioned constraints. Adoption of latest technologies and government policies can be helpful to enhance the market for microbial biopesticides and can secure our crops in sustainable manner.

5.6 Future Perspectives

There are many challenges that are needed to overcome for the performance of microbial biopesticides. Emphasis on the barriers for research patents, use of patented technologies as well as on the availability of publicly funded research results (Boettiger et al. 2006). To implement local production schemes in developing

countries, intervention at the national and international level is very much important. It is also needed to have a look into the ecological relevance vis-a`-vis the use of microbial biopesticides. As such, the effect of microbial biopesticides on microbial communities must be carefully monitored (Gonzalez 2006). For commercial microbial product, three specific criteria for selection should be required, i.e. toxicity, production efficiency, and safety of the product. In order to increase the utility of microbial pathogens in IPM programs, systematic surveys and detailed studies on the properties, mode of action, and pathogenicity of such organisms are required. It is expected that with the advanced microbial research coupled with dedicated efforts from extension specialists, farmers, pest management regulators, and general public is required. In this way microbial biopesticides could play a prominent role in future pest management programs (Mocali 2010).

According to the researchers, in the case of *B. thuringiensis* (Bt) it has been studied that, insecticidal toxin present in Bt and its survival may not always depend on insect pathology. It can colonize seedlings from spores in the soil, exchange genetic information on the phylloplane, and an appreciable multiplication can occur in the frass of insects that it did not kill. Therefore, longer-term studies in nature and their survival in soil and plants in the presence of susceptible and non-susceptible invertebrates are required (Ravensberg 2011). Spores in the frass could be a source of recolonization from the soil and be transferred to other plants. These findings illustrate a possible cycle, not dependent on insect pathology, by which *B. thuringiensis* diversifies and maintains itself in nature. The mechanism of resistance, specifically for Cry proteins, is a matter of concern. Recently, a database consisting of 12,519 high-quality sequences have been developed from the larval gut of European corn borer. This obviously can provide basis for future research to develop gut-specific DNA microarrays to analyze the changes of gene expression in response to *B. thuringiensis* protoxins/toxins and the genetic difference(s) between Bt resistance and susceptible strains. In fact, 52 candidate genes have been identified that may be involved in Bt toxicity and resistance. For instance, out of selected genes, five genes with decreased expression and ten with increased expression in Cry1Ab-resistant strain of European corn borer may help in identifying the genes involved in Bt resistance that could provide new leads into the mechanism of Cry1Ab resistance in these insects (Bizzarri and Bishop 2008). Commercialization is the final and most difficult step in the development of a microbial product. Costs amount to US$14–21 million for a new entrepreneur and its introduction time to market including registration is not less than 5–7 years. Therefore, to examine all these critical factors in the developmental process and successful commercialization of microbial biopesticides are the important parameters for the production of any effective microbial biopesticide (Khajuria 2009).

5.7 Conclusion

Presently biopesticides are being used everywhere in the world, it is also known that developed countries seem to be ahead in their wider application but their situation still remains in dilemma for the effective use over the synthetic pesticides. It is also

believed that biopesticides may be less vulnerable to genetic variations in plant populations that cause problems related to pesticide resistance and their use is not overly complicated. Developing countries have huge possibilities for using biopesticides as the production can be less expensive and labor is cheap in comparison to developed nations. Microbial biopesticides are expected to provide predictable performance and it must do so in an economically viable manner for their better acceptability and adaptability. But various challenges as the efficacy of the microbial activity, survival of microorganisms, delivery systems, determining host range, avoiding injury to non-target organisms, consistency, performance in field conditions, economics, government regulations, and confidence among the end users need to be properly overlooked. However, awareness among the farmers, manufacturers, government agencies, policy makers, and the common men, training on production and quality control to manufacturers, and organizational training to extension workers and farmers to popularize biopesticides may be essential for better adoption of the technology.

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Arbuscular Mycorrhizal Fungi (AMF) for Improved Plant Health and Production

6

Syeda Asma Bano and Bushra Uzair

Abstract

Agricultural production must increase due to increase in population. Inorganic fertilizers are used to promote growth of plants. The use of inorganic fertilizers is rising day by day, which is very expensive and is a huge cause of environmental pollution. We need to find out strategies to improve our agricultural productivity by using environmental friendly approach. Arbuscular mycorrhizal fungi form symbiotic association with majority of plants and provide the plants with essential nutrients especially phosphorus; hence, there is less need for inorganic fertilizers. Most of the species of AMF belong to sub-phylum Glomeromycotina. Four orders of arbuscular mycorrhizal fungi (Glomerales, Archaeosporales, Paraglomerales, and Diversisporales) have been identified in this sub-phylum. There are transport proteins located in the fungus and plant plasma membranes, which help in the transport of different nutrients. Roots of different plants release certain exudates after contact with AM fungi, these fungi also respond to plant's exudates by releasing certain compounds such as sesquiterpenes, hence after a mutual dialogue, AM fungi associate itself with the roots of plants. Identification of AMF involves the use of DNA markers such as smallest subunit (SSU) rRNA gene, the internal transcribed spacer (ITS), and the large subunit (LSU) rRNA gene. Plants having AM association are more tolerant to metals, drought, salinity, heat, and adverse environmental conditions. Soil structure and soil nutrients are improved due to AMF association. Plants having mycorrhizal association can better cope with the biotic and abiotic stress conditions than the non-mycorrhizal plants and can be well adapted to the changing environment conditions. There is a positive impact on the stability of ecosystem due to the presence of mycorrhizal

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plants. AMF plays important role in sustainable development of agriculture and may also increase resistance of plants to pathogens attack.

Keywords

Arbuscular mycorrhizal fungi · Bio-fertilizers · Arbuscules · Fungal hyphae · Glomeromycota · Symbiosis

6.1 Introduction

The term mycorrhiza has been derived from the Greek words for "fungus" and "root." Fungi of phylum Glomeromycota and majority of terrestrial plants show the arbuscular mycorrhizal symbiosis (Schüssler et al. 2001). Mycorrhiza is associated with the roots of over 90% of all plant species. An extensive hyphal network is developed by mycorrhizal fungi in soil, connecting the plant communities and offering a horizontal transfer of nutrients (Prasad et al. 2017). Symbiotic fungi associate with the roots of most of the plants to form mycorrhiza, which plays an important role in the acquisition of nutrients from the soil and therefore plant nutrition is improved. The life cycle of fungi requires association with host roots, as hyphal growth is very limited in the absence of host plant. The AM symbiosis results in bidirectional nutrient exchange: the fungus gets food material by plant photosynthates, and plant nutrition, mainly phosphate is enhanced by the fungus (Smith and Read 1997). There is extensive hyphal branching of AM fungi near the host roots before the development of appressorium. Appressorium is the structure which is used to penetrate the plant root. Some signaling molecules are released by host roots, which stimulate the hyphal branching. Akiyama et al. (2005) had isolated a branching factor named strigolactone (5 deoxystrigol) from the root exudates of *Lotus japonicus*. For the parasitic weed striga and orobanche, strigolactones were known as seed germination stimulants. Plants having mycorrhizal association are drought tolerant. They are more resistant to pathogens attack and there is reduction in irrigation and fertilizer requirement. Hence healthy plants are produced as a result of mycorrhizal association. There is beneficial effect on soil chemistry and soil biology besides improved nutrients supply to plants as a result of mycorrhizal association. Generally speaking there are two types of mycorrhiza, ectomycorrhiza and endomycorrhiza. In endomycorrhizal association, the fungus colonizes the host plant's root tissues intracellularly as in arbuscular mycorrhizal fungi (AMF) or extracellularly as in ectomycorrhizal fungi. The association is sometimes mutualistic when both fungi and plant partners are benefitted. Mycorrhiza may have a parasitic association with host plants in some specific conditions (Johnson et al. 1997). Signaling pathway between plant and mycorrhizal fungi has also been described and several genes have been identified.

6.2 Types of Mycorrhizae

There are seven types of mycorrhizae:

1. Ectomycorrhizae
2. Endomycorrhizae
3. Orchid mycorrhizae
4. Arbuscular mycorrhizae
5. Ericaceous mycorrhizae
6. Arbutoid mycorrhizae
7. Ectotrophic mycorrhizae

Among them endo- and ectomycorrhizae are the most abundant and widespread.

6.2.1 Ectomycorrhizae

It is a type of mycorrhizal relationship that is found between a mycobiont, fungal symbiont, and roots of various plant species. Ectomycorrhizae produce a Hartig net surrounding the root (Fig. 6.1a). Fungi do not penetrate their cell walls of host.

6.2.2 Endomycorrhizae

Ectomycorrhizae form a symbiotic relationship with the roots in which fungi penetrate their cell walls of root cortex (Fig. 6.1b).

6.2.3 Orchid Mycorrhizae

These are symbiotic relationships between the roots of plants of the family Orchidaceae and a variety of fungi. All orchids are myco-heterotrophic at some point in their life cycle. It is the type of symbiotic relationship in which fungal hyphae penetrate into the root cells and form pleotons (Fig. 6.1c).

6.2.4 Arbuscular Mycorrhizae

It is a type of mycorrhizae in which symbiont fungus penetrates the cortical cell of the root of vascular plant and forms vesicle arbuscules and hyphae in the root cortex (Figs. 6.1d, 6.2, 6.3). The arbuscular mycorrhizal (AM) symbiosis occurs between fungi of the Glomeromycota (Schüssler et al. 2001) and the majority of terrestrial plants.

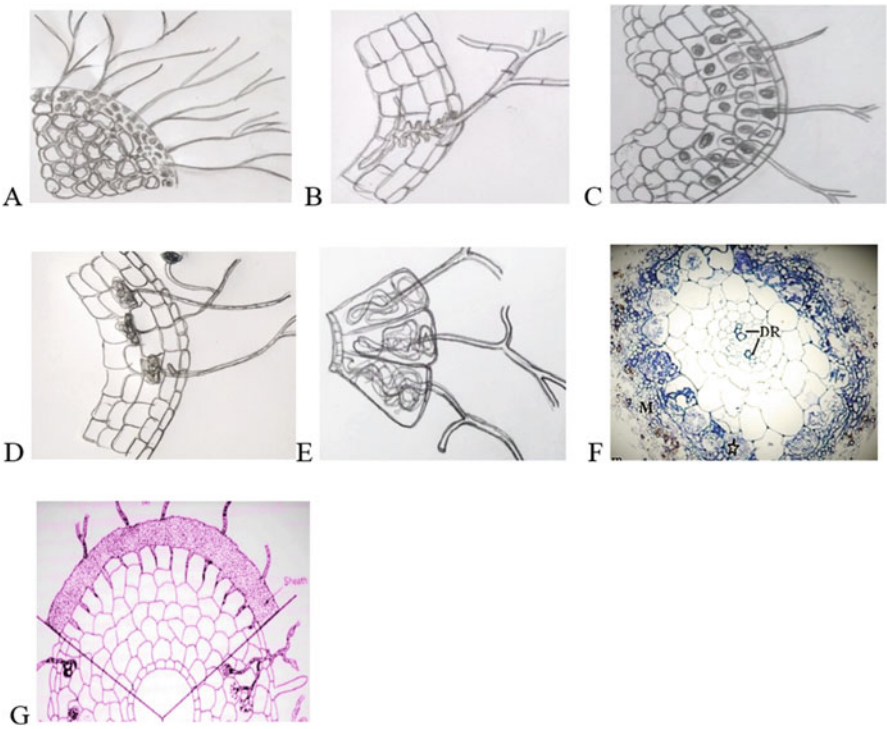

Fig. 6.1 (**a**) Ectomycorrhizae, (**b**) endomycorrhizae, (**c**) orchid mycorrhizae, (**d**) arbuscular mycorrhizae, (**e**) ericaceous mycorrhizae, (**f**) arbutoid mycorrhizae, (**g**) ectotrophic mycorrhizae

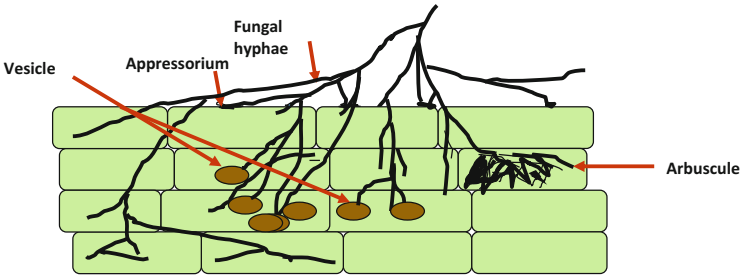

Fig. 6.2 Colonization of plant root by fungal hyphae, vesicles, and arbuscules

6.2.5 Ericaceous Mycorrhizae

The members of the plant family Ericaceae and several types of mycorrhizal fungi form a mutualistic relationship called ericoid mycorrhiza (Fig. 6.1e). These mycorrhizas are characterized by fungal coils in the epidermal cells of the fine hair roots of ericaceous species. The plant's cell membrane remains intact and fits over

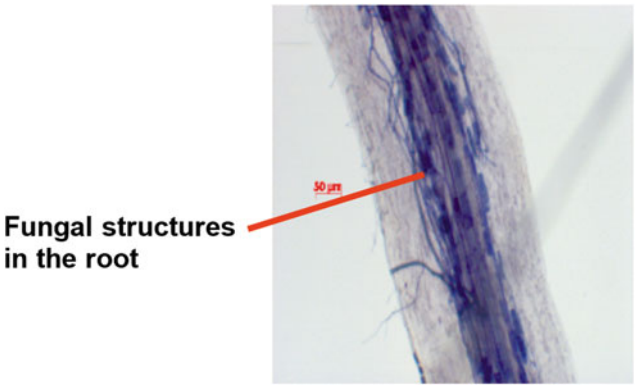

Fig. 6.3 Growth of fungal structures in plant roots (roots were stained by Pelikan ink staining method)

the coils of hyphae. These coils of hyphae serve to increase the surface area available for nutrient exchange.

6.2.6 Arbutoid Mycorrhizae

Hyphae of arbutoid mycorrhizae penetrate in the outer cortical cell and fill them with coil (Fig. 6.1f). The main feature of this symbiotic relationship is mantle sheath, Hartig net, and intracellular coils. Plants of genera Arctostaphylos and Arbutus exhibit association with arbutoid mycorrhizal fungi. The fungi that form arbutoid mycorrhizal relationships are basidiomycetes. Most fungal species that form ectomycorrhizal associations are also basidiomycetes.

6.2.7 Ectotrophic Mycorrhizae

Ectotrophic mycorrhizae is a type of symbiotic relationship in which fungi form pseudo-parenchymatous sheath around the root and send branches inward as well as outside in the soil.

6.3 Arbuscular Mycorrhizal Fungi as Plant Growth Stimulators

Arbuscular mycorrhizal fungi (AMF) are one of those micro-organisms which promote plant growth and nutrition. Fungi of phylum Glomeromycota develop arbuscular mycorrhizal association. Most of the phosphorus is present in unavailable form. AMF help in the acquisition of phosphorus for the plant. Besides the phosphorus other nutrients like sulfur, nitrogen, and other micro-nutrients are transported

to the plant. AMF also enhance tolerance of plants to several environmental stresses and plant pathogens. These plant pathogens cause huge loss of crop yield worldwide. AMF protect plants against these pathogens. AMF improve plant growth and productivity by improving mineral nutrients uptake; hence, plant biomass and productivity increases (Bona et al. 2016; Fiorilli et al. 2013). Plants having AM association can cope with the biotic and abiotic stress conditions more efficiently as compared to non-mycorrhizal plants (Augé 2001; Fiorilli et al. 2013). Mycorrhizal association can enhance the absorption of roots and resistance of plants to external stress factors is also improved, especially in high-pressure open-pit mines, they can promote plant growth and improve the vegetation recovery in the mine reclamation area (Song et al. 2020). The mechanisms of adaptation of AMF for abiotic stresses are generally linked to increased hydromineral nutrition, production of osmolytes, gene regulation, ion selectivity, and synthesis of antioxidants and phytohormones. As far as the biotic stresses are concerned, AMF are involved in pathogen resistance including improvement of the plant's defense system and competition for colonization sites (Diagne et al. 2020). AMF also improve plant growth under reduced water supply conditions (Posta and Duc 2019). AMF regulate plant growth under different stress conditions. AMF colonization by *R. irregularis and F. mosseae* can significantly reduce the intensity of infection by *N. ditissima* which causes apple canker—a major pathogen (Berdeni et al. 2018). Few volatiles were found to be specifically emitted in response to the symbiont or pathogen (Dreher et al. 2019). Specific volatile organic compounds emission in response to different organisms could be based on the action of different receptors at the plasma membrane, such as receptors for specific factors of AM fungi leading to CSSP activation and receptors that recognize general fungal presence inducing a PAMP-induced defense response.

Enhanced photosynthetic rate along with water and mineral nutrients has been observed in plant having AM association. Soil structure and fertility are also improved by AM association with plants. AM fungi associate with cereals, vegetables, fruit trees, and many other crop plants, therefore play an important role in sustainable agriculture.

The main benefit of AMF to the host plant is the acquisition of phosphorus. Presence of high phosphorus in soil suppresses the development of AM fungi, as there is transcriptional alteration. Several researchers demonstrated the benefit of AMF in improving the plant health and yields (Mäder et al. 2000; Rouphael et al. 2015; Hijri 2016). Another important aspect of AMF is the enhancement of root system development (Gutjahr and Parniske 2013). High value crops could be inoculated with suitable AMF strain for more yield and better growth. A significant increase in tuber production was observed as a result of inoculation with *Rhizophagus irregularis* (Hijri 2016). Improvement of health of plant due to AM inoculation has already been demonstrated clearly by many researchers (Schubert and Lubraco 2000; Balla et al. 2008).

Application of AMF in micropropagation of plants in nurseries is highly successful (Azcón-Aguilar and Barea 1997; Jeffries et al. 2003; Kleinwächter et al. 2012; Maronek et al. 1981). AMF could also improve nutrients uptake and growth of fruit

tree at transplant (Lovato et al. 1992; Schubert and Lubraco 2000). Several factors interfere with the AM symbiotic relationship with plants. High phosphorus contents in soil hinder this association besides that extensive plowing also reduces AMF (Douds and Millner 1999; Mäder et al. 2000; Grant et al. 2005; Hartmann et al. 2015). Specific biocides and non-host plants such as chenopodiaceae and Brassicaceae also affect this association. Mycorrhizal plants also improve soil fertility due to release of Glomalin, which contains 30 to 40% C and its related compounds which improve the water holding capacity of soil (Sharma et al. 2017).

6.4 Mechanisms of Arbuscular Mycorrhizal Symbiosis

Plant derived and fungal signaling molecules are responsible for arbuscular mycorrhizal symbiosis (Gutjahr and Parniske 2013). The signaling pathway starts from the strigolactones which are released from plant roots. These strigolactones then stimulate the AMF (Akiyama et al. 2005; Besserer et al. 2006; Kretzschmar et al. 2012). Next AMF release lipochitooligosaccharides which activate further pathway in plant that is shared with root nodule symbiosis (Harrison et al. 2002; Gutjahr and Parniske 2013) (Fig. 1.3). Hundreds of gene are activated in host cell as a result of AM symbiosis (Liu et al. 2003; Güimil et al. 2005; Hohnjec et al. 2005; Fiorilli et al. 2013; Gomez et al. 2009; Guether et al. 2009; Breuillin et al. 2010a, 2010b).

AMF also produce glomalin in soil which is a glycoprotein and it is very important in the stabilization of soil aggregation (Singh et al. 2013). The soil is protected from erosion due to hyphal network of AMF, hence enhancing plant growth and improving the mineral nutrition acquisition for the plant. The water retention capacity of soil is also increased due to the effect of arbuscular mycorrhizal fungi on soil quantity. Land ecosystem remains intact due to reduced leaching of nutrients from the soil (Cavagnaro et al. 2015). Querejeta (2017) also showed the better water holding capacity of mycorrhizal soil. The pre-symbiotic signaling and hyphopodium formation is affected by the supply of high phosphorus concentration. This is due to the inhibition of the synthesis of strigolactones by the plants. Strigolactones play a very important role in the development of pre-symbiotic stages between AM fungi and plant roots. Strigolactones also stimulate spore germination and cell proliferation of AMF by activating mitochondria. A transcriptomic study showed that the genes encoding enzymes for the biosynthesis of carotenoid and strigolactone are found downregulated under high level Pi supply. In mycorrhizal plants two phosphate uptake pathways were suggested, one is the direct uptake and other is mycorrhizal uptake pathway. The direct uptake pathway is controlled by Pht1 members which is non-mycorrhiza regulated, while the mycorrhizal pathway was induced by specific arbuscular mycorrhiza related phosphate transporters. The examples of two of these AM related phosphate transporters are *LjPT3* and *MtPT4* in *Lotus japonicus* and *Medicago truncatula* which are required for mycorrhizal symbiosis. Many phosphate transporter genes are expressed in roots under phosphorus deficient conditions. Phosphorus transporter genes are also an important part of the Pi starvation signaling pathway. MYCS (mycorrhiza transcription factor binding

sequence), a novel cis-elements, was found to be also required for AM-induced or -specific expression of the AMR PT genes. Plant hormones also play a role during the establishment of AM symbiosis except other complex factors. However the reports explaining the correlation between AM development and plant hormones other than strigolactones are very limited, although several phytohormones, e.g., abscisic acid (ABA), auxins, jasmonic acid, and ethylene have already been described.

The lateral root development is altered by modulating the sensitivity of auxins by Pi availability. This process involves TIR1 auxin receptor. The development of lateral roots is induced due to AM colonization. These lateral roots are the favored sites of AMF colonization. Strigolactones could interact with auxins and the outgrowth of lateral roots is accelerated under phosphate starvation, while the lateral root development is suppressed by providing GR24 to the roots of Pi-replete plants. The mechanisms showing the cross-talk between the signaling of AMF, Pi, and phytohormones are yet to be explored.

A genetic program is responsible to control the development of arbuscular mycorrhizal symbiosis as well as nodulation (Harrison 2005; Paszkowski 2006; Reinhardt 2007; Stacey et al. 2006). Defective plant mutants in both mycorrhiza and nodule development were isolated; hence, it was suggested that mycorrhizal symbiosis shares a common signaling pathway with the nodulation pathway (Marsh and Schultze 2001; Oldroyd and Downie 2006). Many genes that are expressed during nodulation are also induced during mycorrhization, providing some evidence about the functional overlap between root symbiosis (Albrecht et al. 1998; Journet et al. 2001).

Two Nod-factor receptor kinases (nodulation specific), NFR1 and NFR5 have been known to affect the earliest Nod-factor responses (Radutoiu et al. 2003), but not the AM symbiosis (Wegel et al. 1998), suggesting that the fungal signaling factor (Kosuta et al. 2003) is different from Nod factor. Little is known about the mechanism of association between the fungi and the plant; however, some plant signaling components that play a role in symbiosis are already known. These include a predicted ion channel (Ané et al. 2004; Imaizumi-Anraku et al. 2005), a receptor like kinase (Endre et al. 2002; Stracke et al. 2002), and calcium and calmodulin dependent protein kinase (Levy et al. 2004; Mitra et al. 2004), controlling the common bacterial and fungal symbiotic pathway. Genes encoding these proteins are called the common SYM genes. These genes control the process of symbiosis (Kistner and Parniske 2002). Seven genetic loci have been identified so far in Lotus japonicas for their participation in the common symbiotic pathway. Calcium spiking (oscillations in cytoplasmic calcium level due to certain signaling process of symbiosis) was observed in several legumes (Kosuta et al. 2008; Oldroyd and Downie 2006; Wais et al. 2000) showing nodulation and mycorrhizal development. Membrane intrinsic protein MtAqp1 was induced during mycorrhizal symbiosis (Krajinski et al. 2000), which was predicted to play its role during AM association. A lipid transferase protein gene was expressed in epidermal cells and it may be linked with appressorium formation (Breuillin et al. 2010a, b), as the transcript level increased when the fungus formed appressoria and penetrated the roots. Pumplin

et al. (2010) indicated a gene encoding VAPYRIN protein required for arbuscular mycorrhizal symbiosis.

Saito et al. (2007) found that Nucleoporin 85 is required for mycorrhization and nodulation. Nup85 encodes a nucleoporin 85 which is a family of proteins forming nuclear pore complex, which together with nucleoporin 133 may help in controlling symbiosis. This gene is required for calcium spiking during symbiotic signaling process. Nuclear pore complex mediates mRNA export and protein import. Nod factor failed to show calcium spiking in Nup85 mutant so symbiosis was affected (Saito et al. 2007). Study of a M. truncatula 6 k root interaction transcriptome (Mt6k-RIT) revealed the identification of 752 genes upregulated in mycorrhizal tissues and also involved in nodulation (Küster et al. 2004; Manthey et al. 2004). Seven Lotus japonicas genes (SYMRK, CASTOR, POLLUX, SYM3, SYM6, SYM15, and SYM24) were identified to be required for bacterial and fungal symbiosis (Kistner et al. 2005). Contrary to these common SYM genes, not many genes involved specifically in mycorrhiza formation have been characterized through mutational studies although Zhang et al. (2010) have reported a mycorrhizal specific ABC transporter gene. Another phosphate transporter which is indispensable for mycorrhizal symbiosis has been demonstrated by Javot et al. (2007). A widespread mutualistic association of land plants and fungi is arbuscular mycorrhizal symbiosis (AMS) which is suggested to have originated early in the evolution of land plant (Bidartondo et al. 2011; Taylor et al. 1995). Most of the genes required for AMS have similarities with the evolutionary nitrogen-fixing rhizobium legume symbiosis (RLS) or by reverse genetic analysis of differently expressed candidate genes (Gutjahr and Parniske 2013). Genes required for AMS are conserved and are present in only in host plants and absent from non-host species. For example, two ABC transporters (STR and STR2), a GRAS transcription factor (RAM1), a phosphate transporter (PT4), and a lipid biosynthetic enzyme (RAM2) are all required for AMS and are found only in AMS host plant (Harrison et al. 2002; Wang et al. 2012).

AM symbiosis is very ancient as compared to rhizobial symbiosis (Parniske 2008; Remy et al. 1994). This gives an indication that the AM signaling pathway had developed first and rhizobial symbiosis later (Sprent 2007); hence, many genes became common between the two symbiotic pathways, performing their functions in mycorrhizal and in nodulation processes. Predicted functions of these genes include membrane transport, defense and stress responses, primary metabolism, and regulation of gene expression. Two genes, MtC93310 and MtC50410, were identified to be induced during arbuscular mycorrhizal symbiosis. MtC50410 belongs to Gras family of transcription factors and it is a homologue of RGA1 and GAI from *A. thaliana* (Manthey et al. 2004; Peng et al. 1997; Pysh et al. 1999; Truong et al. 1997). Transcription factors perform their function alone or with other proteins in a complex by activating or blocking the recruitment of RNA polymerase (an enzyme that performs the transcription of genetic information from DNA to RNA (Latchman 1997)). Previously two GRAS transcription factors NSP1 and NSP2 were identified to play their role during symbiosis (Smit et al. 2005; Kaló et al. 2005; Maillet et al. 2011).

6.5 Signal Exchange and Recognition

Exchange of signals occurs between the plant and fungi, as a result of which the plant and AM fungal interaction is initiated (Harrison 2005). Certain signal molecules are released by plant roots which are called the branching factors (BFs). AM fungi develop branching as a result of these signal molecules. Akiyama et al. (2005) isolated a branching factor from the root exudates of a model legume *Lotus japonicus*, and it was identified as 5, deoxystrigol (strigolactone). These were previously isolated as seed germination stimulants, from the parasitic plants Striga and Orobanche (Bouwmeester et al. 2003). The germination of fungal spores can occur in the soil in the absence of the plant signals; however, extensive growth of the fungal hyphae occurs due to the root exudates of the plants especially the strigolactones.

The molecular and cellular events are triggered in AM fungi due to release of strigolactones from the host roots, which help in stimulating the fungal growth (Akiyama et al. 2005; Besserer et al. 2006). The expression of mitochondrial related genes is also induced due to these root exudates; hence, there is activation of fungal respiratory activity (Tamasloukht et al. 2003). A few examples of natural strigolactones are: 5, deoxystrigol, strigol, strigyl acetate, sorgolactone, orobanchol, alectrol. GR24 and GR7 are examples of synthetic analogs. Strigolactones have been isolated from root exudates of many monocots like maize, millet, sorghum, and dicots including cotton, cowpea, red clover, *Menispermum dauricum,* and *Lotus japonicus* (Akiyama et al. 2005; Akiyama 2007; Cook et al. 1966, 1972; Hauck et al. 1992; Muller et al. 1992; Siame et al. 1993; Yasuda et al. 2003; Yokota et al. 1998). Their characterization is difficult due to their instability and very low concentration. Flavonoids are also present in plant root exudates and are important in the symbiotic rhizobium legume interaction. They are inducers of rhizobial nodulation genes, involved in the synthesis of lipochitooligosaccharide signals called Nod factor (Perret et al. 2000). Flavonoids also stimulate the growth and branching of fungi (Becard et al. 1992; Gianinazzi-Pearson et al. 1989; Tsai and Phillips 1991).

The stimulatory effect of flavonoids on AMF hyphal growth depends on the chemical structure of the compound (Becard et al. 1992; Chabot et al. 1992; Scervino et al. 2007). The flavonoid pattern was altered in mycorrhizal roots and it may be due to the developmental stage of the AM symbiosis as demonstrated by Harrison and Dixon (1993) and Larose et al. (2002). Fungi release diffusible symbiotic signals which are called Myc factors (Maillet et al. 2011). These myc factors are similar to Nod factors in their structure and are a mixture of sulfated and non-sulfated simple lipochitooligosaccharides (LCOs). This diffusible factor is recognized by the plants and certain genes are activated in the plant roots which may help in the development of AM symbiosis. A mycorrhiza specific factor (Myc) induced the expression of MtENOD11 in the roots of *Medicago truncatula* (Kosuta et al. 2003). Chabaud et al. (2002) also reported the activation of MtENOD11 gene in epidermal and cortical cells in response to inoculation by *Gigaspora rosea*. Mycorrhizal colonization was also reported to be increased as a result of Nod factor (Olah et al. 2005). About hundred genes expressed in mycorrhizal roots have been

isolated. Inoculation with different fungi *Glomus mosseae* and *Glomus intraradices* resulted in overlap of genetic program (Hohnjec et al. 2005). One member of an AM-induced gene encoding blue copper binding proteins (MtBcpl) was expressed in arbuscule-containing cells, indicating some role of this gene in mycorrhizal symbiosis (Hohnjec et al. 2005). Recently two mycorrhiza specific blue copper binding genes were identified in *M. truncatula* Jemalong 5 (Parádi et al. 2010). It is possible that these copper binding genes play some role in mycorrhizal symbiosis. To understand the mechanism of association of functional AM symbiosis, detailed analysis of the promotor can also help in the identification of upstream regulatory mechanism of AM. By using the promotor reporter gene fusion the expression pattern driven by mycorrhiza specific promoters of *M. truncatula* was identified as described by Krajinski and Frenzel (2007).

6.6 Inoculation of AM Fungi in Contaminated Soil

AMF protect the plant from polluted soil by accumulating or sequestering the toxic metal ion (Weissenhorn et al. 1995; Diaz et al. 1996; Gonzalez-Chavez et al. 2004). Inoculation of suitable strains of AMF may help in bioremediation of the contaminated soil. Several different types of heavy metals contaminate the soil. AMF could help in promoting growth of plant in heavy metal contaminated soil (Bano and Ashfaq 2013; Leyval et al. 2002; Turnau et al. 2006; Khade and Adholeya 2007; Sheoran et al. 2010). AM inoculation also improves the yield of crop as well as survival of trees in dry conditions, due to the presence of specific strain of vascular arbuscular mycorrhizal fungi.

6.7 Molecular Identification of AMF

Analysis of DNA of AMF involves the use of various markers. The use of these markers began in the early 1990. The smallest subunit (SSU) rRNA gene, the internal transcribed spacer (ITS), and the large subunit (LSU) rRNA gene are currently the most commonly used DNA markers. Ecological study used the SSU region, while taxonomic construction of the Phylum Glomeromycota utilizes ITS and LSU region.

6.8 Steps of AM Symbiosis

AM symbiosis increases the area of soil from which the plants can access mineral nutrients as the fungal hyphae grow in areas which the plant roots have not accessed (Smith and Read 2008). After the germination of fungal spores, some morphological changes (e.g., fan like structures of hyphae and increase in hyphal length) occur in the hyphae which increase the possibility of contact between hyphae and host roots (Fig. 6.4). The biochemical (chemical composition) and topographical properties

(configuration of the surface) of the host root cell wall help in the formation of AM fungal appressoria (Giovannetti et al. 1993; Nagahashi and Douds 1997). The fungal hyphae grow through the plant roots by passing through the root epidermal, exodermal, and cortical cell layers to reach the inner cortex (Fig. 6.4). The arbuscules, the symbiotic functional units are formed in the cortex. The host plasma membrane invaginates and proliferates around the arbuscule (Alexander et al. 1989; Bonfante-Fasolo 1984; Gianinazzi-Pearson 1996; Harrison 1999). These studies suggested a 3.7-fold increase in host plasmalemma as a result of which the periarbuscular membrane is formed. In this way a new apoplastic space is created between periarbuscular membrane and the arbuscule, called the periarbuscular space (Bonfante and Perotto 1995; Harrison 1997). This creates a symbiotic interface which helps in the transport of nutrients to the plant roots and photosynthates from plant to the fungus. Schoknecht and Hattingh (1976) and Cox et al. (1980) suggested that the phosphate transport between the fungus and the plant occurs at the periarbuscular membrane. The movement of phosphorus from the soil to the plant occurs through fungal hyphae (Pearson and Jakobsen 1993; Sanders and Tinker 1971; Smith and Gianinazzi-Pearson 1988) and the phosphate is translocated as polyphosphates. Before flowing outward from arbuscule to the periarbuscular space, the polyphosphates are degraded to phosphate (Cox et al. 1980; Solaiman et al.

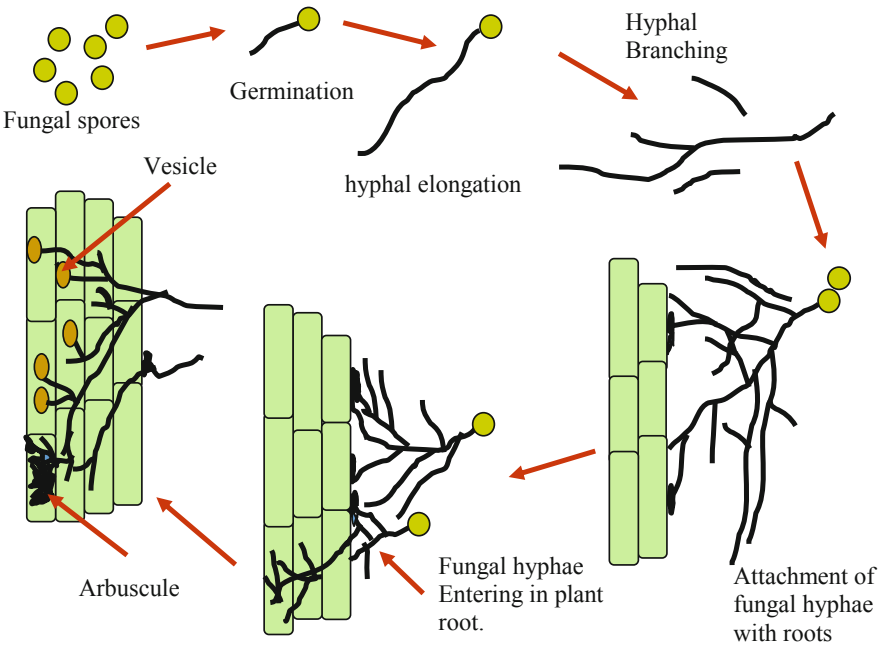

Fig. 6.4 Developmental stages of the arbuscular mycorrhizal association. 1: fungal spores, 2: spore germination, 3: hyphal elongation, 4: hyphal branching, 5: hyphal attachment to plant root, 6: penetration of plant roots by fungal hyphae, 7: arbuscules and vesicles formation

1999). Different stages of development of arbuscular mycorrhizal symbiosis are shown in Fig. 6.4.

6.9 Mechanism of Nutrient Transport in AM Symbiosis

The symbiotic interfaces for the transport of nutrients are developed during the colonization process of host plant root (Smith and Smith 1990). Two different types of symbiotic interface are found: (1) intercellular in which fungal hyphae occur within the intercellular spaces of root cortex and (2) intracellular in which fungal hyphae penetrate the walls of the root cells. These interfaces along with symbiotic structures (arbuscules and hyphae) play important role in nutrient transport (Gianinazzi-Pearson et al. 1991). The transport of nutrients between the two partners of the mycorrhizal association involves two processes: (1) The solutes flow out from donor organism as passive transport process into the interfacial apoplast. (2) These nutrients are taken up by active uptake by the receiver organism (Smith and Read 2008). Woolhouse (1975) hypothesized the existence of active mechanisms involved in the transfer of carbon and phosphate. The passive nutrient transport system involves the transport of nutrients to and from the plant along a concentration gradient without the use of metabolic energy. Diffusion of nutrients to the plant occurs, when these nutrients are low in concentration in the plant cells, while being higher concentration in plants, the carbon compounds are transported from the leaves to the fungal cells as fungi need carbohydrates to fulfill their food requirements.

It was proposed that the nutrient transport processes in the AM association are linked to transport proteins which are present in the plant and fungal plasma membranes (Harrison 1999). Various cytochemical studies have indicated that the periarbuscular membrane shows H + -ATPase activity; hence, a proton gradient is created which facilitates the transport of nutrients to the plant. H + -ATPases belong to a large family of pumps which are called P type ATPases. All these are energized by ATP and form a phosphorylated aspartyl intermediate during the reaction cycle, hence the name P type. The plasma membrane H + -ATPase is a single subunit protein of approximately 950–1000 amino acid residues (Geisler and Venema 2011).

The intracellular and extracellular pH is also maintained as a result of activity of H + ATPases (Smith and Raven 1979). H + -ATPases are widespread in plants, for example, they are present in guard cells and function in stomatal opening. H + ATPase in root hairs functions in the transport of nutrients. They are highly concentrated in phloem and help in long distance transport. Their main function is energization of transport of nutrients. H + -ATPases are integral membrane proteins that move metabolic solutes across the membranes against their concentration gradient. These are called transmembrane ATPases. Several genes encoding H +- -ATPases in different plants have been identified previously. Gianinazzi-Pearson et al. (2000) demonstrated the induction of two H + -ATPase genes in arbuscule-containing cells by using promoter β-glucuronidase (GUS) fusions. AM fungi regulate the expression of H + -ATPase genes in tomato (Rosewarne et al. 1999).

Ferrol et al. (2002) also indicated the presence of transcripts of H + -ATPase genes in wild type while they were absent in the mycorrhiza defective mutants plants of tomato. This indicates that H + -ATPases may be associated with mycorrhizal symbiosis.

6.10 Requirement of Phosphorus for Plants

Phosphorus is found in two forms in soil, which are organic and inorganic. A few examples of organic phosphorus are plant residues, manures, and microbial tissues. The amount of organic phosphorus in soil ranges from 5 to 90% of total soil phosphorus. Sources of inorganic phosphorus (Pi) include complexes of iron and aluminum phosphate, phosphorus absorbed on clay particles, and apatite (the original source of all phosphorus). These organic and inorganic phosphorus compounds exhibit reduced solubility in water. The phosphate concentration in the soil solution can be less than 10μM due to rapid adsorption of phosphate ions to clay and organic matter (Bieleski and Ferguson 1975; Holford 1997). Hence a lesser amount of phosphorus is available for absorption by roots, while the Pi concentration in the living plant cells is within millimolar range. Two ionic forms of phosphorus that can be absorbed by plants are $H2PO4-$ and $HPO42-$. These phosphate ions have more reactivity with the soil particles and hence become part of soil particles by fixation process (Bache 1964). The locking up of nutrients in the soil is the driving force for symbiotic relationship. This fixed form of phosphorus cannot be used by the plants. Mycorrhizal fungi help in the acquisition of phosphorus for the plants that support this symbiotic association (Bolan 1991; Smith and Read 2008). Organic sources of phosphorus (e.g. phytic acid and nucleic acids) are also acquired by mycorrhizal fungi to convert it to the available form for the plants (Jayachandran et al. 1992). The phosphatase enzyme released by the extraradical arbuscular mycorrhizal hyphae breaks down the bonds which are present between various elements of the organic phosphorus (Joner et al. 2000). Hence the majority of the plants having mycorrhizal association are better in growth as compared to non-mycorrhizal plants (Feng et al. 2003; Smith and Read 2008; Tarafdar and Marschner 1995).

6.11 Phosphate Transporters

Plants having AMF association have extraradical hyphae in the roots, which increase the area for absorption of minerals especially phosphorus (Jackobsen 1999). These extraradical hyphae have phosphate transporters, which help to absorb phosphorus from the soil (Harrison and Buuren 1995; Harrison and Dixon 1993). This explains that fungal hyphae are initial sites of phosphate uptake. Two different groups of phosphate transporters have been identified and cloned in recent years. The high affinity transporters as the name suggests show a high affinity for phosphate and operate in the micromolar range, while the low affinity transporters work at the concentrations in the millimolar range (Bieleski and Ferguson 1975). The

low-affinity phosphate transporters show sequence similarity with the eukaryotic sodium dependent phosphate transporters (Daram et al. 1999). A low-affinity phosphate transporter is present in the chloroplast membrane and it also influences the allocation of phosphorus within the plant (Versaw and Harrison 2002). High affinity phosphate transporters are expressed during phosphorus starvation conditions and have been cloned from the roots of various plant species, for example, *Medicago truncatula MtPT1* and *MtPT2* (Chiou et al. 2001; Liu et al. 1998b); tomato *LePT1* and *LePT2* (Daram et al. 1998; Liu et al. 1998a); Arabidopsis (*AtPT1* and *ATPT2*) (Muchhal et al. 1996; Smith et al. 1997). Expression of the phosphate transporter in tomato root suggested its contribution in phosphate uptake (Daram et al. 1998).

Two genes *MtPT4* in *M. truncatula* and *StPT3* in potato were found to be expressed in the arbuscule-containing cells (Harrison et al. 2002; Rausch et al. 2001). This suggests the role of these genes in symbiotic association for the transport of phosphorus. Similarly a rice phosphate transporter *OsPT11* was identified to be expressed during the arbuscular mycorrhizal symbiosis. Its activation was independent of the soil phosphate and nutrient availability, while it was strictly correlated with the degree of root colonization by *Glomus intraradices* (Paszkowski et al. 2002). Another tomato phosphate transporter had been identified that may be involved in the uptake of phosphorus. This was suggested by obtaining the high transcript levels in arbuscule-containing cells (Rosewarne et al. 1999). Chiou et al. (2001) showed that *MtPT1* protein levels decrease in roots during development of a symbiosis and it was not detected in roots colonized by AM fungi indicating that this transporter may not be involved in symbiotic phosphate transport. The activity of phosphate transporters is linked to H^+-ATPases, for the production of a proton gradient to provide a force for the transport of nutrients between fungi and plant (Schachtman et al. 1998). One member of H^+ ATPase gene family was expressed in roots of *M. truncatula* in arbuscule-containing cells (Krajinski et al. 2002) on AM colonization. The association of phosphate transporters with the periarbuscular membranes suggests the uptake of phosphorus through the mycorrhizal pathway.

6.12 Conclusion and Future Prospects

Application of AMF may replace the use of industrial fertilizers as these improve the growth of plants and protect plants under abiotic and biotic stress conditions. AMF inoculation also provides an environmental friendly approach to nourish plants. There is a need to produce different arbuscular mycorrhizal bio-fertilizers on large scale for food security. Lab experiments should be extended to different biogeochemical zones for field applications. Researchers, private and public sectors must participate to increase the production of AMF and extend its use in different countries. In the future there is need to identify genes which are responsible for AMF mediated growth of plants under salinity, drought, and other such environmental stress conditions. In this context both plant and arbuscular mycorrhizal fungus genes could be explored.

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Plant–Soil–Microorganism Interaction Involved in Natural Suppression of *Take-All* Disease

7

Paola Durán and María de la Luz Mora

Abstract

Take-all disease is the most important root disease in wheat caused by the fungus *Gaeumannomyces graminis* var. *tritici*. Considering economic importance of wheat, the disease is a serious problem worldwide. The effective and economically feasible control of the disease is a major problem around the globe. Strategies based on chemical control of take-all have been inefficient due to that the control of soil-borne pathogen is depending on the use of soil fumigants of broad-spectrum gaseous as methyl bromide, chloropicrin, metam sodium which are unacceptable in agriculture. The discovery of suppressive soils involving major plant–microbe interactions resulted in some significant advances, particularly in elucidating the role of the enzymes. These microbes through several mechanisms including the biocontrol, antibiosis, systemic resistance in plants (ISR) have made advanced progress in identifying major factors involved host range and pathogenicity determining as well as recognizing the mechanism that explains disease suppression. Moreover, the high-throughput sequencing techniques open new avenues for microbial control of plant disease considering, for example, the engineering plant microbiome to improve the plant health and food security.

Keywords

Wheat · Root disease · Soil-borne pathogen · Biological control · *Gaeumannomyces graminis*

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7.1 Introduction

Take-all disease is caused by the soil-borne pathogenic fungus *Gaeumannomyces graminis*. This pathogen causes the most important root disease of wheat (*Triticum aestivum* L.) worldwide (Hornby 1983; Cook 2003). However, Ggt can affect another cereal plants as rye (*Secale cereale* L.) and triticale (×*Triticosecale,* hybrid of wheat and rye). This fact affects significantly the cultural control of the pathogen, due to agronomic rotation is a better alternative that consists of the culture with non-susceptible crop hosts for 1–2 years (Cook 2003).

The discovery of suppressive soils limiting the proliferation or damage of the pathogen opened new alternatives for environmentally friendly techniques for soil-borne disease biocontrol. This is very important due to the soil-borne pathogen could increase their incidence as a consequence of climate change (Delgado-Baquerizo et al. 2020).

Soil suppression is defined as the ability of a natural soil to reduce or suppress the activity of plant pathogens, mostly due to the presence and activity of soil microorganisms. Their presence increases the ecosystem resilience by creating redundancy in ecosystem services, making soil less vulnerable to short-term changes in the environment (Wall et al. 2012). The suppressiveness could be achieved indirectly by creating a physical environment that limits the survival, spread, or infectivity of the pathogen, or favors the plant over the pathogen; or directly by supporting the proliferation of antagonistic microorganisms (Löbmann et al. 2016). For example, several studies showed the relation of *Pseudomonas* spp. and Ggt suppression due to the production of 2,4-diacetylphloroglucinol (DAPG) (Weller et al. 2002; Garbeva et al. 2004; Mavrodi et al. 2007; Yang et al. 2014).

Aspects of suppressiveness are still debated, as the relation between pathogen density and disease incidence. In the case of *Fusarium* sp. suppression, early studies reported no relation between these parameters (Amir and Alabouvette 1993). Mazzola (2002) defined suppressive soils as those in which disease development is minimal even in the presence of a virulent pathogen and a susceptible plant host. On the other hand, authors showed that the magnitude of suppression of take-all is largely dependent on the amount of the pathogen present in the soil relative to the natural antagonists, the cropping history, and the soil types, likely resulting in differing capability to suppress take-all (Cook 2003; Chng et al. 2015). In *Gaeumannomyces graminis*, recent studies revealed that no differences in fungal concentration between suppressive and conducive soils were found, confirming that suppressive soils had low disease incidence despite Ggt DNA concentration (Duran et al. 2018). Similarly, Chng et al. (2015) evidenced low disease severity coupled with high Ggt DNA concentrations in roots. Thus, despite that suppressive soils have been studied for over 100 years (Chandrashekara et al. 2012) and have been demonstrated for a wide range of soil-borne plant pathogens including bacteria, nematodes, oomycetes, and fungi (Table 7.1). Techniques to take advantage the important niche of suppressive soils have not developed.

Actuality, considering that recombinant DNA techniques have provided a solution to obstacles associated with the use of culture-dependent techniques generating

Table 7.1 Soil-borne pathogen suppression

Strain	Country/ source soil	Plant	Cause	Reference
Fungi				
Rhizoctonia solani, Fusarium sp	Brazil/ pasture, fallow ground, forest	Common bean	Abiotic (hydrolysis of fluorescein diacetate, CO_2) Biotic (total microbial activity)	Ghini and Morandi (2006)
Rhizoctonia solani	Egypt	Sugar beet	Plant growth promoting (PGP yeast), *Candida valida, Rhodotorula glutinis, Trichosporon asahii*	El-Tarabily (2004)
Rhizoctonia solani	India	Rice	*Pseudomonas spp*	Rangarajan et al. (2003)
Rhizoctonia solani, Pythium aphanidermatum, fusarium oxysporum	Belgium	Mungbean	PGP rhizobacteria (*Brevibacillus brevis, Bacillus subtilis*)	Li et al. (2005)
Rhizoctonia solani, Macrophomina phaseolina, Fusarium solani	Pakistan	Tomatoes	PGP rhizobacteria (*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bradyrhizobium japonicum*)	Siddiqui and Shaukat (2002)
Rhizoctonia solani	Germany	Sugar beet	Abiotic (pH) Biotic (*Actinomyces, Bacillus, Pseudomonas*)	Latz et al. (2016)
Rhizoctonia solani	Netherlands	Sugar beet	*Proteobacteria, Firmicutes, Actinobacteria*	Mendes et al. (2011)
Fusarium sp.	Substrate	Cucumber	Sludge compost: Sewage sludge (pig manure), sawdust, matured sludge compost	Huang et al. (2012)
Fusarium sp.	Substrate	Chrysanthemum	Composted sewage sludge into the Pinus bark-based substrate	Pinto et al. (2013)
Fusarium sp.	Substrate	Tomatoes	Sewage sludge and yard wastes	Cotxarrera et al. (2002)
Fusarium spp.	China	Peanut	Intercropping of peanut with *Atractylodes lancea*	Li et al. (2018)

(continued)

Table 7.1 (continued)

Strain	Country/ source soil	Plant	Cause	Reference
Pythium ultimum	Sweden	Wheat	Permanent soil cover and a balanced nutrient	Löbmann et al. (2016)
Fusarium oxysporum	Algeria	Palm groves	Soil abiotic factors (i.e., clay addition to sandy soil)	Amir and Alabouvette (1993)
Fusarium oxysporum	Korea	Strawberry	Actinobacteria	Cha et al. (2016)
Fusarium oxysporum	Brasil	Common bean	Pseudomonadaceae, Bacillaceae, Solibacteraceae, and Cytophagaceae	Mendes et al. (2018)
Fusarium solani	Pakistan	Tomatoes	PGP rhizobacteria (*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bradyrhizobium japonicum*)	Siddiqui and Shaukat (2002)
Gaeumannomyces graminis	Chile	Wheat	Soil microbiome	Andrade et al. (2011)
Gaeumannomyces graminis	Chile	Wheat	Endophytic microbiome	Durán et al. (2017, 2018)
Gaeumannomyces graminis	Australia	Wheat	Stubble retention and reduced tillage	Donovan et al. (2006)
Bacteria				
Ralstonia solanacearum	Japan	Tomato	Soil bacteria	Shiomi et al. (1999)
Xanthomonas oryzae	India	Rice	*Pseudomonas spp*	Rangarajan et al. (2003)
Streptomyces spp	USA	Potato	Lysobacter, Acidobacteria	Rosenzweig et al. (2012)
Nematode				
Heterodera avenae	UK	Oat	*Verticillium chlamydosporium, Nematophthora gynophila*	Kerry et al. (1982)
Meloidogyne javanica	Belgium	Mungbean	PGP rhizobacteria (*Brevibacillus brevis, Bacillus subtilis*)	Li et al. (2005)
Meloidogyne javanica	Pakistan	Tomatoes	PGP rhizobacteria (*Pseudomonas fluorescens, Pseudomonas aeruginosa, Bradyrhizobium japonicum*)	Siddiqui and Shaukat (2002)

independence on cultivating those organisms in the laboratory (Foo et al. 2017). Additionally, with the sequencing of RNA that includes full-length cDNA analyses, serial analysis of gene expression (SAGE)-based methods, and noncoding RNA improvement, the next-generation sequencing as "meta-omics' tools have been improved widely the progress in research that involved the study of microbiomes, defined as microbiota, metagenome, and surrounding environment of a microbial community (Sheth et al. 2016).

Here, we review new research horizon in agriculture to improve plant health by engineering microbiome from conducive to suppressive soil considering *Gaeumannomyces graminis* as a model to propose the next generation to soil-borne disease biocontrol.

7.2 The Pathogen Causing of Take-All Disease

Take-all is caused by the fungus *Gaeumannomyces graminis* (Sacc.) Arx et Olivier var. tritici (Walker) or Ggt. This fungus is an ascomycete belonging to the family Magnaporthaceae and also affects barley, rye, and related grasses as triticale, but is best known and is most important for the disease it causes on wheat (Cook 2003).

G. graminis can survive saprophytic on infected or dead root and crown debris from previous crops through parasitism causing primary infection (Fig. 7.1a, b), where the pathogen uses these substrata as source of food to infect the next wheat crop (Hornby 1983). Roots come into contact with the ascospores and dark runner hyphae of Ggt colonize the roots superficially and then penetrate directly by hyaline hyphae beneath the hyphopodia into the roots cortex and across the endodermis into the stele obtaining nutrients, carbon, and energy becoming to the secondary infection (Fig. 7.1c, d) (Gilligan et al. 1994; Fang 2009; Weller 2015).

The infection starts as a root rot, causing stunting and deficiency of nutrient in the shoots due to that the mycelia invading causes disrupting water transport and assimilates translocation due to the colonization of vascular tissues causing characteristic black lesions and runner hyphae continue to grow over the root surface, to other roots, and upward to the crown and stem bases (Cook 2003; Weller 2015). The rapid progress of the infection from root to stem basis causes yellowing of lower leaves, stunting, and premature death of plants (Cook 2003; Weller 2015). In fields symptoms appear as chlorotic spot due to the presence of symptomatic plants (Fig. 7.1c).

7.2.1 Control Methods

Strategies based on chemical control of take-all have been inefficient due to that the control of soil-borne pathogen is depending on the use of soil fumigants of broad-spectrum gaseous as methyl bromide, chloropicrin, metam sodium which are unacceptable in agriculture (Weller 2015), whereas systemic fungicides as triadimefon moves very inefficiently or not at all downward into the roots where the early

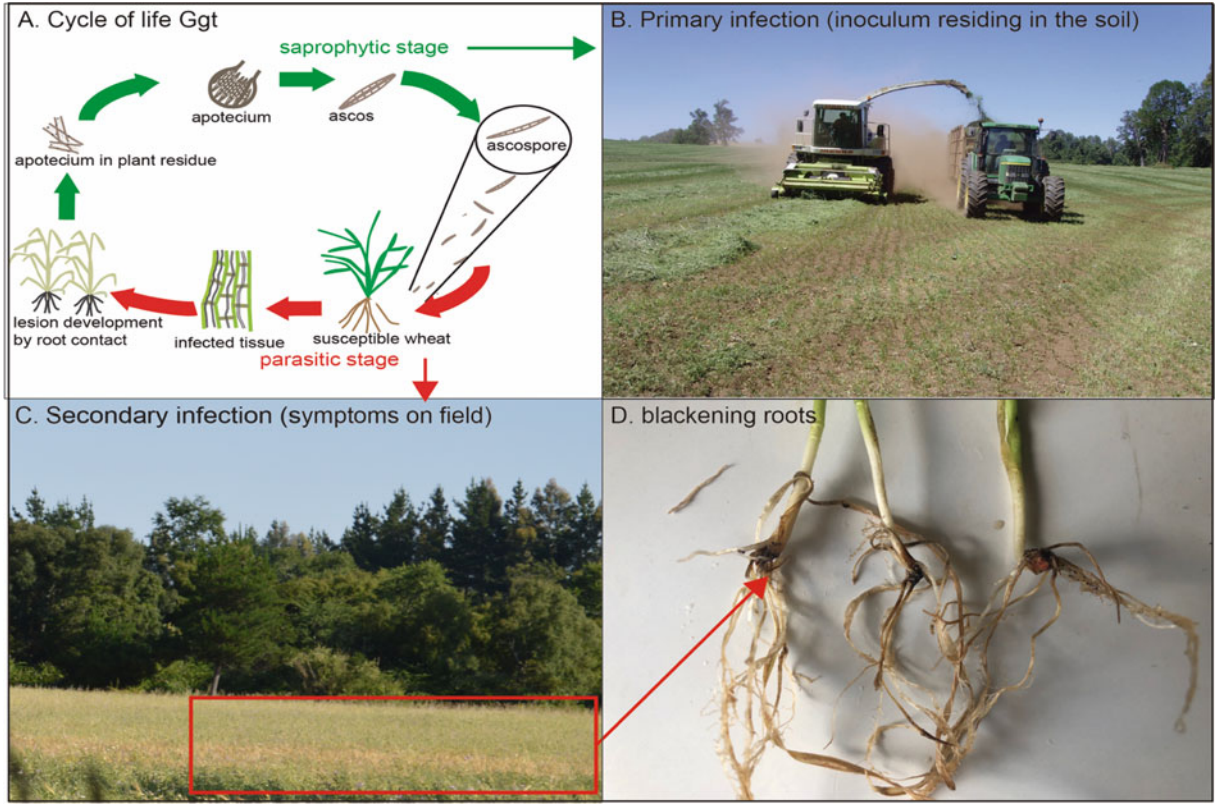

Fig. 7.1 Cycle of life of *Gaeumannomyces graminis* var. tritici (**a**) that present two stages: primary infection (saprophytic stage) where apothecium and residues of infected plants residing in the soil, (**b**) secondary infection where plants are infected by ascospores (**c**, **d**)

protection is needed (Cook 2003). On the other hand, the complexity of the fungal cycle due to the existence of the primary and secondary infection is related with the root structure. Thus, Bailey et al. (2004) showed that the seed treatments are restricted to reduce the infection to the seminal roots by particulate soil inoculum but secondary infection affects seminal and adventitious root systems and consequently not affects the ability of adventitious roots to pass on the disease. For this reason, main cultural practice to take-all control is crop rotation with no susceptible host due to the pathogen is able to survive in crop residue saprophytically as explained above. Contrary, take-all is also controlled by take-all decline (TAD), which occurs naturally with wheat monoculture wheat or barley after a severe outbreak of the disease (Hornby et al. 1998; Weller et al. 2002; Kwak et al. 2012). This phenomenon has been recently called as host-mediated microbiota engineering (Rodriguez and Durán 2020).

7.2.2 Biological Control

Biological control of take-all has been poorly studied and the most of studies has been realized under in vitro conditions. In the last years, studies related with Ggt biocontrol has been restricted to *Pseudomonas fluorescens* producers of 2,4-diacetylphloroglucinol (2,4-DAPG) (Mazzola 2002; La Fuente et al. 2004; Validov et al. 2005; Jamali et al. 2009; Kwak et al. 2009, 2012). However, in natural soil system *Pseudomonas* rhizosphere microorganisms comprise only 1–10% of the total culturable bacteria (Mavrodi et al. 2007) and culturable bacteria represent only a small portion (1–10%) of total bacteria in the rhizosphere (Nannipieri et al. 2003). In addition, *Pseudomonas* strains are highly sensible to desiccation and other adverse factors, thus the dominance and permanence in soil are a limiting aspect (Normander et al. 1999; Liu et al. 2009).

Considering that endophytic bacteria have ecological advantage over rhizobacteria due to plant tissues offer protection against environmental conditions and they have a stronger association with plants than rhizobacteria (Sturz et al. 1999; Reiter et al. 2002; Pathak and Keharia 2013). Several reports have been included endophytic microorganism to be used in agriculture (e.g., soil-borne pathogen control) (Strobel et al. 1996; Zhang et al. 1999; Strobel and Daisy 2003; Babu et al. 2013). In this context, Liu et al. (2009) showed that endophytic *Bacillus subtilis* can successfully inhibit the development of *G. graminis* and other phytopathogens under in vitro and field conditions similar to the treatment with the fungicide triadimefon. In addition, was able to promote the plant growth of wheat seedlings. Similarly, Durán et al. (2014) showed that endophytic strains *Acinetobacter* sp., *Bacillus* sp., and *Klebsiella* sp. inhibited the Ggt mycelia growth in vitro conditions (from 30 to 100%).

Due to cereal plants are able to form symbiotic association with arbuscular mycorrhizal fungi (AMF), Castellanos-Morales et al. (2012) tested the influence of *Glomus mosseae*, *Glomus intraradices*, and *Gigaspora rosea* against *Gaeumannomyces graminis*, demonstrating the influence of AMF on take-all

incidence despite different colonization rates among Glomus species. In contrast, Duran et al. (2018) reported no effect of *Claroideoglomus claroideum* in terms of root infection on wheat plants inoculated with Ggt. However, mycorrhizal plants resulted in an increase in plant biomass. Authors attributed this role to endophytic bacteria (*Acinetobacter* sp. E6.2 and *Bacillus* sp. E5) was able to diminish efficiently the pathogen incidence, confirming the role of these microorganism in order to promote the plant growth and protect against take-all under greenhouse conditions.

7.3 Soil Suppression Against Take-All Disease

Suppression is termed general suppression when it is based in a general antagonist effect of the total soil microbial biomass (Mazzola 2002; Weller 2007). In the general suppression no-specific microorganism or a selected group of microorganisms is solely responsible for the effect (Cook 2003). These specific microbes are recently called key species or core microbiome, driving the microbiome composition and function (Dong et al. 2020). Thus, general suppression is non-transferrable between soils (Andrade et al. 2011; Kwak and Weller 2013). In contrast, the so-called specific suppression, which is specific to a particular pathogenic microorganism and is mediated by specific microorganisms although using mechanisms similar to those operating in general suppression (Cook 2003; Andrade et al. 2011). It has been shown that the addition of 1% (w/w) of natural suppressive soil into sterile suppressive soil inoculated with Ggt is sufficient to transfer the suppression against take-all disease (Andrade et al. 2011; Chng et al. 2015; Durán et al. 2017).

7.3.1 Factors Required for Take-All Suppression

According to Weller et al. (2002) three factors are needed to produce take-all suppression: (1) monoculture of susceptible host, (2) presence of Ggt, and (3) outbreak of take-all (Fig. 7.2). Thus, studies showed that conductive soil where has been developed the disease and wheat monoculture produces a diminution of disease although the pathogen is present in soil "suppression" (Garbeva et al. 2004; Andrade et al. 2011). This phenomenon of take-all decline could be developed during the traditional agronomic practice of wheat monoculture, where the same crop is cultivated in the same soil continuously. Regarding the timing, take-all suppression appeared after 4–6 years of wheat monoculture (Gardener 2004), and even after and showed that soils with 3–4 years of monoculture under relatively high pathogen inoculum concentrations (Chng et al. 2015). In fact, early studies by Baker and Cook (1974) showed that 3 years of successive wheat cropping could be sufficient for the development of specific suppression.

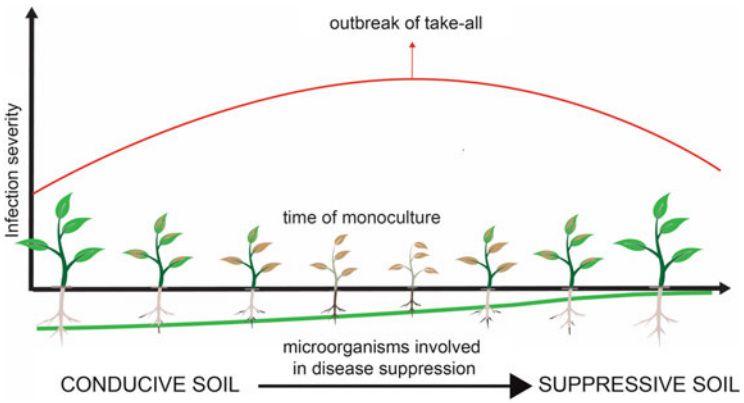

Fig. 7.2 Factors involved in take-all suppression

7.3.2 Abiotic Factors Involved in Take-All Suppression

Abiotic factors as chemical and physical parameters of soil as pH, organic matter, and clay content can influence the soil-borne suppression directly affecting the pathogen, or indirectly through the impact on the soil microbial activity (Mazzola 2002).

7.3.2.1 Soil Chemical Parameters

Physicochemical characteristics such as pH, temperature, chemical composition, texture, and humidity of a soil can influence *Gaeumannomyces graminis* suppression (Whipps 1997). For example, studies shown that *G. graminis* prefers soil with pH from 5.5 to 8.5 (Cook 2003; Freeman and Ward 2004). Thus, the pathogen is less present in soils or rhizosphere soils with less pH, which also can be attributable to the trace nutrients also can be more available in acid than in alkaline soils (Cook 2003). This fact was reported previously by Sarniguet et al. (1992), where showed that N (nitrogen) source is a determinant factor to *Gaeumannomyces graminis* inhibition. Thus, NH_4 treated soils were more suppressive to take-all disease than NH_3 one, causing more acidic on the rhizosphere and favoring the diseases suppression by soil microorganisms. Similarly, Durán et al. (2017) showed that rhizosphere microorganism from suppressive Andisol was directly correlated with soil chemistry mainly P, pH, and Al saturation. However, low knowledge about the influence of physicochemical soil characteristics on the suppression of take-all disease of wheat (Andrade et al. 2011).

7.3.2.2 Rainfall

Take-all is most severe when wheat is grown under high rainfall or irrigation generating a moist ambient, called "Wetland take-all" (Roget and Rovira 1991; Cook 2003). However, take-all can occur in zones with less than 45 cm of annual precipitation called "dryland take-all" (Paulitz et al. 2002).

However, it is important to consider other abiotic factors that could coexist in soils. Therefore, criterion is difficult to apply to suppression mediated by abiotic factors. In fact, studies showed that suppression is more related with soil microbiome (biotic factors) than abiotic factors due to when soils is sterilized (discarding the effects of soil microorganisms) (Durán et al. 2017). Thus, soil suppression could result from biotic and abiotic factors through a diverse and complex set of mechanisms, the biotic aspects, mainly related to the soil microbiota activity.

7.4 Plant–Microbe–Soil Interactions Involved in Take-All Suppression

7.4.1 Microbial Rhizosphere Effect on Soil Suppression

The mechanisms implicated in disease suppression by microbial antagonists include competition for nutrients and colonization sites, antibiosis, synthesis of hydrogen cyanide (HCN), siderophores production, secretion of cell-wall degrading enzymes, production of volatile compounds, lowering ethylene, bacteriophages, interference with the pathogen quorum sensing (quorum quenching), and induction of plant systemic resistance (ISR) (Bakker et al. 2013; Glick 2015). Thus, microbial rhizosphere may act directly or indirectly through parasitism or antibiosis, amensalism or competition for resources (Fig. 7.3).

A study realized by Latz et al. (2016) showed that *Rhizoctonia solani* suppression in potato plants was mediated by rhizosphere bacteria belong to Actinomyces, Bacillus, and Pseudomonas genera (Latz et al. 2016). Trivedi et al. (2017) showed that *Fusarium oxysporum* suppression could be attributed to multiple soil microbial genera where Actinobacteria phyla act as biological indicator of soil suppression against *F. oxysporum* due to inhibit 25% of pathogen growth when the relative abundance of Actinobacteria was above 8%, suggesting this microbial phyla as biological indicator of soil suppression against *F. oxysporum.* Thus, plants could repel or attract (recruit) microbes by using exudates exerting a significant effect on the general health or by managing agronomic practices (Duran et al. 2018; Harkes et al. 2020). Highlighting that microbial selection should consider the origin of the microbes, obtaining and culturing of functional core microorganisms and to optimize the microbial interactions according to their compatibility (Arif et al. 2020).

7.4.2 Antibiosis Influence on Soil Suppression

Antibiosis are commonly the most studied of the mechanisms involved in disease suppression from microbial rhizosphere species. An antibiotic is a secondary metabolite with biocide activity produced by microorganisms to maintain their niche and territory and to enhance survival prospects in competitive environment (Troppens et al. 2013). Their production is a normal part of the self-protective arsenals of multiple microbial species, and consequently these organisms have a great potential

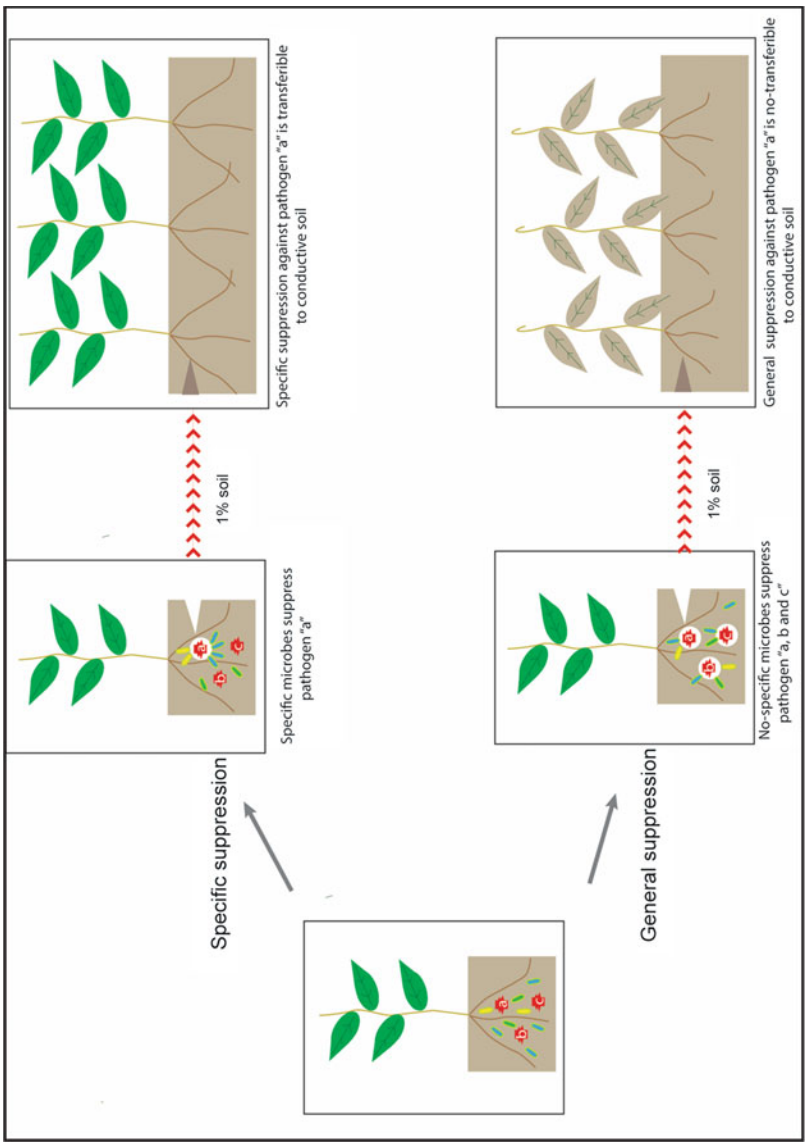

Fig. 7.3 Types of suppression: specific suppression that inhibits the infection of a particular soil-borne pathogen and general suppression or no-specific that inhibits the infection of two or more soil-borne pathogens

for soil conditioning (Pereg and McMillan 2015). Among soil microorganisms, bacteria belonging to genera *Streptomyces*, *Bacillus,* and *Pseudomonas* are particularly prolific producers of secondary metabolites (Troppens et al. 2013). For example, it is well known that several groups of antibiotics are involved in the suppression of fungal phytopathogens by fluorescent *Pseudomonas* spp. like phenazines, pyoluteorin, pyrrolnitrin, and the polyketide 2,4-diacetylphloroglucinol (DAPG) (Yang et al. 2014).

DAPG has been reported as an efficient inhibitor of bacteria, fungi, oomycetes, and nematodes (Troppens et al. 2013). Indeed, this antibiotic is highly capable to inhibit efficiently Ggt and other soil-borne pathogens as *Rhizoctonia solani* (Garbeva et al. 2004; Yang et al. 2014). Early studies carry out by Baker and Cook (1974) showed that repeated monoculture of take-all susceptible host favored the presence of dominant microbial species in the rhizosphere. Later research revealed that microbial activities in the soil were likely responsible for the onset of take-all decline (TAD) (Cook 2003; Weller et al. 2002), as it is the case of populations of 2,4- diacetylphloroglucinol (2,4-DAPG)-producing (Phlþ) *Pseudomonas fluorescens*. Ggt is highly susceptible to the antibiotic 2,4-DAPG, which accumulates in the rhizosphere in sufficient amounts for disease control when the bacteria reach a (above a threshold density of 10^5 CFU g^{-1} root, Weller et al. 2002; Weller 2007). Despite 2,4-DAPG is known to induce systemic resistance (Weller et al. 2012) and the pathogen do not develop tolerance in TAD fields even after decades of wheat monoculture (Kwak et al. 2009). Furthermore, 2,4-DAPG is clearly stable and persistent in the rhizosphere (Kwak et al. 2012), studies realized by Brazelton et al. (2008) reported that 2,4-DAPG altered tomato root morphology and physiology, causing brown roots and inhibition of primary root growth and stimulation of root branching. Later, Kwak et al. (2012) showed similar alterations in wheat roots at final concentration of 10 μg mL^{-1}. Recently, Durán et al. (2017) tested the influence of 2,4-DAPG-producing bacteria by phlD gene occurrence in suppressive soils from Chile, but the presence of these microorganism was only detected in one out of the six suppressive soils.

7.4.3 Plant Defense Against Take-All Disease

Disease occurs when a susceptible plant is infected by a infective pathogen under environmental conditions that favor disease (Surico 2013). However, plants are able to induce defense mechanisms against infectious diseases (basal resistance). These mechanisms can be grouped into pre-existing barriers and post-existing. In the case of pre-existing mechanism against pathogens is well known the structural defense mechanisms (i.e., the wax layer and cuticle, epidermal layer, cytoskeleton) and pre-existing biochemical defense are well known (i.e., phytohormones, phytoanticipins, anti-microbial compounds (i.e., terpenoids, pyrethrins, diterpenoids, saponins) (Doughari 2015).

Saponins are glycosylated triterpenoids (triterpenoids with attached sugar groups) that are present in the cell membranes of many plant species which have deter

properties and act by disrupting the cell membranes of invading fungal pathogens (González-Lamothe et al. 2009). In general, cereals and grasses are deficient in saponins. However oat had been widely described as saponin producer and has been early implicated in the resistance of oats to *Gaeumannomyces graminis* var. avenae (Osbourn et al. 1994). The antifungal activity of avenacin is associated with complexes formation with sterols present in fungal membrane leading to pore formation and loss of membrane integrity (Morrissey and Osbourn 1999). The localization of avenacin is in the epidermal cell layer of oat root tips and in the emerging lateral root initials, suggesting a role as a chemical barrier (González-Lamothe et al. 2009). Moreover, roots of plant also may interact via plants by priming plant defense reactions and rhizodeposits that in turn may select microbial populations in the rhizosphere and soils can influence the interaction among microorganisms themselves (Glick 2015). Thus, microbial through several mechanisms including the suppression of infectious diseases, for example, inducing systemic resistance in plants (ISR) which has been recognized as the mechanism that at least partly explains disease suppression (Bakker et al. 2013).

7.4.4 Induced Systemic Resistance (ISR) as the Mechanism of Disease Suppression

Induced resistance could be triggered by abiotic and biotic (including avirulent strains). In general, induced resistance is of the systemic type due to defensive capacity may be produce in non-infected tissues (Van Loon et al. 1998). Induced systemic resistance (ISR) is a state of enhanced defensive capacity developed by a plant when appropriately stimulated and induced by a PGPB (Van Loon et al. 1998; Glick 2015). However, plants also may develop systemic resistance induced by the pathogen itself is called systemic acquired resistance (SAR). However, induced resistance is not always expressed systemically and only is located in tissues primarily involved (Localized acquired resistance, LAR) (Van Loon et al. 1998). SAR and LAR are similar in terms that could be effective against various types of pathogens. However, SAR is characterized by an accumulation of salicylic acid (SA) that also can be stimulated by exogens application of SA, whereas ISR has been involved with the accumulation of jasmonic acid and ethylene (Glick 2015). Therefore, SA and JA are major hormonal regulators of the plant immune signaling network, where SA is typically effective against infection by biotrophic pathogens, whereas JA is essential for the immune response against necrotrophic pathogens and herbivorous insects (Pieterse et al. 2012).

During the last decades ISR has been recognized as an effective mode of action for a range of microbial that acts as biological control agents. Thus, disease suppression has been evolved with competition for nutrients, antibiosis, and ISR (Bakker et al. 2013). In this context, several studies showed that Pseudomonas can activate a plant defense system by ISR in wheat plants affected by *Gaeumannomyces graminis* by the production of 2,4-DAPG antibiotic (Kwak and Weller 2013). However, the

effectivity of *Pseudomonas* strains as bioinoculant is limited due to their low capacity of survival on soil.

7.5 Conclusions

Studies related with soil disease suppression are numerous, mainly considering biotic factors associated with the disease incidence diminution as microbial composition; however, the mechanisms involved are multiple and complexly interconnected. In fact, studies of plant–microbe–soil interactions guaranty a better understanding of these processes to facilitate their successful applications in biotechnology. Mainly, based in the important niche that offer suppressive soil in terms of microbial effect against to *Gaeumannomyces graminis*, considering that this soil could be lost in the short term due to industrialization and intensive agriculture. The next-generation sequencing opens new alternatives to plant biocontrol, considering, for example, the engineering plant microbiome in order to improve the plant health and food security.

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Improved Practices Through Biological Means for Sustainable Potato Production

8

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Abstract

Potato (*Solanum tuberosum* L.) is the most important non-grain food crop in the world; ranking fourth in terms of total production. It is grown in around 150 countries spread across both temperate and tropical regions at elevations up to 4000 m. Globally, production of potato amounted to approximately 376.83 metric tons. In India, West Bengal is the largest potato producing state. Potato holds a great potential as food for ever increasing population. Potential yields of potato are determined by the characteristics of the crop and various biotic and abiotic factors. Among biotic factors, pathogens like fungal, bacterial, viral, insects, and nematodes play a crucial role leading to overall yield loss of 30–40%, thus threatening its food security. In order to increase the potato production holistic crop protection approach with a range of strategies encouraging natural pest predators, breeding varieties with pest/disease resistance, planting certified seed potatoes, growing tubers in rotation with other crops, and organic composting to improve soil quality are evident. Integrated Nutrient Management (INM) also helps in improvement of quality and quantity of production besides enhancing the sustainability and health of the soil. Proper use of insecticides has proven effective when used as an additional tool in integrated pest management (IPM) practices. Traditional management practices like the use of host-plant resistance, mechanical, biological, chemical, and cultural means of control are not fully expolerd. Conservation farming practices also play important role to

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restore soil and enhancing soil health and play important role to combat climate change issue. The present chapter discusses the importance of improved agronomic practices for sustainable potato production.

Keywords

Solanaceae · Agronomic practice · Pest management · Tuber · PGPR · Sustainable agriculture

8.1 Introduction

Potato, a member of the nightshade family *Solanaceae* and order *Polemoniales*, is an annual herbaceous dicotyledon as far as its vegetative and flowering habits are concerned, but it may be regarded as a perennial as far its capacity for reproduction employing the tubers is considered. Worldwide, potato is the third most important food crop after rice and wheat (FAO 2011). It is grown in 149 countries from latitudes of 65°N to 50°S and from altitudes ranging from sea level to 4000 m (Paul and Ezekiel 2013). It is comprehensively cultivated in China, Russian Federation, Ukraine, Poland, Ireland, Great Britain, Germany, Netherlands, France, Spain, South America, India, and the USA (Mukherjee 2017; Wang et al. 2020). The crop originates from the Peruvian and Bolivian Andes in South America, specifically in Lake Titicaca Basin on the border between Peru-Bolivia. The crop was introduced in India in the mid-seventeenth century probably by Portuguese traders or British missionaries. Potato is one of the principal cash crops of India. The crop covers an area of about 1.86 million ha with an annual production of 41.46 million tons (Mt) with average productivity of 23.12 t/ha in India (2014–2015). West Bengal is the world's highest per day potato productivity state (300 kg/day) and ranks second after U.P in terms of area and productivity (32.96 t/ha). Nutritionally, potato is second to soybean for the amount of protein/ha, with the major storage protein being patatin, one of the most nutritionally balanced plant proteins known and regarded as a wholesome food (Liedl et al. 1987). It contains water (75–80%), carbohydrate (22.6%), starch (14%), sugar (2%), protein (1.6%), fat (0.1%), fiber (0.4%), minerals (0.6%), vitamins (vitamin C rich 17 mg), and energy (97 kcal) (Mukherjee 2017). Besides, it is also a good source of vitamin B (B_1, B_3, and B_6) and minerals such as potassium, phosphorus, and magnesium, and contains folate, pantothenic acid, and riboflavin. It is also a source of essential amino acids like lysine, leucine, tryptophan, and isoleucine. Moreover, potato is low in fat. The crop is also a moderate source of iron, and its high vitamin C content fosters iron absorption. It also contains antioxidants, which also play a critical role in preventing diseases related to aging, and dietary fiber ultimately benefits health.

In the emerging global economic order, the development of agricultural crops is witnessing a rapid transition to the production of agricultural commodities, with potatoes appearing to be a significant crop, ready to sustain and diversify food

production in the new millennium. Temperature and unpredictable drought are the two most important abiotic factors, thus affecting world food securities. In developed countries, especially in Europe and the Commonwealth of Independent States, productivity of potato has decreased by 1% per annum over the last 20 years. However, output in developing countries has expanded at an average rate of 5% per year (Falloon and Betts 2010; Wadas and Dziugieł 2020). Asian countries, especially China and India, stoke up this growth. In the recent past, the developing countries share 52% of global potato output stood surpassing that of the developed world. This is a great achievement, considering the share of potato in global production was little more than 20% twenty years ago in the developing countries (Collier et al. 2008). Globally, production and consumption of potato is steadily increasing than the global population. Fresh potato consumption, once the pillar of world potato utilization, is declining in many countries, especially in developed regions. This is mainly because of the harsh weather condition which alarms the selling price. Thus, it becomes a critical threat to future food security (Rana et al. 2020; Mukherjee 2002).

Increasing the production of potato in adverse conditions would require innovative technology to supplement conventional methods that are unable to prevent yield losses. Various agronomic practices not only improve the soil quality but also enhance the yield of potato. Application of N in two split doses, i.e. half at the time of planting and rest at the time of earthing up to produce higher yields and higher N recovery (Du et al. 2020). Equitable use of major and micronutrients plays an important role in improving the quality of produce besides good yield. Integrated nutrient management must for a crop like a potato. Moreover, the proper use of insecticides has proven effective when used as an additional tool in integrated pest management (IPM) practices. The use of bio-resources such as plant growth-promoting rhizobacteria (PGPR) and other conservation farming practices also play an important role to restore soil and enhancing soil health and play an important role to combat climate change issues.

This chapter focuses on the major factors affecting potato production, various components for its management like integrated nutrient management (INM), integrated pest management (IPM), conservation farming, and cultural practices to improve soil quality that helps to restore degraded soils which leads to enhance its production and yield. It also describes various management practices for sustainable production.

8.2 Factors Constraining Potato Production

Many factors affect the productivity of potato. As potato (edible and reproductive part) is the semi-perishable tuber, there are more chances for disease to accumulate in each planting season which ultimately affects its yielding potential. Other constraints such as traditional potato production system, scarce germplasm resources for cultivar

development, shortage of high-quality seed potatoes, limited knowledge on postharvest handling of the product, and poor technology transfer systems also hinder its productivity (Adane et al. 2010). Moreover, storage and transportation technologies are also affecting potato production, as they are the major constraints for the healthy development of the potato industry. Several factors are affecting the growth and production of potato which are listed in Table 8.1.

8.3 Agronomic Management Practices

An agronomic practice alludes to the scientific investigation of soil management and crop production. It includes the water system and the use of herbicides, pesticides, and compost. Agronomy stresses staple sustenance crops, for instance, corn, rice, potato, beans, and wheat, which are made on a far-reaching scale and address the foundation of our human sustenance supply. Various agronomic practices that help in the sustainable production of potato presented in Fig. 8.1.

8.3.1 Integrated Nutrient Management (INM)

Integrated nutrient management is agronomic practice for the adjustment and maintenance of soil fertility and provides nutrients to the plant at an optimum level for sustaining crop productivity through optimization of all possible resources of plant nutrients in an integrated manner. This practice of nutrient management achieved greater significance in the last few years because of two reasons. First, fertilizer production in India at the present level is not enough to meet the entire plant nutrient requirement to meet productivity. Secondly, long-term experiments (LTEs) conducted in India or elsewhere reveal that neither the organic sources nor the fertilizers in isolation can achieve sustained production under intensive cropping (Serderov et al. 2020). The major components of INM are fertilizers, organic manures, legumes, crop residues, and bio-fertilizers which are explained below.

8.3.1.1 Chemical Fertilizers

Fertilizers contribute to be the most important component of INM. To supply large amounts of nutrients in intensive cropping with high productivity there is increased independence on fertilizers. Moreover, their consumption is not only inadequate but also imbalanced. The N:P_2O_5: K_2O use ratio is quite wide, whereas application of micronutrients and K, S is usually ignored. The domestic production of fertilizer is not sufficient to meet the requirements. On the other hand, problems like global price hike of fertilizers and raw materials would not permit fertilizer import in large quantities leading to a big gap between fertilizer supply and consumption. While organics and bio-fertilizers are expected to bridge a part of this gap, the effective use of fertilizers in narrowing the nutrient supply gap also needs greater emphasis.

Table 8.1 Various factors affecting potato production

Factors	Description
Biological characteristics	The biological characteristics of potato are itself a big constraint. The characteristics like low multiplication rates of seed tubers, costs related issues for maintaining seed quality through successive multiplications, and other technical difficulties, owing to the potato's susceptibility to soil and seed-borne insect pests and diseases
Lack of efficient seed systems	For regular multiplication and distribution of certified seed tubers and the rapid deployment of new and improved varieties many developing countries lack efficient systems. Factors includes lack of managerial expertise, limited technical capacity, and inadequate resource allocations to seed systems
Diseases and insect pests	Diseases and insect pests are another major constraint. New strains of late blight have continued to spread in many developing countries. Late blight constitutes the most serious threat to potato production. Second to late blight is bacterial wilt found particularly in warmer, more tropical regions also pose severe threat to potato production. The impact of insect pests varies between regions and seasons of the year. Major insect pests include aphids, tuber moths, leaf miners, Colorado potato beetle and Andean potato weevil
High production costs and lack of credit	In comparison to other food crops, production of potatoes is capital-intensive. With limited access to credit and few means of mitigating the risks, small-scale farmers find it difficult to compete in potato production. The current global financial crisis could leave a great number of farmers with little money and no credit to invest in production
Price instability	Small-scale potato growers are susceptible to abrupt changes in input and output prices. Year-to-year and seasonal price changes can affect small growers who lack the financial resources and resilience of larger producers and cooperatives
Inefficiency of local markets	Potato prices are usually decisive by supply and demand. It is a crop of low-income farmers and consumers to ride out episodes of food price inflation. However, its profitability totally depends on efficient local markets
Limited access to higher value markets	Rapidly growing processing segment as well as to potato export markets helps the small-scale potato growers to earn profit
Inadequate capacity building initiatives	Programs should be carried out in order to upgrade the skills of potato growers need to be matched by government efforts like monitoring and enforce regulations on pesticide use
Lack of support to farmer organizations and entrepreneurs	Support for local entrepreneurship and potato farmer groups is lacking in many developing countries to improve seed quality and promote variety development. In Argentina, efforts are being made by public and private sector to transfer technology for integrated crop management to its contract growers

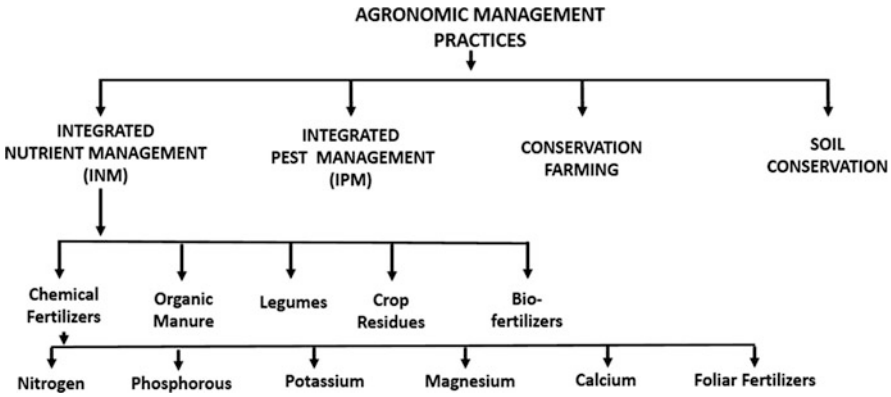

Fig. 8.1 Agronomic management practices for sustainable potato production

Fertilizer utilization by the crops varies from 30 to 50% in the case of N, 15–20% in the case of P, and less than 5% in the case of micronutrients. Thus a significant amount of applied nutrients is lost through various pathways. Increased nutrient use efficiency should be a prioritized area of research for restoration and improvement of soil health and minimizing the cost of crop production.

Nitrogen

The amount of nitrogen applied to a potato crop varies from 100–300 kg/ha depending on the soil characteristics and purpose of the crop. However, excessive or high nitrogen doses stimulate haulm growth, delay tuber formation, and ultimately affect tuber quality (low dry matter content, high reducing sugar content, and high protein and nitrate content). However, a split application might be preferred if there is a risk of leaching (i.e., heavy watering on light soils) or scorching (application of large quantities of fertilizer under dry conditions).

Phosphorus

Phosphorus imparts to the early development of the crop and tuberization. It enhances the crop's dry matter content and ameliorates the tuber's storage quality. Usually, more than 100 kg/ha is applied, while on phosphorus-fixing soils much higher doses are preferred.

Potassium

Potassium not only boosts yields but also improves tuber quality of potato (size, starch content, and storability). An ample supply of potassium can also help to minimize internal blackening and mechanical damage and has been associated with increased stress tolerance.

Magnesium

Attention should be paid to magnesium requirements, especially when potatoes are grown on light acid soils. Inadequate application of potassium and nitrogen in the form of ammonium reduces the uptake of magnesium.

Calcium

Potatoes are tolerant of soil acidity, hydrogen ion concentration of 4.8 leads to crop failure due to calcium deficiency. Liming may be necessary. Seed potatoes need to be grown in soils with sufficient calcium. Seed tubers which are calcium deficient are failed to sprout properly.

Foliar Fertilizers

Plants take nutrients more efficiently through stomata in their leaves as compared to root. Foliar fertilizers contain both macro and micronutrients. They are absorbed by the leaves and have an immediate effect on plant growth. They may help to overcome apparent nutrient deficiencies, especially micronutrients, and support plant recovery following stress events, such as frost and drought.

8.3.1.2 Organic Manures

Organic manures like FYM, urban compost, crop residues, human excreta, rural compost, sewage-sludge, press mud, and other agro-industrial wastes have large nutrient potential. Traditionally FYM and compost have been the most important manures for maintaining soil fertility and ensuring yield stability. Other organic sources of nutrients such as non-edible oilcake and wastes from various industries are also there. Besides, there are several industrial by-products and municipal wastes with fair nutrient potential. However, these nutrient-carriers have not been properly evaluated to establish their fertilizer equivalents. Thus, there is an urgent need to integrate these sources depending on their availability in different crops and cropping systems. The industrial by-products like spent-wash from a distillery, molasses, press mud, etc., from the sugar industry and wastes from other food processing industries have good manorial value. Sulphitation press mud (SPM) has a great potential to supply nutrients and has favorable effects on soil properties. SPM has assumed great importance as a nutrient supplement in sugarcane-ratoon-wheat and other intensive cropping systems of the sugarcane growing areas. Sewage-sludge and municipal solid wastes (MSW) are also important nutrient sources available for integration with fertilizer inputs, but proper cautions have to be taken to avoid any potential threat of pathogens and heavy metal load. These nutrient sources have lost their relative importance over time in crop production as they are bulky in nature with low nutrient content and short in supply. Although, cost and their limited supply made it necessary to search for alternative and renewable sources of plant nutrients leading to major interest in organic recycling. Less than 50% of the manurial potential of cattle dung is utilized at present, a major proportion is lost as fuel and droppings in non-agricultural areas. Cattle dung and other farmyard wastes recycled back to the soil as manure, substantial nutrients are lost due to inadequate methods of manure preparation and its amount of application. Organic manures not only supply

macro and micronutrients, but it also help in improving the physical, chemical, and biological properties of the soils.

8.3.1.3 Legumes

Legumes are considered as soil fertility restorers because of their ability to obtain N from the atmosphere in symbiosis with rhizobia. Legumes are a major ingredient of INM when grown especially for fodder or grain in a cropping system, or when introduced for green manuring. Legumes grown as green manure, forage, or grain crops improved the productivity of the rice-wheat cropping system (RWCS) and rejuvenated soil fertility (Yadav et al. 2000).

8.3.1.4 Crop Residues

Crop residues have several competitive uses and are considered as an important component of INM. However, in North-West India mechanical harvesting is still practiced and the leftover residue is used in the field as a part of nutrient supply. Moreover, cereal crop residues are valuable cattle-feed and it can be used to supplement the fertilizers. Disposal of rice straw has been a great concern in Trans and Upper Gangetic Plains. In these areas, farmers prefer to burn all these residues in situ which causes environmental pollution on one hand and loss of potential nutrients on the other hand. Although, residue recycling in the field helps to build stable organic matter in the soil and also helps to sustain the yield. Usually, stubbles varies from 0.5 to 1.5 t/ha in traditional harvesting methods. However, its amount is much higher in practices like mechanical harvesting. Stubbles produced from coarse cereals, i.e. sorghum, maize, pearl millet, etc., are difficult to decompose and are normally collected and burnt during land preparation causing significant loss of plant nutrients.

8.3.1.5 Bio-Fertilizers

Bio-fertilizers are the materials containing living or latent cells of agriculturally beneficial microorganisms that play an important role in improving soil fertility and crop productivity due to their ability to fix atmospheric N, solubilize/mobilize P, and decompose farm waste resulting in the release of plant nutrients (Giri et al. 2019). The benefit from these microorganisms depends on their number and efficiency which, however, is governed by soil and environmental factors. Bacterial cultures, i.e. *Rhizobium, Azospirillum*, and *Azotobacter* have the potential to fix atmospheric N which in turn escalates N supply to the crops. Bacterial cultures of *Pseudomonas* and *Bacillus* and fungal culture of *Aspergillus* help in the conversion of insoluble P into usable forms, hence, improve phosphate availability to the crops. Similarly, Arbuscular Mycorrhizae (AM) fungi increase uptake of P with larger soil volume. *Rhizobium* is the primary symbiotic fixer of N and it is the most well-known bacterial species. These bacteria lead to the formation of lumps or nodules where the N fixation takes place by infecting the roots of leguminous plants. The bacterium's enzyme is a rich source of N to the host plant to furnish nutrients and energy for the activities of the bacterium. The *Rhizobium*-legume association can fix up to 100–300 kg N/ha in one crop season and certain situations leave substantial N for

the crop. This symbiotic association could meet 80% of the N requirement of the legume crop. *Azotobacter* free-living N-fixer imparts positive benefits to the crops through a small increase in N input from BNF, development, and branching of roots, production of plant growth hormones, enhancement in the uptake of NO^{-}, NH^{+}, H PO^{-}, K^{+}, and Fe^{+}, improved water status of the plants, increased nitrate-reductase activity, and production of antifungal compounds. In irrigated wheat, a significant response to *Azotobacter* inoculation was recorded in a large number of on-farm trials. *Azotobacter* contribute 20–25 kg N/ha. *Azospirillum* fixes N by colonizes with the root mass. Hence, shows positive interaction with applied N in several field crops with an average response equivalent to 15–20 kg/ha of applied N. Several strains of P solubilizing bacteria and fungi have been isolated, and inoculation with P solubilizing microbial cultures is known to increase the dissolution of sparingly soluble P in the soil. Integrated use of microbial cultures with low-grade rock phosphate might add 30–35 kg P_2O_5/ha. Soil inoculation with *Pseudomonas striata* showed a residual effect in succeeding maize on alluvial soil of Delhi, besides increasing grain yield of wheat.

In recent years, K mobilizing bio-fertilizers (KMB) and Zn solubilizing bio-fertilizers (ZnSB) have been added in order to increase the solubility of K and Zn in soil, respectively. There is an extensive need to assess bacteria which play important role in soil solubility (K and Zn). Also, liquid bio-fertilizers have proved superior over conventional (solid) carrier-based ones. Blue-green algae (BGA) is also another important source of N to wetland rice. As per the estimates, N fixed by BGA inoculation is varied from 20–30 kg N ha-^{1}. Various field studies have also shown that the incorporation of *Azolla* would allow N applications to be reduced by at least 30–40 kg/ha (Dwivedi et al. 2004).

8.3.2 Integrated Pest Management (IPM)

Pest problems may vary from field conditions and seasons because of differences in soil type, cultural practices, cropping history, cultivar, and the nature of surrounding land. Market choice and market conditions also affect the feasibility of management because they determine how a crop must be handled and the value of that crop. Four components are essential to any IPM program: (1) Accurate pest identification, (2) field monitoring, (3) control action guidelines, and (4) effective management methods (Fry 1982).

Almost all pest management tools, including pesticides, are effective only against certain pest species, one must know which pests are present and which are likely to appear. By monitoring, one can get information to make management decisions. Monitoring includes keeping records of weather, crop development, and management practices as well as incidence and levels of pest infestations. Control action guidelines indicate when management actions including pesticide applications are needed to abstain losses due to pests or other stresses.

8.3.3 Conservation Farming

Conservation farming aims at enhancing natural biological processes both above and below ground. The major role of conservation farming is (1) Minimization of mechanical soil disturbances, (2) permanent organic soil cover, (3) diversified crop rotations. By minimizing soil disturbance, it creates a vertical macro-pore structure in the soil, which facilitates the infiltration of excess rainwater into the subsoil and thus improves the aeration of deeper soil layers and further facilitates root penetration.

8.3.4 Soil Conservation

Soil erosion is a major constraint that continues to threaten the sustainability of both subsistence and commercial agriculture. Cultivation of potato requires intensive soil tillage practices throughout the cropping period, which ultimately leads to soil erosion, degradation, and leaching of nitrates. The use of mulch at planting and the "notill" land preparation method is recommended to reduce soil degradation, erosion, and nitrate pollution, and to restore degraded soils aided good potato yields with less requirement of fertilizer. The mulch helps to protect the soil from erosion in the first weeks of the crop. Although mulching reduces the risks of soil erosion and nitrate leaching, it may have some adverse effects (e.g., excessive moisture and reduced soil temperature leading to retarded plant emergence). Hence, it should not be a blanket recommendation. The no-tillage potato is grounded into the soil surface and then covered with a thick layer of mulch, preferably straw, which is fairly stable and does not rot quickly.

8.4 Cultural Practices

Potato tuber develops entirely underground, its quality, shape, disease, and yield are usually influenced by factors like moisture content and humus, texture, and temperature of the soil in which it grows. All these factors render soils for potato production. Therefore, to cope with the adverse effects which affect potato production, it is not only desirable but imperative that proper cultural practices involving strict cognizance of the best methods should be adopted for improving soil conditions. The various cultural practices which enhance potato production are depicted in Fig. 8.2.

Fig. 8.2 Cultural practices for sustainable potato production

8.5 Management Methods

8.5.1 Seed Quality and Certification

Pests can be transmitted in infected seed tubers, including blackleg, bacterial ring rot, late blight, common scab, potato viruses, *Rhizoctonia,* powdery scab, root-knot nematodes, silver scurf, and wilt diseases. To prevent these problems, one must start with healthy stock (Agios 1997). Techniques like micro-propagation and stem cutting have been developed to obtain pest-free potato plants for propagation and production of certified seed tubers. Disease-free stem cuttings or tiny pieces of meristem tissue are cultured and propagated under sterile conditions to produce large numbers of disease-free plantlets or mini tubers (Anonymous 2008).

8.5.2 Biological Control

Any activity of a parasite, predator, or pathogen that keeps a pest population lower than it would be considered as biological control. One of the first assessments that should be made in an IPM program is the potential role of natural enemies and hyper-parasites in controlling pests. Control by natural enemies and hyper-parasites is inexpensive, effective, self-perpetuating, and not disruptive of natural balances in the crop ecosystem.

Bacteria combative to *Erwinia carotovora* are being developed as seed piece treatments for abbreviating seed piece decay and blackleg. Among rhizobacterial *Agrobacterium radiobacter*, *Bacillus subtilus,* and *Pseudomonas* spp. are antagonistic to potato cyst nematodes (*Globodera pallida* and *G. rostochiensis*) though *Pasteuria penetrans* attach PCN (Kerry et al. 2003). Larkin (2007) reported that soil-application of aerated compost tea (ACT) and the combination of ACT with a mixture of beneficial microorganisms reduced stem canker, black scurf and common scab on tubers by 18–33% and 20–23% yield increase in barley/ryegrass rotation, but not in the other rotations. Table 8.2 depicts the effect of organic sources and chemical fertilizers on growth parameters of potato.

Table 8.2 Effect of organic sources and chemical fertilizers on growth parameters of potato

Organic source	Chemical fertilizer	Growth attribute	Reference
FYM	P and K	Potato haulms	Sharma (1986)
Vermicompost	50 per cent RDF	Number of leaves per plant in potato	Patil (1995)
FYM	Inorganic source of nutrients in the ratio of 1:3	Plant height, number of leaves per plant, and leaf area per plant	Sood (2007)
Poultry manure	Reduced RDF	Yield parameters and yield of potato	Md Islam et al. (2013)
FYM	NPK	Yield of potato	Boke (2014)
Cattle manure	NP	Growth rate, and leaf area, average tuber weight, and marketable and total tuber yield	Masrie et al. (2015)
Cattle manure	Mineral NP	Higher tuber yield	Isreal et al. (2018)

8.5.3 Resistant Cultivars

Plant breeding is one of the most important tools available for both the production of the best crop and the management of pests. Pest management is one of the important factors that must be taken into account while choosing cultivars. Cultivars resistant or tolerant of the disease can help reduce losses caused by some soil-borne pathogens and provide long-term, economical protection from conditions that otherwise could inflict severe losses every season (Table 8.3).

Part of every breeding program is the search for resistance to serious diseases, disorders, and nematode pests. Resistance to insect pests is being investigated. New potato breeding selections are assessed for resistance to several viruses, leaf-roll net necrosis, root-knot nematodes, *Verticillium* wilt, scab, blackleg, early blight, and several physiological disorders (Hooker 1983).

8.5.4 Chemical Control with Pesticides

Adequate use of pesticides can not only provide economical protection from pests but also reduce significant losses. In many situations, they are the only feasible means of control. Excessive use of pesticides results in crop damage and hazards to health and the environment. In an IPM, pesticides are used only when field monitoring indicates they are needed to prevent losses (Table 8.4).

Fungicides reduce damage caused by certain foliar pathogens, i.e. late blight, powdery mildew, and severe early blight. Fungicides usually applied before infection occurs or when the disease just begins to develop. Soil fumigants might be used to control nematodes or *Verticillium*.

8.6 Conclusion

The widely-cultivated potato, *S. tuberosum* L., is one of the world's principal food crops. Over the next four decades, the global agriculture industry faces major challenges, as projections suggest that the global population will be between 8.0 and 10.4 billion people, with a median estimate of 9.1 billion. Recently, released studies estimate that worldwide agricultural production will need to grow by 70% over an approximated 45-year interval (between 2005–2007 and 2050), and by 100% in developing countries. The major challenges in sustainable potato production are varying economies of scale, are heterogeneity in soil resources, nutrient availability, pest resistance, weather constraints, demographic changes, and shifts in the availability of arable lands. High-resolution geospatial studies can help in the identification of trends and patterns in local to regional scale commercial production environment, which can in turn encourage the broader adoption of adaptive management strategies that not only increase yield but also promote sustainable land use. Global environmental change (GEC) will lead to elevated temperature in many years, which will in turn involve manipulation of agronomic practices in order to

Table 8.3 Biological control of bacterial and fungal disease of potato

Biocontrol agent	Effective against	Disease	Reference
Antagonistic isolate BC8	*Pseudomonas solanacearum*	Bacterial wilt	Ciampi et al. (1989)
Bacillus subtilis BS 107	*Erwinia carotovora* subsp. *Atroseptica* and *Erwinia carotovora subsp. carotovora*	Blackleg and soft rot	Sharga and Lyon (1998)
Bacillus, *Pseudomonas*, *Rahnella*, and *Serratia*	*Phytophthora infestans* (strain US-8)	Late blight	Daayf et al. (2003)
Bacillus sp. sunhua	*Streptomyces scabiei*	Scab	Han et al. (2005)
Biocine S2HA	*Ralstonia solanacearum*	Brown rot	Kabeil et al. (2008)
Basidiomycetes	*R. solanacearum*	Brown rot	El-Fallal and Moussa (2008)
Burkholderia cepacia	*Fusarium sambucinum*, *F. oxysporum* and *F. culmorum*	Potato dry rot	Recep et al. (2009)
Pseudomonas spp. StT2 and StS3	*Rhizoctonia solani*	Potato black scurf	Tariq et al. (2010)
Pseudomonas koreensis	*Phytophthora infestans*	Late blight	Hultberg et al. (2010)
Lactic acid bacteria	*Phytophthora infestans*	Late blight	Axel et al. (2012)
Pseudomonas fluoresces (Pf2), *Bacillus subtilis* (Bs3) and *Rahnella aquatilis*(Ra39)	*Pectobacterium atrosepticum*	Blackleg	Hoda et al. (2016)
Pseudomonas and *Bacillus* genera	*Dickeya* sp. and *Pectobacterium* sp.	Blackleg	Raoul et al. (2016)
Rhizobacteria	*Globoderaro rostochiensis*	Golden nematode	Salinas et al. (2016)
Brevibacillus formosus strain DSM 9885, and *Brevibacillus brevis* strain NBRC 15304	*Alternaria alternata*	Brown leaf spot	Ahmed (2017)
Bacillus amyloliquefaciens Ba01	*Streptomyces* species	Potato common scab	Lin et al. (2018)

Table 8.4 Detailed description of potato diseases (bacterial, fungal, and viral) and their management

Disease	Causative agent	Symptoms	Management
Bacterial disease			
Blackleg	*Pectobacterium* spp.	Soft rot of seed pieces Black to brown discoloration of the stem, stunting and wilting of affected stems	Cleaning seed handling, planting, and cutting equipment is important
Aerial stem rot/aerial blackleg/aerial soft rot or bacterial stem rot	*Pectobacterium carotovorum* subsp. *carotovorum* (syn. *Erwinia carotovora* subsp. *carotovora*) *Pectobacterium atrosepticum* and *Dickeyadianthicola* (syn. *Erwinia chrysanthemi*)	Water-soaked lesion on the stem, shrivelled stems	Use whole tubers, or allow cut seed pieces to suberize, or cork over, before planting Avoid over irrigation and fertilization
Soft rot	*Pectobacterium carotovorum (*subsp. *carotovorum, odoriferum) Pectobacterium atrosepticum, Dickeyadianthicola Pseudomonas, Bacillus and Clostridium*	Tuber becomes infected, foul smelling odor, non-emergence of plants, wilting, browning of tissues, haulm desiccation and plant death	Avoid harvest when temperatures are >65–75 °F, particularly when conditions are wet Provide protection for harvested tubers from sunscald, heating or desiccation Avoid bruising during harvest and handling Maintenance of soil calcium level
Ring rot	*Clavibacter michiganensis* subsp. *sepedonicus*	Shortened internodes, slight discoloration	All tissue cultures should be tested by PCR before propagation
Brown rot	*Ralstonia solanacearum*	Prominent milky ooze when an infected lower stem is placed in water	Plant disease-free seed in non-infested soil and crop rotation
Common scab	*Streptomyces scabies*, *S. acidiscabies* and *S. turgidiscabies*	Initial infections result in superficial reddish-brown spots on the surface of tubers	Maintain high soil water levels Avoid planting scabby seed tubers Scab-resistant varieties are useful Maintain soil pH levels at 5–5.2
Fungal disease			
Alternaria Brown rot	*Alternaria alternata*	Small, dark round necrotic lesions, leaves	Cultural practices and foliar fungicides

(continued)

Table 8.4 (continued)

Disease	Causative agent	Symptoms	Management
		may be affected, drying up	Provide adequate fertilization Fungicides are very efficient for controlling brown leaf spot
Early blight	*Alternaria solani*	Small, black lesions. Spots enlarge, and by the time they are one-fourth inch in diameter or larger, concentric rings in a bull's eye pattern can be seen in the center of the diseased area	Crop rotation and destruction of plant debris and weed hosts are used to reduce the sources of inoculum Rotation, avoid over irrigation Fungicide programs are the most effective means to control the disease
Late blight	*Phytophthora infestans* (Mont.)	This disease damages leaves, stems, and tubers. Affected leaves appear blistered as if scalded by hot water and eventually rot and dry out Affected stems begin to blacken from their tips, and eventually dry out Affected tubers display dry brown-colored spots on their skins and flesh	Good field drainage and proper plant spacing for optimal air Proper sanitation is necessary At planting, seed treatment fungicides exist Deep hilling can be used to protect tubers from sporangia washing off leaves Avoid excessive fertilization to prevent canopy overgrowth Fungicide application is considered an integral part of late blight management
Powdery mildew	*Erysiphe cichoracearum*	Disease begins with brown flecks on the leaves. These flecks can coalesce into larger, water-soaked regions that may appear black Powdery mildew forms distinctive white, powdery patches on leaves and stems Leaves, beginning at the base, yellow then become necrotic. Left unchecked, the plant may die	Elemental sulfur applied as a dust or spray is sufficient to control the disease if treated before the pathogen is widespread If the disease is widespread, there are multiple fungicides labeled for use

(continued)

Table 8.4 (continued)

Disease	Causative agent	Symptoms	Management
Viral disease			
Mild mosaic	Potato virus A	Chlorotic mottling, slight crinkling	Kill vines in seed plots early
Yellow dwarf	Potato yellow dwarf virus	Plants often produce small, misshapen tubers, cracks are common	Plant disease-free seed potatoes, rogue diseased plants control insect vectors
Stem-end browning	Unknown; virus suspected	Stem-end browning	Plant resistant varieties
Witches' broom	Virus suspected; possibly a mycoplasma	Produce many marble-sized tubers	Plant disease-free seed and practice careful roguing

improve crop efficiency. Worldwide, various researchers believed locally-to-regionally specified sustainable and environmentally responsible potato production systems will help meet the challenges for long-term and country-driven food security and poverty alleviation.

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9 Bacterial Plant Diseases and Their Management: Conventional Versus Modern Approaches

Nayab Ali, Abbas Ali, and Muhammad Ali Syed

Abstract

Annually, significant loss occurs to crops due to bacterial plant pathogens. This poses a major constrain on global food production. Various factors remain a hurdle in effective disease management, like the use of disease susceptible cultivars by the growers and the development of the pathogen by the favorable prevailing environmental conditions. Emergence of new bacterial plant diseases is another problem of concern. Due to shortage of arable land, higher yield, stable and safe food supply remains a major necessity of increasing world population. To overcome these short comings, devising and implementation of novel treatment options for bacterial diseases of plants is a grand challenge. Another serious problem is the emergence of the antibiotic resistance to the currently used antibiotics. Implementation of molecules like antimicrobial peptides and nanoparticles represent a best option to overcome the problem of antibiotic resistance. Also understanding bacterial pathosystem is critical for identifying potential targets in a pathogen. For sustainable and effective disease management in future, research should be carried to identify potential pathogen targets, new strategies and novel delivery methods should be explored. In this chapter, we will summarize some bacterial diseases of cereals, fruits, and vegetables and their management. We will also discuss some novel approaches as their exploitation would be effective in disease control.

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9.1 Introduction

Plant pathology refers to the study of plant diseases. Both biotic and abiotic factors are involved in plant diseases. Biotic component includes microorganisms and parasitic plants, while environmental factors are included in abiotic components (Keswani et al. 2019). Bacteria are unicellular microorganisms ranging in size from 1 to 2 μm that can be seen with the aid of microscope. Among microorganisms, bacteria successively inhabit various plant surfaces and tissues. All plants harbors microbes either on surfaces or inside their tissues (Vidaver and Lambrecht 2004). The term epiphyte is used when microbes are present on plant surfaces while endophytes are microbes living inside plant tissues. Bacteria associated with plant may possess beneficial or detrimental effect for plant. Both as a pathogen or to possess beneficial effect for the plant, high number of bacteria are required. Usually 106 CFUs (Colony forming unit) or more than this is required to confer pathogenic or beneficial effect to a plant (Meena et al. 2002). About 325 years back, individual bacteria for the first time was visualized by a microscope and about 100 years ago first bacterial plant pathogen was identified. These bacteria were responsible for fireblight of pear and apples in New York and Illinois, USA (Burrill 1878; Khan et al. 2012). When visualized with microscope, bacterial plant pathogens appear in several morphological forms. Initially these plant pathogens were differentiated from each other on the basis of morphology. They may be spherical (cocci), rods (bacilli), spiral shape (spirilla), or have tendency to change their shape or pleomorphic (Vidaver and Lambrecht 2004).

Worldwide plant pathogenic bacteria are associated with many serious plant diseases. However they cause less damage in comparison to fungal or viral pathogens (Kennedy and Alcorn 1980; Rezzonico et al. 2009). In warm blooded organisms including humans, bacterial pathogens are more dominant than fungi. But in case of plants, bacterioses are less frequent than mycoses (Egli and Sturm 1981; Rezzonico et al. 2009). The reduced parasitic nature of bacteria for plants is because of their morphology. Unlike fungi with a vegetative body structure, bacteria are unicellular organisms and their division results separation into individual cells. It is difficult for a single cell to overcome an obstacle such as cell wall. Thus the unicellular properties of bacterial pathogens make it difficult for them to penetrate and spread inside the host plant. Invasion of plant tissue by fungal pathogens takes place by means of their germinal hyphae (Kennedy and Alcorn 1980; Cole and Hoch 2013). Whereas no such structure is present in bacteria which makes it difficult for them to fix itself to the outer cutinized layers of plants. For bacterial pathogens only two ways exist for penetration purpose. First one is invasion of plant through non-cutinized parts such as lesions, root hairs, etc., and the second is its entrance

through natural openings such as nectarines and stomata, etc. (Egli and Sturm 1981; Rezzonico et al. 2009).

In order to classify plant diseases, several parameters are taken like infected plant type, infected organ, disease symptoms, and phytopathogen type. Phytopathogen type is considered to be more useful criterion for classification of plant diseases as it identifies the causative agent, complication, and possible control measure for disease management (Daly 1984; Hotson and Mudgett 2004).

In human and veterinary science, pathologists examine various body fluids and tissues, etc., to determine the effects and nature of the disease. The job of medical doctor is to diagnose, treat, and prevent the disease. Plant pathologist do all previously described work. However they have much to do than that of human and veterinary pathologists. Most of their studies related to plant pathology do not include the host alone. Studies on vectors, pathogens survival strategies in soil, etc., are also an essential part in plant disease research (Bruehl 1991; Hotson and Mudgett 2004).

In the proceeding section we will discuss some common bacterial diseases of plants and their possible management.

9.2 Bacterial Diseases of Cereal Plants

9.2.1 Bacterial Leaf Blight of Rice

Rice is an essential staple food consumed in various areas of the world particularly in Asian countries (Pérez-Montaño et al. 2014). Bacterial leaf blight is the destructive disease of rice that is present worldwide affecting plant leaf. *Xanthomonas oryzae* pathovar *oryzae* is the causative agent of the disease that cause severe losses in rice yield (Arshad et al. 2017). The bacterium is aerobic, non-spore forming Gram negative rod that grows best at 25–30 °C (Ou 1985). The host plant is infected by the bacterial pathogen at maximum tillering stage. This results in higher yield loss. In 1884 the disease was observed for the first time by the Japanese farmers (Tagami and Mizukami 1962). Initially water soaked stripes appear on leaf blades that increase in length and width with the passage of time and may cover the entire leaf blade. On young lesions, drops of bacterial exudates may be observed. In case of severe infection, various small circular lesions appear on the glumes with water soaked margins. Fewer and lighter grains were produced by the infected plant that is of poor quality (Saha et al. 2015). The disease can be controlled by using conventional and non-conventional chemicals, via modification of cultural practices, biological control, and through the use of botanical extracts or natural products (Ou 1985; Jiang et al. 2009).

9.2.2 Bacterial Leaf Blight of Wheat

Bacterial leaf blight of wheat is a common worldwide disease caused by the bacterium *Pseudomonas syringae* pv. *syringae* (Young 1992). When plant reaches the boot stage, the disease appears on the upper leaves. Smaller water soaked lesions appear which expand with time. Initially necrotic lesions appear which then change from gray-green to tan white color. In wet weather slimy droplets appear on leaves. The disease can be controlled by cultural management practices while no biological control method has been observed for the disease management (McCulloch 1920; Kazempour et al. 2010).

9.2.3 Bacterial Stalk Rot of Maize

The causative agent of bacterial stalk rot of maize or corn is *Erwinia caratovora f.* sp. *Zeae* (Thind and Payak 1985; Yanan 2006). The disease exists in two forms, i.e. top rot and basal rot. In case of top rot, the rot develops on upper top parts and extends downward while in case of basal rot, the disease symptoms develop on lower basal parts and then extends to upward region. In case of top rot, the tips of whorl middle leaves are wilted and dried out. Throughout the stalk the decay spreads and the plant soon droop down. In basal rot, yellowing of the leaves takes place. Internally the stalk becomes soft and a foul odor is produced because of tissue disintegration. Finally the stalk dried up into disjointed fibrous tissue as the disease progresses. No biological control method is available and management of the disease is aimed at chemical control (Sinha and Prasad 1977; Yanan 2006).

9.2.4 Kernel Blight of Barley

Kernel blight of barley is the bacterial disease of barley crop that exists in two forms, i.e. spot kernel blight and basal kernel blight (Gross 1991; Braun-Kiewnick et al. 2000). Basal kernel blight is the most common form of the disease. Symptoms of basal kernel blight include dark brown discoloration of the embryo at the kernel end. On the other hand, spot blight kernel has well defined spots on the kernel lemma thus differentiating it from basal kernel blight. Both forms of the disease are caused by different pathovars of *Pseudomonas syringae*. Spot kernel blight is caused by *Pseudomonas syringae* 554 while the causative agent of basal kernel blight is *Pseudomonas syringae* pv *syringae*. Wet moist conditions favor disease development. Use of disease free seed is effective in disease management (Martinez-Miller 1994).

9.2.5 Bacterial Top and Stalk Rot of Sorghum

The bacterium *Erwinia chrysanthemi* is the causative agent of top and stalk rot in sorghum. In this disease the upper four to five leaves in the whorl become dead. This is the most common symptom of the disease. When the infected plant is spliced longitudinally, the interior of the stalk appears to be reddish in color and may be water soaked. No effective control measures are available for disease management. If the disease is present in considerable amount in the field, the grain sorghum should not be planted next year (Doggett 1970; Hseu et al. 2008).

9.3 Bacterial Diseases of Fruits

9.3.1 Fire Blight of Apple

Erwinia amylovora is the causative agent of fire blight. In Northern America, the disease first appeared and then it spread slowly around the world. All trees parts can be effected by the disease such as shoot tips, flowers, and rootstock crowns (Peil et al. 2009). Major symptoms of the disease are browning of leaves and black necrosis of the shoots. Antibiotics are effective in disease management but the treatment of antibiotic resistant *E. amylovora* strains is a major problem. Use of antagonists is an alternative strategy for disease management (Beer et al. 1983; Khan et al. 2012).

9.3.2 Bacterial Black Spot of Mango

In South Africa the disease was first time described by Doidge in 1909. All aerial parts of mango plants can be affected by the disease (Fig. 9.1). Route of entrance for the pathogen is natural openings like stomata, etc., and wounds (Gagnevin and Pruvost 2001). Most commonly leafs and fruits are affected but in case of severe

Fig. 9.1 Bacterial black spot on mangoes (Source: https://www.intechopen.com/books/horticultural-crops/mango-diseases-impact-of-fungicides; Reproduced with permission)

infection branch cankers may occur. On leaves water soaked spots develop which then raises and turns black. Leaf abscission is the consequence of the severe infection. In case of fruits water soaked spots develop on lenticels. Premature fruit drop is the consequence of severe infection in fruit. The causative agent of the disease is *Xanthomonas campestris* pv. *Mangiferaeindicae* (El-Goorani 1987). Chemical control is an effective disease management strategy (Alizadeh et al. 1997).

9.3.3 Bacterial Citrus Canker

Bacterial citrus canker is one of the devastating diseases of citrus crops and is caused by *Xanthomonas axonopodis* pv. *citri*. The disease severity differs with prevailing climatic conditions, species, and varieties of citrus. Disease symptoms include necrotic lesions on various parts of plant like lesions on twigs, leaves, and fruits. The most important economic loss due to citrus canker occurs when disease develops on fruit (Gabriel et al. 2000). The main source of inoculums is cankerous twigs, leaves, and fruits but primarily the pathogen survives on naturally occurring lesions. Management of the citrus canker is a difficult task but the use of disease resistant cultivars is effective to minimize the infection (Das 2003).

9.3.4 Citrus Greening

Citrus greening is one of the most devastating vector borne diseases of citrus, caused by a non-culturable bacterium known as *Candidatus*. The species of the genus *Candidatus* were named according to the origin. In Asia the species of the genus were named as *Candidatus* Liberibacter *asiaticus*, while in Africa the causative agent responsible for citrus greening was known as *Candidatus* Liberibacter *africanus* (Texeira et al. 2005). The disease is characterized by leaf mottling, yellowing of shoots, stunted plant growth, malformed fruits, and finally plant death (Fig. 9.2) (Fujikawa and Iwanami 2012). No cure for the disease exists and disease management includes some traditional methods like using chemicals to control insect vector and removal of infected plants or planting material (Alvarez et al. 2016).

9.3.5 Bacterial Canker of Apricot Trees

Bacterial canker of apricot is caused by *Pseudomonas syringae* pv. *syringae*. Characteristic symptoms of the disease include various water soaked small lesions on young leaves, blossoms, and twigs. As the disease progresses, twig dieback, attachment of dried leaves to trees, blossom blast, trunk cankers, and bark necrosis may occur (Kotan and Şahin 2002). Application of copper hydroxide is effective in order to reduce disease symptoms (Wimalajeewa et al. 1991; Kotan and Şahin 2002).

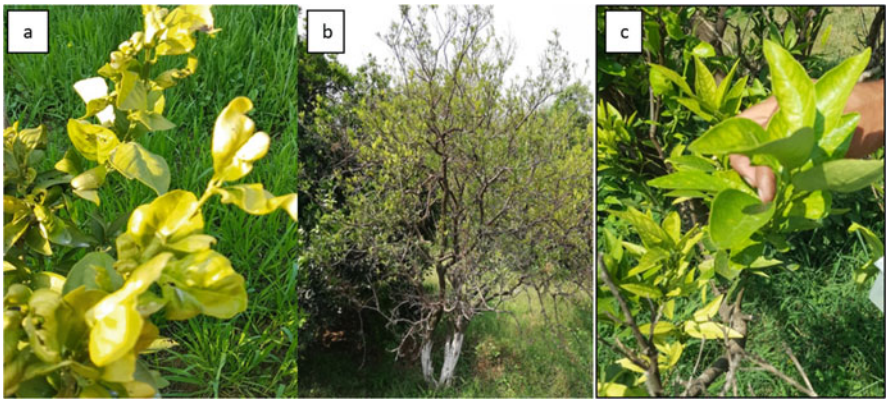

Fig. 9.2 Symptoms of citrus greening: (**a**) Mottled leaves-asymmetrical pattern, (**b**) effected tree yellow dragon-die back, (**c**) tree with yellow shoot and leaves (self-images)

9.4 Bacterial Diseases of Vegetables

The following is a brief overview of some bacterial diseases of vegetables.

9.4.1 Bacterial Wilt of Tomatoes

In tomatoes bacterial wilt is incited by the pathogen Ralstonia solanacearum. Bacterial wilt is the most devastating disease in vegetable crops present throughout the world with a broad host range. Apart from tomatoes, various other vegetables may also be affected by the same pathogen. Other vegetable host of the pathogen includes chilli, eggplant, and several solanaceous crops. Severe yield losses occur due to broad host range of the pathogen (Aslam et al. 2017). Initially terminal leaves of the infected plant wilts that is then followed by permanent wilt in 2–3 days. During the initial stage of the infection, the vascular system of the infected plant becomes light brown in color and in the final stage turns to dark brown. When the plant becomes completely wilted, the pith and cortex become dark brown near the soil. When the infected plants are suspended in water, the bacterium will ooze out as a milky white stream. The pathogen then survives in soil and enters new young plants via their roots (Khokhar and Hri 2013). Various methods are employed for the disease control like use of disease free planting material, chemicals, crop sanitation, and crop rotation. However these methods can be employed alone and have several drawbacks (Aslam et al. 2017).

9.4.2 Bacterial Blackleg and Soft Rot of Potato

Erwinia carotovora (Ec) is responsible for blackleg and soft rot in potatoes (Fig. 9.3). The bacterium is flagellated Gram negative facultative anaerobic. Potatoes can be infected by the two subspecies of *Erwinia carotovora* designated as *E. c.* subsp. *carotovora* (Ecc) and *E. c.* subsp. *atroseptica* (Eca). Major cause of black leg is Eca that results in blackening of stem base when originated from mother tubers. Aerial stem rot is caused by Ecc (Ali et al. 2012). Soft rot symptoms include wet, soft tan colored tissues. Initially rot involves tuber surface and then progresses inwardly. During the early stages of decay the infected tissue are odorless but in the later stages foul odor is produced upon secondary invasion of infected tissue. Plants with blackleg usually have stunted growth. At the margins leaflets roll upward. Wilting of plants take place. An inky black decay is exhibited by the stem of infected plant. Chemical treatment, use of disease free seeds, and pathogen free tubers may help to control disease occurrence (Bonde 1950; Czajkowski et al. 2011).

9.4.3 Bacterial Blight of Peas

In peas the pathogen responsible for blight disease is *Pseudomonas syringae* pv. *pisi*. High yield loss occurs due to the presence of the pathogen in the field. Bacterial peas blight is the seed borne disease as primarily seed is infected and represent the source of infection. On leaves and stipules water soaked spots develop. In warm weather the spots are dark brown while black in cool weather (Hollaway et al. 2007). The pathogen attacks all above ground plant parts. Infected plant pods develop irregular water soaked lesions (Ali et al. 2015). Use of pathogen free seed, crop rotation, crop hygiene, and seed treatment with bactericides can effectively control the disease (Hollaway et al. 2007).

Fig. 9.3 Bacterial soft rot of potatoes. External view of tuber with watery lesion filled with bacterial ooze (Source: https://www.agric.wa.gov.au/potatoes/soft-rot-diseases-potatoes; Reproduced with permission)

9.4.4 Angular Leaf Spot of Cucurbits

This disease has worldwide distribution and is caused by various pathovars of *Pseudomonas syringae* but *Pseudomonas syringae* pv. *lachrymans* is generally associated with the disease in cucurbits (Zitter et al. 1996; Newberry et al. 2016;). Depending on the host and environmental condition the disease symptoms may vary. Circular necrotic lesions develop initially which may become irregular in shape as the disease progresses. Leaf blighting results due to lesion coalescence and finally destruction of leaf canopy occur. In order to control the disease, combination of chemical and cultural practices is required (Newberry et al. 2016).

9.4.5 Black Rot of *Brassica campestris*

Worldwide *Xanthomonas campestris* pv. *campestris* (Xcc) is responsible for black rot in brassica plant. Factors conducive for disease development are application of pathogen infected seeds, use of pathogen susceptible cultivars and weather conditions. Initial symptoms develop as yellowing of cotyledons and on seedlings, deformation of first true leaves. After the fall of diseased leaves at low temperature, plant remains symptomless but infected and on the return of warm conditions symptoms may reappear. Chlorotic spotting is associated with systemic invasion of the pathogen (Ignatov et al. 1998; Vicente and Holub 2013).

9.5 Plant Bacterial Pathogens Management; Conventional Vs Modern Approaches

For plant pathologists, management of bacterial plant diseases remains a grand challenge. Both traditional and modern practices for plant disease management are aimed at inoculum reduction as this represent an effective strategy for disease management. In the following we will briefly discuss both traditional and modern practices for management of bacterial plant diseases.

9.5.1 Use of Chemicals and Antibiotics

In comparison to fungicides, very few chemicals have been marketed that can be used against bacterial plant diseases. While using chemicals for plant disease management, various factors should be considered like effective mode of action, pathogen susceptibility to specific chemical, and the market potential of the chemical in use. Major focus of chemical management of plant diseases includes testing of various available compounds against variety of diseases (Lucas 2020). Introduced in the 1880s, Cu compounds were proved to be effective in bacterial diseases management of plants. These compounds were effective against variety of plant bacterial pathogens like *Xanthomonas spp*, *Pseudomonas spp*, and *Erwinia spp* (McManus

et al. 2002). A Fenton like reaction is catalyzed by copper during oxic conditions that results in lipids peroxidation and damages bacterial proteins (Dupont et al. 2011). However extensive use of these Cu compounds lead to the emergence of resistance in pathogens (Cooksey 1990). In several pathovars of *Pseudomonas syringae*, resistance against Cu is encoded by copper inducible operon designated as cop ABCD (Mellano and Cooksey 1988; McManus et al. 2002).

In the US plant agriculture system the use of antimicrobials is very limited. This is due to various reasons like lack of antibiotic efficacy, environmental concern, and economics (Levy 2001). Oxytetracycline and Streptomycin are the two registered antibiotics that are used in plant agriculture. These antibiotics have prophylactic use that is they will be used on the basis of previous experience or predictive systems. During blossom time, both streptomycin and oxytetracycline may be used prophylactically in spray form. As a prophylactic treatment these both antibiotics can be used every 3–4 days (streptomycin) or 4–6 days (oxytetracycline) in the form of sprays to reduce bacterial disease during blossom. Residues studies related to streptomycin are limited. Various studies have reported that at the time of harvest, fruits have no detectable level of streptomycin residues but leaves still have detectable level of streptomycin (Maxwell et al. 2020). Concerning streptomycin, the 1992 EPA sheet indicates its usage satisfactory related to ecological effects. To freshwater invertebrates, honeybees, and birds streptomycin is non-toxic. However slight toxicity was associated with cold and warm water species of fishes. Oxytetracycline is reported practically to be non-toxic to honeybees, fish, and to aquatic invertebrates. As limited data is available on the usage of tetracycline, therefore, its open application pertaining to environment remains a concern. Streptomycin can be recommended in the concentration ranges from 50–200 ppm (50–200 μg/mL) while oxytetracycline can be recommended in the range of 150–200 ppm (150–200 μg/mL). The recommended concentration depends on the crop type to be treated and the treatment objective (Vidaver 2002).

A few later after the introduction of streptomycin, antibiotic resistance to this antibiotic began to appear in the target plant pathogenic bacteria (Klement et al. 1990; Pohronezny et al. 1994). All genetic forms like plasmids, transposons, and chromosomes, etc., are found to harbor antibiotic resistant genes. In the target pathogenic bacteria, antibiotic resistance to tetracycline has not been reported but has been reported in the phylloplane bacteria (Schnabel and Jones 1999). However, in some regions of the world the use of antibiotics has been discouraged because of the emergence of antibiotic resistance and the transfer of antibiotic resistant genes to clinical pathogens (Sundin et al. 2016).

9.5.2 Crop Rotation

Another strategy to control bacterial plant diseases is crop rotation. Disease management with crop rotation involves growing of non-host plants in the soil until the pathogen will die or its population is reduced to such a level that is unable to cause a disease or can cause a minimum damage to host plant (Kumar et al. 2020). For

successful disease management with crop rotation it is necessary to have knowledge about duration of pathogen survival in the soil, its mode of transmission and reintroduction in the field environment, other ways of survival like persistence of the pathogen between susceptible crops, etc. Those pathogens which can be spread through several ways like those spread by both soil and wind cannot be managed with crop rotation and require other means for disease management (Krupinsky et al. 2002).

After discussing conventional methods, we will briefly focus on some modern approaches of bacterial disease management of plants that can be used as an alternative, incase if traditional methods are less effective or inefficient for disease management.

9.5.3 Biological Control

Beneficial microorganisms or their byproducts or plants/animals byproducts are used for the plant disease suppression. An example of successful disease management with biological control method is the use of the *Agrobacterium radiobacter* strain K84 against crown gall disease caused by *Agrobacterium tumefaciens* (Kerr 2016). However in some situations, disease management with biological control is not effective. Like it is difficult for biocontrol agent to sustain significant population of antagonists in order to protect host specific tissues. Also some bacterial species that can serve as biocontrol agents are opportunistic human pathogens, e.g. *Pantoea agglomerans*, *Burkholderia spp*. Therefore biosaftey concern signifies a need for new biocontrol strains that are safe (Moss et al. 2007). Use of bacteriophages is another novel strategy to control plant disease. Phages are abundant in nature and can easily be isolated from host plant or soil. Various factors limit phage application for disease control like stability in environment and emergence of resistance to phages in the target pathogens (Jones et al. 2007).

9.5.4 Antimicrobial Peptides (AMPs)

These are small amino acid chains typically 50 or smaller amino acid residues and are synthesized by various microbes like fungi, oomycetes, and bacteria. These molecules are cationic in nature and are inserted into cell membrane thus disrupting it or inhibit various cellular processes like protein or nucleic acid synthesis when taken by the cells. For disease management AMPs are suitable candidates but for plant disease management the use of AMPs is still dominated by screening efforts and remains a work in progress (Breen et al. 2015).

9.5.5 Plant Host Resistance

As long as for a decade, breeding crops for disease resistance have been desired for effective disease control. Much of effort has been made to gain knowledge about the key determinants of bacterial host interaction. The introduction of specific disease resistant genes in several crops has resulted in positive momentum for disease control. However this technique is not effective as the effector targets encounter resistance due to which new pathogen races are evolved so the resistance conferred by host plant is not durable. Examples of durable resistance are polygenic, which means that is difficult to be transferred by breeding process (Jones and Dangl 2006).

9.6 Modern Approaches

In the previous section we have discussed bacterial disease management using chemicals, antibiotics, biological agents, or through breeding disease resistant plants. In this section we focus on some recent innovative methods that are less prone to the development of resistance and are environmentally benign.

9.6.1 Type III Secretion System Targeting (T3SS)

For translocation of effector proteins, various plant pathogens like animal pathogens possess type III secretion system which is a needle like structure. In response to the secreted effectors host immune response can be inhibited, thus type III secretion system plays a key role in the pathogenesis mechanism of the pathogens (Stavrinides et al. 2008). In designing novel strategies for disease management, type III secretion system could be an effective target. Upon targeting type III secretion system, pathogenesis of several Gram negative bacterial pathogens can be inhibited.

Plant infection with *Erwinia chrysanthemi* equipped with T3SS genes results in the production of two phenolic compounds t-cinnamic acid (TCA) and o-coumaric acid (OCA). These both compounds are reported as precursor molecules for the production of plant hormone salicyclic acid (SA) that have a role in plant defense system (Yang et al. 2008). This finding suggested phenolic compounds as a potential inhibitor of T3SS. So efforts should be made for the screening of new phenolic compounds to inhibit T3SS in bacterial pathogens.

9.6.2 Biofilm Targeting

For bacteria biofilm represents an effective source of protection from different kinds of stresses such as antibiotics, host defense mechanism, and environmental stresses (Koczan et al. 2009). Developing biofilm inhibitors has become an interesting research area in order to control infection in both plants and animals. Biofilm formation comprises three main stages that are attachment, maturation, and

dispersal. Biofilm inhibitors work by inhibiting any of the stage of biofilm formation. D-amino acids and indole derivatives are biofilm inhibitors. These were applied to biofilms, resulting in inhibition of early biofilm formation. So the symptoms caused by the plant pathogens were reduced and it also enhanced susceptibility of plant pathogens to chemicals like copper and conventional antibiotics (Kolodkin-Gal et al. 2010).

9.6.3 Nanoparticles

Nanoparticles (NPs) 1–100 nm in range have unique physicochemical properties because of their smaller size. They possess high reactivity, have large surface to mass ratio, and unique interactions with different biological systems (Prasad et al. 2016, 2018; Srivastava et al. 2021). These mentioned properties make the NPs excellent antimicrobials and ideal carriers for other antimicrobials (El-Batal et al. 2020). In plant disease management most NPs are evaluated for fungal and oomycete related diseases but for bacterial diseases promising results have also been obtained (Zhang et al. 2017). As antimicrobial NPs may act as photocatalyst damaging bacterial cell envelope or releasing toxic metal ions. NPs are mostly metal oxides such as TiO_2, CuO, ZnO, and Fe_3O_4. Reactive oxygen species (ROS) are generated during photocatalysis in the form of hydroxyl radicals, hydrogen peroxide, and peroxide. Various cellular components of bacterial cell like proteins, lipids, and DNA can be damaged by ROS (Kaushal and Wani 2016; Cheng et al. 2020; Aziz et al. 2014, 2015, 2016, 2019). NPs may have bacteriostatic or bactericidal effect. In plant disease management, titanium dioxide nanoparticles (TiO_2NPs) are reported to be successfully used against bacterial spot pathogen *Xanthomonas perforans*. Ag NPs and ZnO NPs exhibited promising results against *Erwinia amylovora*. Nanoparticles may also serve as an efficient delivery system for several antimicrobials. Carbon nanotubes, hydrogel, liposomes, dentrimers, nano and microemulsions have been studied as effective drug delivery systems (Kang et al. 2012; Prasad et al. 2017).

Since the 1950s, bactericides and pesticides were sprayed on plants using air blast sprays. However this technique was found to be less effective due to the loss of the sprayed material via drift. Beside this only the surface pathogens could be targeted by these chemicals and was not effective against systemic pathogens. In order to successfully combat the pathogens, new drugs and pesticides delivery systems are required that have the potential to target both surface and systemic pathogens (Bhattacharyya et al. 2016). For example, the incidence of fire blight disease in the fruit trees can be reduced significantly with endotherapy approaches than using conventional sprays (Acimovic et al. 2015). So for the effective disease management new approaches similar to the described one should be developed as this would be effective in the management of systemic infections.

9.7 Conclusion and Future Perspectives

A variety of crop types are affected by the phytopathogenic bacteria. Significant economic losses in agriculture are associated with these pathogens and have a negative impact on overall economy. These pathogens pose a threat to global food production. In order to control bacterial diseases of plants, both traditional and modern methods are employed. Mostly farmers follow traditional methods for disease management. Most commonly bacterial diseases are treated with antibiotics and chemical compounds. Although the use of antibiotics and chemicals is effective in disease management, emergence of antibiotic and chemical resistance is one of the major problems concerned with their usage. Also, the chemicals and antibiotics are applied in the form of sprays that can target only the surface pathogens and are not effective in the management of systemic diseases. In order to overcome the problems associated with traditional methods, various novel approaches have been introduced. Use of antimicrobial peptides, biological control, use of disease resistant cultivars of plants, targeting type III secretion system of pathogens, targeting bacterial biofilm and innovation in drug delivery system has been proved to be effective against various pathogens. Application of these novel methods had overcome the problem of resistance in pathogens. Innovations in delivery system also proved to be effective in the disease control. Use of nanoparticles both for treatment and as a delivery system for antibiotics was proved to be effective in many cases of disease management. Moreover these novel approaches offer a promising scenario for effective disease management in comparison to old traditional methods. However, all the innovative strategies that we discussed here are in developmental stages. Therefore growers continue to rely on traditional methods for disease control.

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A Glimpse of Tuber Crop, Their Diseases and Control Mechanisms

10

Deepshikha Thakur and Vineet Shyam

Abstract

Edible tubers are a popular food source due to their vital nutrient and high starch content. These crops are filled with proteins, dietary fiber, minerals such as calcium and potassium, and a certain amount of vitamins like thiamin, vitamin B, and riboflavin, thereby making them a good nutrient source. Most widely used tuber varieties are potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), taro (*Colocasia esculenta*), arrowroot (*Maranta arundinacea*), Indian shot (*Canna indica*), yam (*Dioscorea alata*), crosne (*Stachys affinis*), artichoke (*Cynara cardunculus*), cassava (*Manihot esculenta*), jicama (*Pachyrhizus erosus*). These tuber crops are susceptible to attack by soilborne pathogens that can significantly reduce the yield and quality in the tuber crops. The pathogens that are specific to tubers can survive in soil years after years, affecting the crops consecutively season after season. Major soilborne pathogen groups are fungi, bacteria, viruses, and nematodes. The most familiar diseases caused by soilborne pathogens are rots that affect belowground tissues such as Fusarium dry rot caused by *Fusarium sambucinum* and pink rot caused by *Phytophthora erythroseptica*. However, they are also responsible for causing aboveground diseases such as Verticillium wilt caused by *Verticillium dahliae* and charcoal rot caused by *Macrophomina phaseoli*. A thorough knowledge of the soilborne diseases is very imperative in order to diagnose and manage the soilborne diseases of tubers. The control for soilborne diseases in tubers can be physical, cultural, chemical as well as biological. This chapter will discuss the

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major soilborne pathogens responsible for attacking tubers, their management and control.

Keywords

Tubers · Pathogens · Diseases · Control methods · Management

10.1 Introduction

Edible tubers are a popular food source, due to their vital nutrient and high starch content. Along with starch, these crops are also filled with proteins, dietary fiber, minerals such as calcium and potassium, and a certain amount of vitamins like thiamin, vitamin B, and riboflavin, making them a good nutrient source. Along with its ability to produce high amounts of digestible energy, at the same time it provides income as a cash crop, the major reason for potato being popular with farmers (Kreuze et al. 2020). Most widely used varieties of tubers are taro (*Colocasia esculenta*), arrowroot (*Maranta arundinacea*), yam (*Dioscorea alata*), crosne (*Stachys affinis*), artichoke (*Cynara cardunculus*), potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), jicama (*Pachyrhizus erosus*). These tuber crops are susceptible to attack by soilborne pathogens that can significantly reduce the yield and quality in crops. Major soilborne pathogen groups responsible for these attacks belong to the fungi, bacteria, viruses, and nematode groups. These pathogens survive in the soil for many years, affecting the crops consecutively season after season. The most familiar diseases caused by soilborne pathogens in tubers are rots that affect belowground tissues, for instance, Fusarium dry rot caused by *Fusarium sambucinum* and Pink rot caused by *Phytophthora erythroseptica*. However, they are also responsible for aboveground diseases such as Verticillium wilt caused by *Verticillium dahliae* and charcoal rot caused by *Macrophomina phaseoli*.

Many plant diseases that are caused by soilborne pathogens can be difficult to predict, detect, and diagnose. These pathogens are further difficult to investigate due to the complex nature of the soil environment. Thus, the control of the diseases caused by soilborne pathogens is often very difficult due to the complexity of the interactions between a pathogen and its host, influenced by biotic and abiotic factors of the environment. A thorough knowledge of the soilborne diseases is very imperative in order to diagnose and manage the soilborne diseases of tubers. This chapter will discuss the soilborne pathogens responsible for attacking tubers, their management and control.

10.2 Major Soilborne Pathogen Groups

Soilborne plant pathogens belong to the categories of virus, bacteria, fungi, or nematodes. The plant pathogenic organisms can be resistant and dormant for very long periods even without the hosts, thereby making control and treatment even more problematic.

Fungi are considered as the most significant pathogen group as they are responsible for majority of the tuber diseases. On the basis of morphological and biological characteristics, the five main taxonomic classes of plant pathogenic fungi are: *Plasmodiophoromycetes, Zygomycetes, Oomycetes*, *Ascomycetes, and Basidiomycetes.* Majority of the soilborne fungi producing resilient structures (melanized mycelium, chlamydospores, oospores, and sclerotia) help them to persist in soil for long durations, whereas the thin walled mycelium typical of many fungi survives for only a short time in soil (Koike 2003). Some examples of predominant soilborne fungi are *Sclerotium rolfsii*, *Rhizoctonia solani, Fusarium* sp., *Pythium, Phytophthora*, etc. (Veena et al. 2014).

Another major group of soilborne pathogens is bacteria, which are single celled organisms that have rigid cell walls but lack a membrane bound nucleus. Fewer diseases are caused by soilborne bacterial pathogens than by fungal pathogens. Examples of such bacteria are *Erwinia, Rhizomonas, Ralstonia*, *Agrobacterium,* and *Streptomyces*. Pathogens in the *Pseudomonas* and *Xanthomonas* groups usually persist in the soil for only a short time (Koike 2003).

Viruses are subcellular entities composed of genetic material with the surrounding protein coat. Plant viruses are ubiquitous in nature and are sheltered in the soil from where they find a route to infect the plants, thereby causing significant economic losses to major crops all over the world. Virus disease symptoms include stunting of the plant, tissue discolorations of foliage and fruit. Generally, soilborne viruses survive only in the living tissues of the host plants or in fungal and nematodal vectors that transmit them to plants. Soilborne viruses belong to *Secoviridae*, *Potyviridae*, *Ophioviridae*, *Tombusviridae,* or *Virgaviridae* families (Alison 2014).

Soilborne plant-parasitic nematodes are tiny, non-segmented roundworms, which affect crops by reducing plant vigor and growth. They spend most of their lives in soil, feeding externally either on plant roots or residing inside the roots. In a field affected by the nematodes, part of the standing crop will be heavily infested, whereas others will not be very affected, as a result there will be uneven maturation of the overall crop and quality of the produce will also be lower. *Meloidogyne, Heterodera, Longidorus, Paratrichodorus,* etc. are few examples of soilborne nematodes (Lambert and Bekal 2002).

10.3 Survival and Distribution of Soilborne Pathogens

Soilborne pathogens can be categorized into soil inhabitants, those survive in soil for relatively longer periods, and soil invaders or soil transients, those survive in the soil for moderately shorter periods. Soilborne pathogens are capable of surviving on decaying organic matter as saprobes which under certain favorable conditions turns into pathogenic form (Veena et al. 2014). Saprophytic pathogens are known to survive by taking nourishment from plant debris or soil's organic matter, whereas few other fungal and nematodal pathogens can survive in the soil for long periods even without feeding on organic matter. Survival of such pathogens is aided by their ability to form robust resting structures (cysts, spores, and hyphae), which enable them to survive long periods without a suitable host, or when environmental conditions are unfavorable (www.farmbiosecurity.com). The survival ability of soilborne pathogens partially depends on the biological group to which they belong. Some bacterial pathogens are long term soil inhabitants. Most of these survive as saprophytes on the plant debris or roots for limited periods or directly in the soil. These bacterial pathogen's cells do not produce resilient endospores and the vegetative cells are not particularly resilient in adverse environments. Some species survive by secreting slimy material that dries to form protective layers around the cells, enabling them to withstand unfavorable conditions. Fungal pathogens survive in soil as saprophobes feeding on host plant debris or on other types of organic matter, present in the soil, or as free-living organisms living directly in the soil. Many of these fungi produce resilient survival structures on organic materials, which are released into the soil by tillage operations and through decomposition of the infected material. Survival structures are capable of withstanding extreme temperatures, dry conditions, and also in the absence of suitable hosts. Environmental factors may affect the viability of the survival structures, for instance, the sclerotia of some root infecting pathogens can be sensitive to desiccation. Low soil temperatures can be detrimental to pathogens that are adapted to warmer conditions.

The soilborne pathogen's horizontal and vertical distribution relies on a variety of factors such as production practices and cropping history. On a vertical plane, the root pathogen inoculum mostly lies within the top 10 in. of the soil profile, which consists of host roots, tissues, and other organic substrates. Along the horizontal axis in a field, inoculum distribution is usually amassed in areas where a susceptible crop has been grown (Veena et al. 2014).

The survival and activity of soilborne pathogens are influenced by numerous factors such as soil type, soil texture, pH, moisture, temperature, and nutrient levels. Soils with poor drainage properties are likely to favor the survival of soilborne pathogens such as *Pythium*, *Phytophthora*, and *Aphanomyces*. Similarly, *Fusarium* and *Verticillium* wilts can also be more severe in wet soils than in dry soils. Only a few root diseases are favored by drier soils (for example, common scab of potato caused by *Streptomyces scabies* (Veena et al. 2014)).

10.4 Soilborne Diseases of Tubers

Tuber crops are one of the major food crops in the world and it will certainly feed a big part of the global population in the next years. The economical outlets for these crops are great; however, numerous diseases either soil- or airborne can cause huge losses in the production. The occurrence and development of soilborne diseases depend on very diverse factors affecting either the pathogen or the plant. Potato, Jerusalem artichoke, yam, sweet potato, Colocasia, arrowroot are some of the common tubers consumed globally.

10.4.1 Potato

Potato (*Solanum tuberosum*) is the starchy tuber that is native to America. It is a popular food source and culinary ingredient all over the world. Around the globe, approximately 40 soilborne diseases affect the potato tubers, which are the most economical part of the tuber crop (Oerke et al. 1994). Regrettably, the conditions which are provocative for the development of potato diseases are occasionally the same as required for the potato growth, that is, temperature between 10 °C and 25 °C, high humidity, neutral pH, etc. (Fiers et al. 2014). Soilborne diseases affecting crop development comprise Rhizoctonia canker, black dot, potato early dying (Verticillium wilt), and numerous nematodes. Soilborne diseases affecting tuber quality comprise various pathogens such as viruses, bacteria, nematodes, and fungi (Gudmestad et al. 2007).

Fungal pathogens are probably the most prevalent type of soilborne pathogen. The fungi belonging to *Fusarium* species are the plant pathogens causing huge economical agricultural production damage throughout the globe (Bentley et al. 2006). Symptoms caused by several *Fusarium* pathogens are cortical decay of roots, root rot, wilting, yellowing, rosette, and premature death on infected plants. Dry rot of seed tubers caused by *Fusarium* can reduce crop establishment by killing developing potato leading to crop losses up to 25% (Wharton et al. 2006). Other soilborne fungi affecting potato are also further discussed. One of the most underestimated diseases is powdery scab of potato, caused by the *Spongospora subterranea* (Falloon et al. 2016), in which initially small, light-colored, blister appears that later becomes large, dark, and open pustules. Another major soilborne fungi *Rhizoctonia solani* has otherwise a wide host range and is also responsible for causing damping-off, stem canker, and black scurf in potato (Fritz 2008). In temperate and high altitude tropical regions, *Synchytrium endobioticum* wart or black wart causes considerable yield loss in which galls are produced on several plant parts (Jeger et al. 1996). Whereas, in arid regions, *Erysiphe cichoracearum* is responsible for causing powdery mildew of potato, wherein, the leaves turn black, then die and drop from the plant. *Phytophthora infestans* causes most serious fungal disease, i.e., late blight. In this, water-soaked lesions on foliage appear that later becomes necrotic, turning brown or black. Early blight caused by *Alternaria solani* is worldwide in distribution and is one of the most important foliage diseases. On the

leaves and stems, brown, angular, necrotic spots marked internally by a series of concentric rings form (Vijvera et al. 2020). The soil- and seed-borne disease pink rot is caused by *Phytophthora erythroseptica* wilt with stem decay and leaf chlorosis. *Sclerotinia sclerotiorum* causes white mold that affects potato mainly in the cool tropics and temperate zones. *Sclerotium rolfsii* causes stem rot also known as southern blight, which is worldwide in occurence in potato, particularly under hot and moist conditions. Initially, daytime wilting and yellowing result which progresses to a white mycelium growth on stems, tubers, or soil, often in fan-like mats. *Rosellinia* sp. that causes Black rot may cause heavy potato yield losses in moist soils rich in organic matter. Diseased plants are stunted and wilt. *Verticillium albo-atrum*, *V. dahliae* causing Verticillium wilts may be a serious problem in tropical and subtropical regions and irrigated deserts where water deficiency may be severe (Arora and Khurana 2004).

Soilborne bacterial pathogens contribute to the tuber diseases mainly causing wilts and rots. Although fewer diseases are caused by soilborne bacterial pathogens than by fungal pathogens. Examples of such pathogenic bacteria are *Erwinia, Rhizomonas,* and *Streptomyces*. Pathogens belonging to the groups of *Pseudomonas* and *Xanthomonas* groups are usually short persisting in the soil. *Pseudomonas solanacearum* causes bacterial wilt in potato also known as brown rot which is the most serious bacterial disease problem of potato in warm regions of the world. In this, mild yellowing usually accompanies wilting. Grayish white beads exude from the usually darkened vascular ring of cut stems or tubers. Bacterial soft rot can result when wound sites of plant roots are infected with bacteria of the *Erwinia spp*. This results in a slimy rot that can affect any part of the plant, including heads, curds, edible roots, stems, and leaves. Blackleg and soft rot blackleg of potato are also caused by *Erwinia* spp. These are the widely distributed diseases that are especially harmful in humid climates. *Erwinia carotovora* ssp. *carotovora* usually occurs in warm climates, *E. c. ssp. atroseptica* in cool climates, and *E. chrysanthemi* only in hot climates. Bacterial ring rot is caused by *Clavibacter michiganensis ssp. Sepedonicus*, in potato which is a recurring disease problem in temperate regions. It affects the lower leaves causing appearance of pale-yellow color between major veins, further followed by plant death. Another common bacterial defect of tuber that affects the quality of potato is Scab, that is caused by *Streptomyces scabies*. Several types of lesions develop that can be superficial or reticular, deep or pitted or protuberant (Arora and Khurana 2004; Göre 2017).

Virus diseases in potatoes can often be diagnosed by mosaic patterns on leaves, stunting of the plant, leaf malformations, and tuber malformations. Several common viral diseases of potatoes are further discussed. PLRV, i.e., potato leafroll virus is the most important potato virus and is common in all countries that can cause yield losses in highly susceptible cultivars up to 90%. Primary symptoms caused by current season aphid-transmitted infection are rolling of upper leaves, especially of leaflet bases. Late infections may not cause symptoms. Potato viruses Y (PVY) is the second most important potato virus that can cause yield loss of up to 80%. It is perpetuated through infected tubers and transmitted by aphids in a nonpersistent manner (Kumar and Jeevalatha 2014). Rugosity, bunching, twisting of leaves,

downward turning of leaflet margins, stunting, necrosis of leaflet veins, necrotic spotting, leaf necrosis, and stem streak are typical. Mosaics virus symptoms may be caused in potato leaves by several different viruses (PVX, PVS, PVM, also PVY and PVA), singly or in combination, that may cause yield losses above 10%, with the extent varying according to strain and potato cultivar (Kreuze et al. 2020). Transmission is through infected tubers and by contact (not by aphids) and normally causes mosaic. Potato mop-top virus (PMTV) occurs in areas with cool, damp conditions that favor the spread of its fungus vector, *Spongospora subterranea*. Symptoms consist of rings on the surface, sometimes brown and necrotic, extending as arcs into the tuber flesh. The importance of Calico and Aucuba disease virus (AMV, PAMV, TRSV, PBRSV, TBRV), which usually occur under cool conditions, depends on the causal virus and the cultivar. Symptoms of Alfalfa mosaic (AMV), potato aucuba mosaic (PAMV), tobacco ringspot (TRSV), potato black ringspot (PBRSV), and tomato black ring (TBRV) consist of bright yellow markings on leaves as spots, blotches, flecking, and/or yellowing around veins (Kumar and Jeevalatha 2014). Potato yellow vein disease is apparently caused by a virus transmitted by the whitefly *Trialeurodes vapor*ariorum. Soon after infection, bright yellowing of minor veins (tertiary) is evident and as disease progresses, secondary veins and leaf lamina become yellow, usually leaving primary veins green (Kreuze et al. 2020).

Several species of nematodes can also cause economic damage to tubers. Nematode injury varies among species, but can include galls on roots and tubers (root-knot), necrosis of roots (root-lesion), or a subtle stubby-appearance (stubby-root) (www.farmbiosecurity.com). Nematode lesions further increase the chances of infection attack by soilborne bacteria and fungi. *Globodera rostochiensis* (potato cyst nematode, PCN) infects Solanaceae, such as potato and eggplant. Cyst nematodes *Globodera pallida* and *G. rostochiensis* are serious diseases that also increase the incidence of infection by bacterial and Verticillium wilt. Root-knot nematodes *Meloidogyne* spp. *Polyphagous* attacks increase incidence of infection by bacterial wilt, *Verticillium*, and other pathogens. In warm climates, nematode attacks are even more prevalent. Weak top growth and small, chlorotic leaves that wilt quickly in warm weather are typical. Depending upon the severity of the damage, the infected roots show knots or galls of different sizes. False root-knot nematodes *Nacobbus aberrans* cause severe damage under conditions of heavy infestation. Infested plants are weak, strings of galls in a beadlike fashion are typical of infected roots (Inserra et al. 2004). Root-lesion nematodes, *Pratylenchus penetrans*, and no fewer than ten other related species are found in temperate climates. Root-lesion nematodes are migratory endoparasites. High populations cause brown necrotic lesions in the cortical root tissue. Infected tubers show purple-brown pimples, pustules, or wartlike protuberances that lower their market value (Table 10.1).

Table 10.1 Major microbial pathogens of potato

Bacteria	Fungi	Viruses	Nematodes
Streptomyces ipomoea, Ralstonia solanacearum, Streptomyces scabies, Erwinia carotovora ssp., Pseudomonas solanacearum, Pectobacterium atrosepticum, Dickeya sp, P. carotovorum, Streptomyces scabies, Clavibacter michiganensis, Pseudomonas syringae, Erwinia chrysanthemi, Sphaceloma batatas	*Phytophthora infestans, Sclerotium rolfsii, Rosellinia sp., Spongospora subterranean, Rhizoctonia solani, Verticillium albo-atrum, Phoma exigua* var. foveata, *Fusarium sp., Synchytrium endobioticum, Phytophthora infestans, Phytophthora erythroseptica, Sclerotinia sclerotiorum, Sclerotium rolfsii, Alternaria solani, Fusarium spp., Macrophomina phaseolina, Phoma foveata, Phytophthora infestans, Rhizoctonia solani, Spongospora subterranea, Synchytrium endobioticum, Erysiphe cichoracearum, Verticillium spp.*	PLRV, potato leafroll virus, Potato virus Y, PVNn potato virus, PVA potato virus A, Mosaics virus (PVX, PVS, PVM), Andean potato mottle virus (APMV), Andean potato latent virus (APLV), AMPV Sweet potato virus disease (SPVD), Sweet potato feathery mottle virus (SPFMV), Yam mosaic potyvirus	*Globodera rostochiensis, Globodera pallida. Meloidogyne chitwoodi, Nacobbus aberrans, Pratylenchus penetrans, Pratylenchus spp, Meloidogyne spp. Scutellonema bradys, Pratylenchus coffeae, Meloidogyne spp.*

10.4.2 Jerusalem Artichoke

Jerusalem artichoke (*Helianthus tuberosus*), also is known by other names such as earth apple, sunroot, sunchoke, or topinambour. It is a species of sunflower native to eastern North America. Powdery mildew (due to *Erysiphe chicorianum* f. sp. *helianthi*) has been reported from Bulgaria. Common diseases of sunflowers

(*Helianthus annuus*) such as downy mildew, septoria leaf spot, rust, and sclerotinia wilt are regarded as potential problems. The tubers are susceptible to the development of rots during storage. Fungi *Botrytis cinerea, Rhizopus stolonifer,* and *Sclerotinio sclerotiorum* are reported to be fairly common; the last two can cause severe losses even when the tubers are stored at low temperatures. In addition, the tubers are sometimes infected by a fusarium rot, probably caused by *Fusarium acuminatum*. *Sclerotinia* wilt and stalk and tuber rot have been reported on Jerusalem artichoke, that infection produces basal cankers, root rot, tuber rot, and wilt symptoms (Mordue and Holliday 1976). Downy mildew caused by *Plasmopara halstedii* affects the mature foliage of Jerusalem artichoke. Powdery mildew appears as a powdery white growth on the surface of the stems and leaves (McCarter and Kays 1984). Rust caused by *Puccinia helianthi Schwein* has been a serious disease of Jerusalem artichoke in the Southeastern United States. The first symptom of rust usually noted is the production of uredinial pustules on the foliage and, occasionally, on the stem (Zimmer and Rehder 1976). Bacteria *Pseudomonas syringae* causes Apical chlorosis. The disease can reduce plant stands by as much as 50%. Newly emerged shoots exhibit a yellowing of the growing tips (apical chlorosis) which can spread downward over most of the shoot (Gulya et al. 1982). Jerusalem artichoke can grow well even when root-knot nematodes are present (http://www.missouribotanicalgarden.org).

10.4.3 Yams

Yams are the plant species in the genus Dioscorea (family Dioscoreaceae) cultivated for the consumption of their starchy tubers. *Dioscorea alata* (white yam), *Dioscorea bulbifera* (potato yam), *Dioscorea cayenensis* (yellow yam), *Dioscorea esculenta* (Asiatic yam), and *Dioscorea batatas* (Chinese yam) are some of the yams that are grown for their edible tubers (Bridge 1982; Mohamed and Mantel 1976). Fungal disease that attacks yams is Anthracnose (Scorch) caused by *Colletotrichum gloeosporioides* (Arunachalam et al. 2011). The disease causes small, dark brown spots, or black lesions on leaves which may be surrounded by a chlorotic halo, leaf necrosis, dieback of stem, withered leaves, and scorched appearance. During storage of the yam tubers, severe losses are caused by rotting due to soilborne fungi *Botryodiplodia theobromae, Aspergillus spp., Rosellinia bunodes, Lasiodiplodia sp., Fusarium oxysporum, F. solani,* and other *Fusarium sp*. (Bridge 1982).

Virus diseases have been reported world-wide in Yams and can cause up to 40% loss in yield. Most are of the mosaic type causing leaf mottling, and most are serious only when the infection occurs early and is severe, leading to stunting and sometimes causing the production of numerous basal shoots, giving the plant a bushy appearance. Yam mosaic disease caused by Yam mosaic potyvirus has the common symptoms of infected leaves show yellow and green patterns (called mosaics) between the veins or may show vein banding (narrow green strips bordering the veins). In the severity of the disease, is the leaves show shoe-string symptom (long, thin and strap shape). Transmission of virus is through aphids and tubers/setts

Several species of nematodes also attack yams. The yam nematode, *Scutellonema bradys*, is widely distributed in both Old and New World tropics and causes "dry rot" of the tubers. *Pratylenchus coffeae*, causing rather similar lesions, has been reported to attack yams in Puerto Rico, Jamaica, and the Solomon Islands, while the root-knot nematodes, *Meloidogyne spp*. of worldwide distribution, sometimes attack this crop. Various species of *Dioscorea*, *Dioscorea alata*, *Dioscorea esculenta* also attacks the yams. Dry rot disease caused by yam nematode, *Scutellonema bradys* shows dry rot of 1–2 cm on the infected tubers. Initially this dry rot is of cream and light-yellow lesions appear just below the outer skin without any external symptom. With progress in disease lesion spreads deeper (maximum up to 2 cm). At later stage the rot becomes light and dark brown to black in color and tubers may show external cracks. Entry of fungus through these wounds causes further decay of tubers in storage. There is no aboveground symptom with yam nematode infestation. Root-knot nematode *Meloidogyne incognita* infects the yams and the infected plants are stunted with poor growth. The leaves turn yellow in color (Inserra et al. 2004). Tubers and feeder roots are galled. Tubers are deformed and develop abnormal rootlets called crazy roots.

10.4.4 Sweet Potato

Sweet potato (*Ipomoea batatas*) is an herbaceous perennial in the family Convolvulaceae grown for its edible storage roots. Sweet potatoes are subject to a number of diseases caused by soilborne pathogens both in the field and in storage.

Out of wide range of fungal diseases affecting the crop stand, several that are of importance are: Stem rot (due to *Fusarium oxysporum f. baratas*) can destroy 10–50 per cent of the crop of susceptible cultivars and has been reported to kill 99 per cent of infected plants in certain circumstances. Black rot (caused by *Ceratocystis fimbriata*) can develop in stored tubers as well as affecting the plants in the field. Scurf rot or soil stain (caused by *Monilochaetes infuscans*) is widespread and produces a brown or black discoloration on the surface of the tubers, which considerably reduces their market value. Footrot (due to *Plenodomus destruens*) frequently affects plants raised from transplants and infected plants often produce no tubers although they make reasonable vine growth. Other field diseases of sweet potatoes (and their causal organisms) are root rot (*Phymatotrichopsis omnivora*), mottle necrosis (*Pythium spp.*), phyllosticta leaf blight (*Phyllosticta batatas*), septoria leaf spot (*Septoria bataticola),* and white rust (*Albugo ipomoeae*-panduratae). Other storage rots affecting sweet potatoes (and their causal organisms) are *Erwinia chrysanthemi*, black rot (*Ceratocystis fimbriata*), surface rot (*Fusarium oxysporum*), dry rot (*Diaporthe phaseolorum* var. *batatatis*), charcoal rot (*Macrophomina phaseolina*), and Java black rot (*Botryodiplodia theobromae*), which is often a serious problem in the tropics (optimum growth temperature is about 28 °C). Alternaria leaf spot and leaf and stem blight caused by *Alternaria* spp. cause brown lesions on leaves with concentric rings resembling a target. Black rot caused by *Ceratocystis fimbriata* causes stunted plants, wilting plants, yellowing plants,

dropping leaves, plant death, circular brown-black patches of rot on tubers. *Fusarium solani* causes stem rot in which base of stems is swollen and distorted; deep, dark rot extending deep into tuber and forming elliptical cavities; growth of white mold (Steinsbauer and Kushman 1971).

Among bacteria, *Erwinia chrysanthemi* affects the sweet potato, causing bacterial soft rot. Brown to black water-soaked lesions on stems and petioles expand rapidly and cause large areas of soft rot on the stem; stem may collapse causing several vines to wilt; entire plant may die; storage roots may develop areas of soft rot which is initially colorless, but eventually turns brown with a black margin. In bacterial wilt caused by *Ralstonia solanacearum*, new sprouts wilt and have water-soaked bases which turn yellow-brown to dark brown in color; vascular system of the sprouts is discolored brown; Leaf and stem scab caused by *Sphaceloma batatas* causes small brown lesions on leaf veins which become corky in texture and cause veins to shrink which in turn causes leaves to curl; lesions on stem are slightly raised and have purple to brown centers with light brown margins; scabby lesions form on stems when lesions coalesce. Streptomyces ipomoea causes poor growth of plants due to the production of several phytotoxic secondary metabolites (Bignell et al. 2013).

Virus diseases include those producing internal cork and russet crack in roots, feathery mottle, mosaic, chlorotic spotting and banding in foliage, and little leaf and witches broom. Transmission is usually by aphids, including *Myzus persicae, Aphis gossypii*, whitefly (*Bemisia tabaci*), and others or by the use of infected planting material (Terry 1982). Sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV) causes a disease complex that includes Sweet potato virus disease (SPVD), Sweet potato feathery mottle virus (SPFMV) and Sweet potato chlorotic stunt virus (SPCSV) (Gutiérrez et al. 2007). The symptoms are severe stunting of infected plants, stunting, distorted and chlorotic mottle, or vein clearing of the leaves. It is confirmed that SPCSV enhances the accumulation of SPFMV. The symptoms caused by SPCSV alone are negligible. Whereas symptoms caused by SPFMV are localized, mild, and often asymptomatic and will not cause significant damage to the plant. Common symptom includes appearance of feathery, purple patterns on the leaves. Some of the nematodes are also reported as affecting the crop. *Meloidogyne spp*. causes considerable losses, while the reniform nematodes *Rotylenchus reniformis* also cause severe damage in some areas (Gutiérrez et al. 2007).

10.4.5 *Colocasia esculenta*

Colocasia esculenta (Taro, elephant ear, or cocoyam) is an emergent, perennial, aquatic, and semi-aquatic herbaceous species of the Araceae family, native to Asia. As a root vegetable, plant is grown primarily for its edible corms. Nowadays, *C. esculenta* is considered the fifth most consumed root vegetable worldwide. Taro is also used as an ornamental plant. Fungal and oomyceteous plant pathogens of taro have been reported to cause losses in taro fields. Taro leaf blight disease, caused by *Phytophthora colocasiae*, foliar oomyceteous diseases agent, is a major

limiting factor in taro production worldwide (Brooks 2008). One of the major fungi that can be associated with taro root rots is black rot (*Ceratocystis fimbriata*) causing rot of storage corms of taro worldwide (Harrington et al. 2011). Root rot of taro may also be caused by any one or a combination of several common soilborne microorganisms such as *Phytophthora citricola, Phytophthora nicotianae, Pythium* spp., *Fusarium oxysporum, Fusarium solani, Sclerotium rolfsii,* and *Rhizoctonia* sp. (Ooka 1994). The relative importance of these pathogens and their effect on yield vary among countries or disease conditions. Pythium root and corm rots are probably the most widely distributed disease of taro (Ooka 1994). *Pythium carolinianum* is mostly associated with wetland taro rather than dryland taro. In wetland situations, while *P. carolinianum* is known to infect taro under favorable conditions, it is a less aggressive pathogen than other species such as *Pythium myriotylum* (Liloqula 1993) that are more commonly associated with corm rot. Pythium root rots usually develop into corm rots where the interior of the corm is progressively transformed into a foul smelling soft mass (Jackson and Gerlach 1985). Spread occurs via zoospores that are carried in irrigation water and are attracted to chemical exudates from the root tips (Jackson and Gerlach 1985). *Pythium* species can also be transferred to new areas on infected vegetative planting material (Sibel et al. 2014).

10.4.6 Arrowroot

Arrowroot also known as *Maranta arundinacea L.* is not normally subject to serious attacks by pests or diseases. In parts of the Caribbean, particularly in wet districts, arrowroot sometimes suffers from a rot caused by *Rosellinia bunodes*. Two leaf blights, caused by *Rhizoctonia solani* and *Pellicularia filamentosa*, are reported to infect arrowroot in India. A condition known as "cigar roots," in which the rhizomes become elongated and very fibrous, has also been reported from the Caribbean but is thought to be due to nutritional deficiencies (Maiti et al. 1980). Banded leaf blight caused by fungus *Thanatephorus cucumeris* shows a chlorotic band of the leaves with ultimate browning and rotting (Reddy 2015). A vascular wilt disease of arrowroot was reported in Brazil in 1962 that shows the presence of whitish small dots and narrow streaks. Control methods include rouging, controlling aphids, planting healthy planting material, and controlling weed (Reddy 2015).

10.5 Control of Soilborne Pathogens

An effective control method against the pathogens is essential to ensure good production and yield stability. A number of methods such as chemical, physical, cultural, and biological techniques have been developed for the control of plant disease by soilborne pathogens (Singleton et al. 1992). Although there is no general perfect method to be used in all instances of soilborne pathogens control, but different methods (Fig. 10.1) can be suitable in different situations, as discussed below.

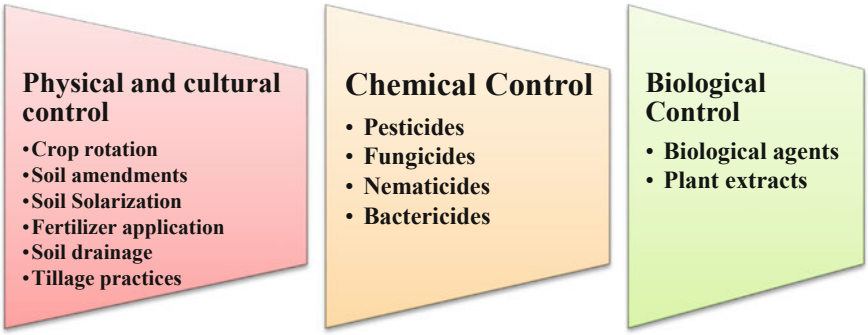

Fig. 10.1 Effective control methods and strategies against soilborne pathogens

10.5.1 Physical Methods and Cultural Control Practices

Due to the negative effects of the chemical methods, there is growing interest in physical methods and cultural practices for disease control for the management of soilborne pathogens. Physical and cultural methods of control include heating the soil or propagation material, irradiation, crop rotation, fallow, flooding, deep plowing, flaming, soil solarization, adjusting planting date, irrigation, fertilization, compost, weed control, herbicide application, sanitation, tillage, and others (Jaacov 2000).

10.5.1.1 Crop Rotation

The use of crop rotations in the farming system allows time for the soil microbiota to displace, weaken, or destroy the propagules of soilborne pathogens of any one crop while another, usually unrelated crop is growing. In general, soilborne plant pathogens multiply in the presence of their preferred host plant(s) and decline when the host plant is absent. Most of these pathogens could survive in the soil in the absence of the host plant if it were not for the combined action of competition, antibiosis, and predation/parasitism imposed by the associated soil microbiota (Veena et al. 2014). Oat-potato, annual ryegrass-potato, or clover-potato crop sequences have been found to reduce both *Rhizoctonia solani* inoculum levels in soil and suppress subsequent disease development in a potato crop (Johnston et al. 1994). Barley and clover demonstrated reductions in Rhizoctonia canker and black scurf through the first couple of rotation cycles, after which these diseases increased to levels comparable with continuous potato (Larkin et al. 2010). Full-season rotation crops (barley, ryegrass, canola, and rapeseed) in two to three-year rotations with potato were reported to significantly reduce Rhizoctonia outbreak in potato (15–50%) (Larkin et al. 2012). Wiggins and Kinkel (2005) reported that cropping sequences involving potato had an intense effect on soil microbial community but crop rotation was ineffective in controlling potato scab disease.

10.5.1.2 Soil Amendments

Plants and their products like organic amendments, compost, crop residues, green manures, fish meal, blood meal, biochar, chitosan-based products, etc. can significantly decrease the incidence of soilborne diseases (Bailey and Lazarovits 2003). Application of organic amendments like sawdust, straw, oil cake, etc., can successfully cope with the diseases caused by pathogens such as *Pythium*, *Phytophthora*, *Verticillium*, *Macrophomina, Phymatotrichum,* and *Aphanomyces* and plant-parasitic nematodes (Corato 2020; Rosskopf et al. 2020). The application of amendments to adjust the pH of the soil can be beneficial. The application of lime (2500 kg/ha) reduces the clubroot of cabbage by increasing soil pH to 8.5 (Utkhede and Guptha 1996). Similarly, the application of sulfur (900 kg/ha) to soil brings the soil pH to 5.2 and reduces the incidence of common scab of potato caused by *Streptomyces scabies* (Davis et al. 1974). Many herb plants contain essential oils, including terpenes, as well as phenols, alcohols, organic acids, and other compounds with potentially biocidal activity (Paret et al. 2010). Application of castor cake and neem leaves helps to reduce the foot rot of wheat (Utkhede and Guptha 1996). Incorporation of de-caffeinated waste and water hyacinth in management of root-knot nematode in carrot has been reported by Davis and Das (1998). Although organic amendments such as composts may be useful for the management of soilborne diseases, they are not widely implemented due to concerns about potential side-effects (non-selective activity, cost effectiveness, and scale practicality) (Yulianti et al. 2006; Colla et al. 2012). Compost dwelling microorganisms produce some chemical compounds (e.g., siderophores, tannins, phenols) which are antagonistic to various soilborne pathogens and also produce plant growth hormones. The use of composted softwood and hardwood barks gave reproducible control of *Pythium ultimum* in lettuce, *R. solani* in cucumber, radish, and bedding plants under greenhouse conditions (Stephens and Stebbins 1985). In regard to soilborne pathogens, another organic amendment, i.e., biochar's suppressive capability has been reported for the following species: *F. oxysporum f. sp. asparagi, F. oxysporum f. sp. radicis-lycopersici, Fusarium proliferatum, Pythium aphanidermatum, Phytophthora cactorum, Phytophthora cinnamomi,* and *R. solani* (Elmer and Pignatello 2011; Jaiswal et al. 2014). Conn and Lazarovits (1999) revealed that the application of fresh chicken manure was highly effective in reducing the incidence of potato scab, Verticillium wilt, and parasitic nematodes.

10.5.1.3 Soil Solarization

Soil solarization or solar heating is the pre-planting method and a good way of controlling soilborne pathogens (Panth et al. 2020). The aim of soil solarization is to harness solar energy to raise the temperature of moistened soil which can result in the control of soilborne pathogens, especially *Fusarium* species. Soil solarization is a useful practice which is able to reduce soil pathogen populations which is achieved by covering the soil with plastic films. Actually, light plastic films (LPFs) are nowadays widely used especially in open and greenhouse vegetable crop cultivations in some countries as they are able to raise soil temperature more than 20 °C above air temperature (Gonzalez et al. 1993; Kumar and Sharma 2005). In

general, solarization appears to be an effective practice able to control nematodes, even though it may cause stress on the soil microbial biomass. In addition, it is demonstrated that the organic amendments exert a protective role keeping soil microbial biomass and enzymatic activities protected from the detrimental effect of heating. In a study by Kumar and Sharma (2005), soil disinfestations were carried out in warm climate, through soil solarization method for the relative control of *Fusarium* pathogens. In this method, infested soil was thoroughly plowed, irrigated deeply for better heat transmission into the soil. Afterwards, the moistened soil was covered with transparent polyethylene sheet to raise soil temperatures to a high range (more than 10 °C above air temperature). The edges of the sheets were buried to ensure the plastic is held in place. The plastics were left on the soil for 6 weeks during the hottest part of the summer. The method came out to be a successful practice to control soilborne fungi, as well as *Fusarium* species. In another study, the count of *Fusarium oxysporum* in the upper 15 cm of a naturally infested soil was also reported to be reduced by soil solarization (Gonzalez et al. 1993). During the 9 months following treatment, the *F. oxysporum* population stabilized at a low level in soil solarized for 2 months, but fluctuated in soil solarized for 1 month. The amount of *Fusarium* wilt on plants was also revealed to be generally low after soil solarization treatment. The sub-lethal doses of increased temperatures due to soil solarization also render the pathogen propagules further susceptible to attack of biological control agents.

10.5.1.4 Fertilizer Application

Nutrient manipulation through fertilization or modification of the soil environment to influence nutrient availability is an important cultural control for plant disease and an integral component of production agriculture. Fertilization decreases soilborne diseases by maximizing the inherent disease resistance of plants, by facilitating disease escape through increased nutrient availability or stimulated plant growth and by altering the external environment to influence the survival, germination, and penetration of pathogens. It is clear that the severity of most diseases can be decreased and the chemical, biological, or genetic control of many plant pathogens enhanced by proper fertilization. Breeding nutrient-efficient or disease-tolerant crops and establishing cultivar requirements should further improve production efficiency (Huber 1990). Application of ammonium bicarbonate reduces the viability of sclerotial bodies of *S. rolfsii* (Punja and Grogan 1982). Application of phosphatic fertilizers also influences the host resistance by increasing the production of phytoalexins (Gottstein and Kuc 1989). Management of *Pythium* and *Phytophthora* by application of phosphoric acid has also been reported (Asha et al. 2018).

10.5.1.5 Soil Drainage

Good soil drainage depends on the management of irrigation in order to minimize the dispersal of soilborne pathogens through water. This irrigation and drainage management is capable to monitor disease incidence by avoiding the soilborne pathogens to other areas. When diseases occur, timely removal of dead or infected plants can reduce the potential for inoculum build-up. Good soil drainage practices

are known to decrease the number and activities of certain oomycetes pathogens (e.g., *Pythium*) and nematodes. Irrigation also helps to reduce the soilborne disease charcoal rot caused by *M. phaseolina* (Kendig 2000).

10.5.1.6 Tillage Practices

Tillage practices refer to the soil preparation carried out between the harvest and following sowing/cultivation operation. Soil preparation before sowing aids in reducing pathogen population by two main methods that is either through burial of inoculum deep into the soil or drying of the inoculum when exposed in the top layers. In a study by Singh (2001), when the sub-soiling was done preceding the planting of root rot susceptible and tolerant cultivars of green pea, in the soil infested with *F. Solani f. sp.* and *Pythium ultimum*, sub-soiling was reported to increase the yield of green pea.

Along with the all of the above-mentioned cultural practices, some other practices are also used such as roguing (useful particularly for the control of viruses), planting only high quality seed free from pathogens, adequate but not excessive irrigation and fertilization, incorporation of green manure crops (sudangrass, sesame, rapeseed, white mustard, or perennial ryegrass). Simple measures such as the painting of the cut surfaces with limewash or Bordeaux mixture or coating with wood ash can control Yam rotting due to fungi *Botryodiplodia theobromae, Aspergillus spp., Rosellinia bunodes, Lasiodiplodia sp., Fusarium oxysporum, F. solani,* and other *Fusarium s*pp. (Bridge 1982). Rotting during storage may be minimized by treating cut or bruised surfaces of the harvested tubers in the same manner (Bridge 1982; Twumasi and Moses 2014).

10.5.2 Chemical Control

Chemical control is the most reliable when it comes to the choice of farmers as the alternatives for chemical control against soilborne pathogens are not adequately effective for controlling the current disease incidence. Eventually, farmers turn to one or more of the known chemical alternatives such as methyl bromide for fumigation of soil (Labrada 2008). Although most chemical pesticides are used to protect plants from infection or to eradicate a pathogen that has already infected a plant, there are some chemical treatments, which aim at eradicating or greatly reducing the inoculum even before it comes in contact with the plant. Fungicides can be applied to the soil as dusts, liquid drenches, or granules in order to control damping-off, seedling blights, crown and root rots, and other plant diseases. In irrigated fields the fungicide is sometimes applied through irrigation water, particularly in sprinkler irrigation. Metalaxyl, diazoben, pentachloronitrobenzene (PCNB), captan, and chloroneb are the fungicides used for soil and seed treatments. Soil fumigation with methane sodium is reported to control powdery scab caused by *Spongospora subterranean*.

Most soil treatments, however, are aimed at controlling nematodes, and the materials used are volatile gases or produce volatile gases (fumigants) that penetrate

the soil throughout (fumigate). Some nematicides, however, are not volatile but, instead, dissolve in soil water and are then distributed through the soil. Among alternative chemicals, only dazomet has been registered as a broad-spectrum soil fumigant in most countries, including Serbia. Products that effectively reduce soilborne pathogens of some crops by soil and plant applications are fungicides in the dicarboximide, benzimidazole, and triazole chemical groups (Vatchev and Maneva 2012). Azoxystrobin fungicides are widely used to control *R. solani* (Sundravadana et al. 2007). An effective disease measure against vascular pathogens could also be the application of imidazoles, benzimidazoles, and triazoles either to soil or to plants (Everts et al. 2014). Fungicides based on cyprodinil and fludioxonil are recommended against *S. sclerotiorum* (Benigni and Bompeix 2010). Propamocarb-hydrochloride, fosetyl-Al, metalaxyl, and azoxystrobin are fungicides that are commonly used to control Pythium spp. and Phytophthora spp. on pepper (Rekanović et al. 2011). For yams, sanitation by removal of crop debris and fungicide treatment: maneb, benomyl, benomyl + propineb, zineb, and mancozeb have all been reported to give reasonably good results in disease control (Thompson et al. 1977).

However, it is very important to emphasize that long term use of pesticides has a negative influence on microbial growth and activity, leading to reduced soil fertility and productivity (Wang et al. 2006). The decrease in number of nitrogen fixing, phosphorus-solubilizing microorganisms, and inactivation of soil enzymes is observed in pesticide-contaminated soils (Antonious 2003). Similarly, many studies have shown that pesticides reduce the activities of soil enzymes that are key indicators of soil health.

10.5.3 Biological Control

Biological control of pathogens, i.e., the total or partial destruction of pathogen populations by other organisms, occurs routinely in nature. In an attempt to reduce the use of pesticides, there is an increasing interest in introducing biological agents and putting to use plant compounds as natural commercial products for managing soilborne pathogens (Cook 1993). Although there are number of bottlenecks in the usage of biocontrol such as formulation and delivery, variability in performance, and problems with poor efficacy under optimum conditions for disease development, but simultaneously there are many benefits associated with them. Various mechanisms are involved in the biological control of fungal pathogens. These mechanisms include: the production of secondary metabolites (antibiotics, siderophores, hydrolytic enzymes, volatile extracellular metabolites, hydrogen cyanide), parasitism, competition for nutrients, promotion of plant growth and, finally, induced resistance within the plants (Moeinzadeh et al. 2010). Unpredictable performance coupled with this extreme variability represents one of the greatest obstacles to the implementation of biological disease control practices in agriculture (Nelson 2004). Researchers have increased their efforts to take advantage of such natural biological antagonisms

and to develop strategies by which biological control can be used effectively against several plant diseases.

Numerous studies have shown suppression of disease incidence in different crops after supplementing soils with fungal or bacterial antagonists. It is important to point out that bioagents can reduce harmful effects of some pathogens below a certain threshold with no substantial changes in the soil microbiological balance (Neshev 2008). It has been indicated that several established biocontrol agents, including strains from the genera *Bacillus, Pseudomonas, Sphingomonas, Stenotrophomonas,* and *Serratia*, can suppress vascular or soilborne fungal pathogens (Bhattacharjee and Dey 2014). Fungi belonging to the genus *Trichoderma* and bacteria such as *Pseudomonas* spp., or *Bacillus subtilis*, on the one hand are the promising biocontrol agents due to their unique antimicrobial activities, including the production of antibiotics and toxins to compete with pathogenic organisms and, on the other hand, they stimulate plant growth (Mukry et al. 2010). *B. subtilis, Trichoderma harzianum,* and *T. virens* have been reported as biocontrol agents against soilborne potato diseases (Brewer and Larkin 2005).

Plant extracts and especially the volatile essential oils from medicinal plants have been reported to possess antimicrobial activity against a variety of plant pathogens and pests (Kalemba and Kunicka 2003). Essential oils and their components are gaining in interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose use (Jobling 2000). Oregano, fennel, and laurel oils demonstrated antimicrobial activity against soilborne fungi of bean under laboratory conditions (Turkolmez and Soylu 2014). In addition, cinnamon, thyme, basil, and fennel essential oils showed fungicidal effects on *Pythium sp*., *F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. pisi, Verticillium albo-atrum,* and *Rhizoctonia* sp. (Tanović et al. 2013). Strong activity was also recorded for lavender oil when tested against *F. oxysporum* at the dosage of 60 μL oil (Kadoglidou et al. 2011).

Considerable research has been directed toward biological seed treatments for control of a variety of soilborne plant diseases. Biological seed treatments have proven to be effective, in many cases, as fungicide seed treatments for the control of several soilborne plant pathogens including the major genera *Pythium, Rhizoctonia, Fusarium, Gaeumannomyces, Phytophthora, Verticillium,* and *Thielaviopsis* (Whipps 1987).

10.6 Suppressive Soils

Some of the soils known as conducive soils are known to exaggerate the severity of the diseases caused by soilborne pathogens, such as *Fusarium oxysporum* (the cause of vascular wilts), *Gaeumannomyces graminis* (the cause of take-all of wheat), *Phytophthora cinnamomi* (the cause of root rots of many fruit and forest trees), *Pythium* spp. (a cause of damping-off), and *Heterodera avenae* (the oat cyst nematode), whereas on the other hand, in suppressive soils they develop much less and cause much milder diseases. The mechanisms by which soils are suppressive to

different pathogens are not always clear but may involve biotic and/or abiotic factors and may vary with the pathogen. In most cases, however, it appears that they operate primarily by the presence in such soils of one or several microorganisms antagonistic to the pathogen. Such antagonists, through the antibiotics they produce, through lytic enzymes, through competition for food, or through direct parasitizing of the pathogen, do not allow the pathogen to reach high enough populations to cause severe disease. Numerous kinds of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and disease suppression has been shown to be caused by fungi, such as *Trichoderma, Penicillium,* and *Sporidesmium*, or by bacteria of the genera *Pseudomonas, Bacillus,* and *Streptomyces*. If the suppressive soil is mixed with conducive soil it reduces the disease incidence by introducing the antagonistic microorganisms against the pathogen. For example, soil amended with soil containing a strain of a Streptomyces species antagonistic to *Streptomyces scabies*, the cause of potato scab, resulted in potato tubers significantly free from potato scab. Suppressive, virgin soil has been used, for example, to control *Phytophthora root* rot of papaya by planting papaya seedlings in suppressive soil placed in holes in the orchard soil, which was infested with the root rot *Phytophthora palmivora* (George 2005).

10.7 Conclusion

Tubers are the significant crop of the global food platter which certainly feeds a large part of the world's population and will continue to do so in the subsequent years. But then tubers are highly susceptible to soilborne pathogens such as bacteria, viruses, fungi, and nematodes. Soilborne pathogens are responsible for causing severe damages to the tubers, the economically most important part of the plant. The occurrence and development of soil-borne diseases depend on very diverse factors affecting either the pathogen or the plant. A better understanding of disease etiology collaborated with choosing good control methods such as physical or cultural methods, chemical methods, and biological methods will help in the disease suppression and occurrence, resulting in the healthy and increased tuber yield.

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Soil Borne Fungal Diseases and Their Control in Below Ground Crops

11

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Abstract

Soil docks a variety of diseases impacting crop health and yield potential of several agricultural crops. Fungal pathogens are in the forefront responsible for number of wilts, rots, rot and blight diseases through the accumulation of the mycotoxin into edible parts which also affect human health as well on consumption. Fungal pathogens spread from plant to plant making diseases very difficult to manage with highly heterogeneous incidence. Practices dealing with adequate knowledge about their dissemination and survival, environmental conditions and culture practices, detailed information about host resistance and susceptibility can lead to effective control of these soil borne fungal pathogens. In recent years, studies based on plant–microbe interaction have led to exploitation of various breeding and biocontrol strategies to develop crop resistant against various fungal diseases. Thus, the detailed study on mechanisms such as survival, dissemination of soilborne pathogens; effect of environmental conditions role of cultural practices, host resistance and susceptibility screwed up with biological interactions will play a major role in disease management in soil matrix.

Keywords

Soilborne diseases · Fungal pathogens · Root and tuber crops · Cultural practices · Biological control

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11.1 Introduction

Soilborne diseases are caused by pathogens that prevail in the soil matrix and its residues serving as reservoir of inoculums for the pathogens. Fungal pathogens can either survive in soil for shorter durations (soil transients) or possess the ability to survive for longer durations (soil inhabitants) as well. Also there are some who live on dead and decaying matter (saprobes) and can turn into pathogenic form under suitable congenial conditions. Certain conditions can also modify these pathogens to remain in non-pathogenic form in the soil (Singh 2001). Many factors including production practices, cropping history, etc. influence the horizontal and vertical distribution of these fungal pathogens. Vertical distribution deals with the prevalence of fungal inoculum in the top layer of soil where host roots, tissues and other organic matter are present on the other hand horizontal distribution is where the susceptible crop is grown (Barhoom and Sharon 2007). Based on morphological and biological features, plant based fungal pathogens are divided into five main taxas, namely Basidiomycetes, Ascomycetes, Plasmodiophoromycetes, Oomycetes and Zygomycetes. Apart from this there is one more separate class of asexually producing spores, Fungi imperfecti, which are the spores produced by some species of Ascomycetes and Basidiomycetes. Most crucial soil borne Fungi imperfecti are *Verticillium*, *Fusarium*, *Rhizoctonia,* etc. (Butler 1918). Major soil borne Plasmodiophoromycetes are the *Spongospora subterranean* which is the causal agent of powdery scab of potato. Similarly, *Pythium*, *Phytophthora*, *Aphanomyces* and *Bremia* are major Oomycetes. Ascomycetes include fungal pathogenic agents like *Sclerotinia* and *Monosporascus*. The major reason behind these fungal pathogens' prolonged survival in the soil is that they produce resilient stable structures like chlamydospores, sclerotia, oospores, mycelium, etc. (Taro 1929).

A majority of crop losses are encountered due to fungal pathogens, tuber and root crops being the topmost affected one. Reason behind the major losses of tuber crops is that they are rich in starch with high soil moisture (60–90%) content favouring the growth and spread of the fungal pathogens (Fig. 11.1). Following cereal crops, tuber crops are the most cultivated one rich in nutrition and high energy source along with other health benefits such as possessing antimicrobial, antioxidative, hypoglycaemic, immunomodulatory activities, etc. (Thankappan and Nair 1990). Although tuber crops such as potato, cassava, sweetpotato, yam, taro, aroids, etc. belong to different botanical families but are clubbed together as all of them produce underground food. Root and tuber crops are vegetatively propagated crops including stem cuttings (cassava), stolons or cornhead (cocoyam and taro), tubers (potato and yam) and vine cuttings (sweet potato). They store starch either in rhizomes, tubers, roots, stems and corms. Based on that potato and yam come under tuber category, cassava and sweet potato in storage roots, taro and cocoyams originate from corms, underground stems and swollen hypocotyls and arrow roots from edible rhizome (Chandrasekara and Kumar 2016). It is important to detect the attack of fungal pathogens at an early stage because most of the initial attacks of soil pathogens are hidden in the soil and are only visible until the above ground parts are affected and show symptoms like wilting, chlorosis, stunting and gradually death. These

Fig. 11.1 Factors favouring the activity of soil borne fungal pathogens

pathogens have wide host range including weeds and can survive for longer duration in soil without a crop host as well (Utkhede and Guptha 1996). Moreover their microscopic nature, nonspecific infection symptoms and inappropriate diagnostic methods make it a difficult task to control them. Agriculture soil being the most exploited one due to fungal pathogens possess the major concerns regarding eradication and control of the soil borne fungal pathogens (Freeman et al. 1998). Practices dealing with adequate knowledge about their dissemination and survival, environmental conditions and culture practices, detailed information about host resistance and susceptibility can lead to effective control of these soil borne fungal pathogens.

11.2 Fungal Diseases of Major Tuber Crops

Tubers such as potato, sweet potato, yam, taro and cassava are the most economically important crops prone to soil borne fungal diseases leading to severe crop damage and yield loss. Table 11.1 enlists the major fungal disease of these crops and their symptoms along with their causal organisms.

11.2.1 Potato

Potato ranks fourth among the most important food crops after maize, wheat and rice and ranks third in terms of consumption after wheat and rice (Ezekiel et al. 2013). Potato production is limited by various biotic and abiotic diseases. Where abiotic stress deals with zinc deficiency, high temperature, pH, salinity, etc., high starch and soil moisture content favours the growth and spread of soil borne fungal pathogens (Saeed et al. 2020). In some cases low moisture can lead to wilting in potato. Among other biotic pests (viruses, bacteria, nematodes, etc.) fungal pathogens possess the serious threat to the potato cultivation (Abbas et al. 2013). Late blight, leaf roll and ring rot are the major destructive fungal diseases of potato causing s yield losses out of which late blight is the most severe one which is discussed as follows.

11.2.1.1 Late Blight

Late blight of potato is the major fungal disease that is worldwide in its distribution. Potatoes grown in hilly areas are more prone to late blight as high temperature and drought are unfavourable for the fungal growth. Prolonged cool and humid environment can act as indicator also for the occurrence of the disease as well. Late blight can cause severe damage to potato crop by reducing tuber size and weight thereby lowering the yield and in severe cases there is complete loss of the crop as well (Fry and Goodwin 1997).

11.2.1.2 Causal Organism

Late blight is caused by *Phytophthora infestans* having aseptate and branched mycelium. Overwintering of mycelium takes place in infected tubers and also the hyphae are both intercellular and intracellular.

11.2.1.3 Symptoms

The upper part of the plant is infected first where purple to black lesions appear on the tips and margins of leaflets, petiole, and stem. With increase in fungal growth, the lesions spread to whole leaf surface. The first attacking zone of the fungus is leaf and gradually spread towards other parts and if not controlled, then foliage destruction occurs along with curling of leaves. A characteristic odour may be omitted. After the top infection, tubers get infected leading to brownish discolouration of skin (Andrivon 1996).

Table 11.1 The major fungal disease of tuber crops and their symptoms along with their causal organisms

Disease name	Major tuber crops affected	Causal organism	Symptoms
Leaf spot	Sweet potato	*Phomopsis ipomoea batatas*	Newly emerged shoots from the infected plants collapse suddenly including circular spots and brown lesions
	Taro	*Phyllosticta colocasiophila*	
	Cassava	*Cercospora henningsii, Cercospora caribaea*	
	Yam	*Sclerotium rolfsii*	
Alternaria brown spot	Potato	*Alternaria alternata*	Small, dark and round necrotic lesions Black pits on the surface of tuber
Late blight	Potato	*Phytophthora infestans*	Water soaked irregular lesions appear on young leaves Dark brown or black lesions on stem surface
	Taro	*Phytophthora colocasiae*	
Dry rot	Potato	*F. coeruleum, F. eumartii, F. oxysporum* and *F. sulphureum*	Brown, dry and powdery rot but in later stages can turn watery Brown patches on tuber surface
	Taro	*Fusarium solani*	
	Yam	*Botryodiplodia theobromae*	
Early blight	Potato	*Alternaria solani*	Dark brown spots resembling bull's eye
Pink rot	Potato	*Phytophthora erythroseptica*	Off white texture of infected tubers vinegar like smell watery fluid excretion
Gangrene	Potato	*Phoma exigua*	Appearance of thumb marks and large cavity lines on infected tubers
Powdery scab	Potato	*Spongospora subterranea*	Appearance of under skin spots and deformed growth of infected tubers
	Sweet potato	*Elsinoe batatas, Sphaceloma batatas*	
Wilting	Potato	*Fusarium oxysporum*	Yellowing and dullness of leaves and followed by wilting and death of vine
	Sweet potato	*Verticillium alboatrum*	
Charcoal rot	Potato	*Macrophomina phaseolina,*	Lower surface of stem appears dark like a black leg
	Taro	*Ceratocystis fimbriata*	
Stem canker	Potato	*Rhizoctonia solani,*	Appearance of brown cankers on underground stem
	Sweet potato	*Ceratocystis fimbriata*	
Wart disease	Potato	*Synchytrium endobioticum*	Appearance of rough warty outgrowths or protuberences on stolon, leaf and stem
Silver scurf	Potato	*Helminthosporium solani*	Shrunken tubers with silver sheen on it

(continued)

Table 11.1 (continued)

Disease name	Major tuber crops affected	Causal organism	Symptoms
Skin spot	Potato	*Polysecytalum pustulans*	Appearance of light brown lens on underground parts
Watery wound rot	Potato	*Pythium ultimum* and *P. debaryanum*	Black and yellow lines on the tubers
Chlorotic leaf distortion	Sweet potato	*Fusarium lateritium*	White waxy mucilaginous layer on young leaves spread first which then pass on to stem and veins later
White rust	Sweet potato	*Albugo ipomoea panduratae*	Presence of chlorotic and yellowish blotches
Scurf	Sweet potato	*Monilochaetes infuscans*	Light brown spots on roots
Alternariosis	Sweet potato	*Alternaria bataticola*	Brown lesions on the leaves and black lesions on petioles and stem
Cassava ash disease	Cassava	*Oidium manihotis*	Appearance of white mycelium over leaf surface
Anthracnose (wither tip)	Cassava	*Glomerella manihotis Colletotrichum manihotis*	Pale brown shallow depression bearing spots on normal green tissue
	Yam	*Colletotrichum gloeosporioides*	
Elephant foot yam disease	Yam	*C. siamense*	Pale to tan spots on leaves
Soft rot	Yam	*Rhizopus spp. Rhizoctonia solani S. Rolsii*	Fungus ramify the tissue which turn brown and then become soft
Violet root rot	Sweet potato	*Helicobasidium mompa*	Plants become chlorotic and violet colouration of roots appear along with strong alcoholic smell
Collar rot	Sweet potato and yam	*Sclerotium rolfsii*	Sudden wilting of sprouts, followed by rotting and finally death of crop
Rhizopus rot	Taro	*Rhizopus stolonifer*	Appearance of white to cream colour rot with yeasty smell
Black rot	Taro	*Ceratocystis fimbriata*	Charcoal black rot with banana like odour to it
Spongy black rot	Taro	*Botryodiplodia theobromae*	Cremish to grey spots gradually turning dark
Pythium rot	Taro	*Pythium aphanidermatum*	Stunted plants with curled leaves along with yellowish spots
Stem rot	Cassava	*Glomerella cingulata Botryodiplodia spp.*	Infected stem pieces show brown discolouration
Root rot	Cassava	*Phytophthora spp. Sclerotium rolfsii*	Swollen roots damage

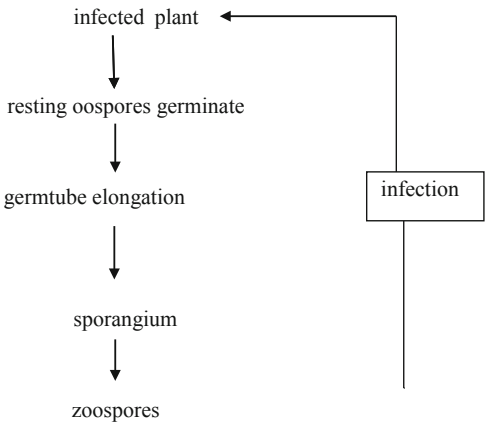

Fig. 11.2 Disease cycle of *Phytophthora infestans* causing late blight in potato

11.2.1.4 Disease Cycle

Primary source of infection are the infected tubers where fungal pathogen overwinters as dormant mycelium. During planting, the resting oospores that are in ample amount in infected tubers, germinate and usually end in terminal sporangium (Fig. 11.2). This sporangium further divides into zoospores which infect the healthy sprouts. Formation of sporangia requires an optimum temperature range of about 18–22 °C with 91% relative humidity. During warm conditions only direct germination occurs where no zoospores are formed and sporangium functions as conidium and directly puts an infection thread whereas lower temperature favours indirect germination via zoospores. The infection is dry until the onset of secondary infection from soft rot (Andrivon 1996).

11.2.1.5 Management of Lateblight

Few management strategies to control late blight prevalence in potato crop are listed as follows

- Field inspection of tubers.
- Selection of disease free seed tuber for planting.
- Efficient cold storage is necessary.
- Use of suitable fungicides such as Perenox, Fytolan, Blitox-50, Dithane Z-78, Dithane M 22, etc.
- Growing disease resistant varieties.
- Proper sanitation.
- Dusting foliage with copper-lime dust.

11.2.2 Sweet Potato

Sweet potato (*Ipomoea batatas*) belonging to convolvulaceae family is an herbaceous perennial crop produced for its edible storage roots. Its enlarged roots are

called tubers acting as energy source for the plant (Krochmal et al. 2020). Tubers vary in shape of varied colours (yellow, brown, purple, etc.) and harvested after one growing season (Senthilkumar and Yeh 2012). Although sweet potato contribute to highest yield among the important food crops even in adverse conditions, but still are affected by many biotic and abiotic pests. Insect and viruses are the leading pests of sweet potato, but fungal pathogens also cause serious damage (Hegde et al. 2012). Fungal attack tends to reduce yield by lowering the transport of nutrients to storage roots and reduced photosynthetic area. Many fungal pathogens attack sweet potato, black rot being the serious one which is discussed below.

11.2.2.1 Black Rot of Sweet Potato

Out of all the fungal diseases of the sweet potato, black rot of sweet potato is the most destructive one and is found wherever the sweet potato is grown. It is prevalent in tropics and sub-tropics along with temperate regions. China and Japan contribute to the major black rot affected areas (Thankappan and Nair 1990).

Causal Organism *Ceratocystis fimbriata*

11.2.2.2 Symptoms

- The primary symptom of black rot is the yellowing of leaves followed by its browning and gradually the foliage withers and plant die.
- Sprouts tend to wilt first before turning yellow.
- An early symptom on sweet potato fruit includes slightly sunken and dark brown spots. The rot can spread to the inner part of sweet potato fruit which can lead to destruction of whole root.
- Sunken lesions and cankers may also be visible on underground stem.

Disease Spread Through water, wind, soil, by insects, humans, contaminated tools, etc. Also the fungus enters through wounds

Disease Cycle Ascospores and aleurioconidia are the most common survival units due to their thick walls and can survive in soil for longer durations. Infection is mainly through wounds, natural or manmade and fungus enters through it further causing disease as depicted in Fig. 11.3.

11.2.2.3 Management Strategies

- Crop rotation.
- Planting of disease free seeds.
- Treatment with fungicides such as thiabendizole, Fludioxonil, dicloran, etc.
- Field sanitation.
- Dry storage is necessary.

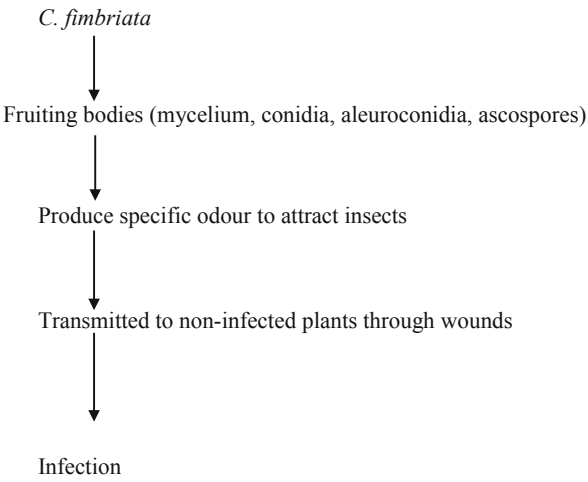

Fig. 11.3 Disease cycle of *C. fimbriata* causing black rot of sweet potato

11.2.3 Taro

Ancient crop belonging to family Araceae is grown in tropics and subtropics. This crop is believed to be of Indian and Malaysian origin. Taro also known as "potato of tropics" or "elephant ear" is an herbaceous perennial plant with leaves resembling a huge "elephant ear" (Bowers 1967). The heart shaped leaves across long petioles emerge from an tuberous rootstock called as "corm". Small tubers or "cormels" are sometimes produced from the sides of main corm. Taro is famous for its edible corm and ornamental properties (Garcia and MonlIor 1971).

Taro is susceptible to many fungal pathogens but do not face severe yield loss except for Phytophthora blight, corm rot and Pythium rot which causes major growth and production loss (Rashmi et al. 2018). Pythium rot as discussed below is the most adverse one and is found wherever taro is grown.

11.2.3.1 Pythium Rot

Pythium rot of taro contributes to the most widely distributed fungal disease probably from the time the crop was introduced. It is the soil borne fungal pathogen attacking roots and underground parts (Carpenter 1919).

Causal Organism *Pythium spp*.

11.2.3.2 Symptoms

- Outer older leaves dry up.
- If not controlled timely, then the disease spread and young leaves are comparatively shorter.
- Roots may or may not be present in infected plants.
- Decline in growth.

11.2.3.3 Disease Cycle

It has a wide host range and during unfavourable conditions, *Pythium* produces oospores that are resistant spores that prevail in soil for longer duration until favourable conditions appear (Fig. 11.4). Waterlogged soil is suitable habitat for *Pythium* to grow and spread (Goyal et al. 1974; Bergquist 1972).

11.2.3.4 Management Strategies

- Site selection.
- Fungicides application, e.g. Metalaxyl.
- Use of resistant varieties such as Pula Sama Sama, Talo vale, Pute Mu, etc.
- Field sanitation.

11.2.4 Cassava

Cassava is the largest producing staple crop of Africa, Asia and Latin America. In Asia and Latin America, cassava is exploited for raw material in industries and animal feed whereas in Africa, it is the major staple food crop (Chanie and Walelign 2020). It is considered as the cheapest source of starch thereby being the most dependable source of food in African countries. The major advantage of growing cassava is that, it can sustain well in poor soils as well, as compared to other tuber crops such as yam, potato, sweet potato, etc. (Blagbrough et al. 2010). But still there exist some factors that limit the cassava production as well; diseases and pests being the major constraints. Bud necrosis, anthracnose, leaf spots and root rot diseases

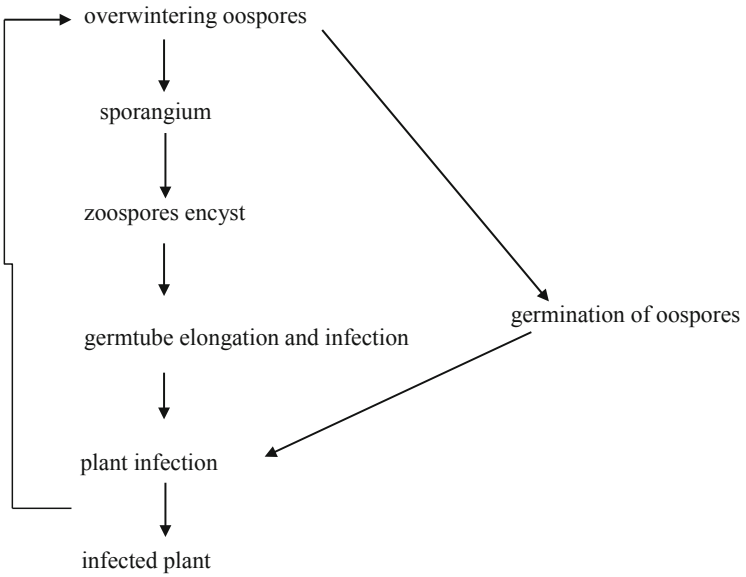

Fig. 11.4 Disease cycle of *Pythium spp.* causing pythium rot of taro

cause serious damage by fungal pathogens to cassava yield and out of these anthracnose being the most destructive one (Zinsou et al. 2017). Hence, it needs to be managed effectively to satisfy industrial, domestic and food security requirements.

11.2.4.1 Cassava Anthracnose Disease (CAD)

Cassava Anthracnose disease (CAD) is economically important disease of cassava which has also attained epidemics in the regions with high rainfall and humidity. Due to high consuming rate of this crop especially in tropical regions, CAD gained a lot of attention for its investigation and eradication (Sharma and kulshrestha 2015).

Causal Organism *Colletotrichum gloeosporioides.*

11.2.4.2 Symptoms

1. Cankers on branches, fruits, leaf spots and stem.
2. Appearance of minute sunken spots on leaves, petioles and stolons.
3. If not controlled, fruiting bodies bearing spores appear on these spots.
4. Stem deformation.

Disease Cycle The fungus produces acervuli which are black fungal fruiting bodies containing conidia (Fig. 11.5). Conidia are responsible for causing infection into the host plant thereby leading to appearance of cocentric rings of conidial masses around lesions that appear on stolons and petioles (Alahakoon et al. 1994).

11.2.4.3 Management Strategies

- Crop rotation.
- Chemical control.
- Fungicide control such as use of copper fungicides.
- Use of resistant cultivars, such as TME 30001, 30,211,91/00313,91/00684.
- Proper drainage of soil.

11.2.5 Yam

Yam is herbaceous perennial or annual plant with trailing vines. The name (yam) is given to various species of genus Dioscorea including *Dioscorea cayenensis* (yellow yam), *Dioscorea alata* (white yam), *Dioscorea esculenta* (Asiatic yam), *Dioscorea bulbifera* (potato yam), *Dioscorea batatas* (Chinese yam), etc.

These are grown for their edible tubers that extend from stolons from a central corm (specie specific). Leaves are oval containing petioles of the same length or even longer and tubers are either cylindrical or curved of brown, pink or black skin colour (Liu et al. 2007).

Yam is rich in carbohydrate content and serve as the major staple crop of tropics and Nigeria being the largest producer of yam, still its yield and quality is affected by diseases and pests to a greater extent. Yam plants are prone to infection right from

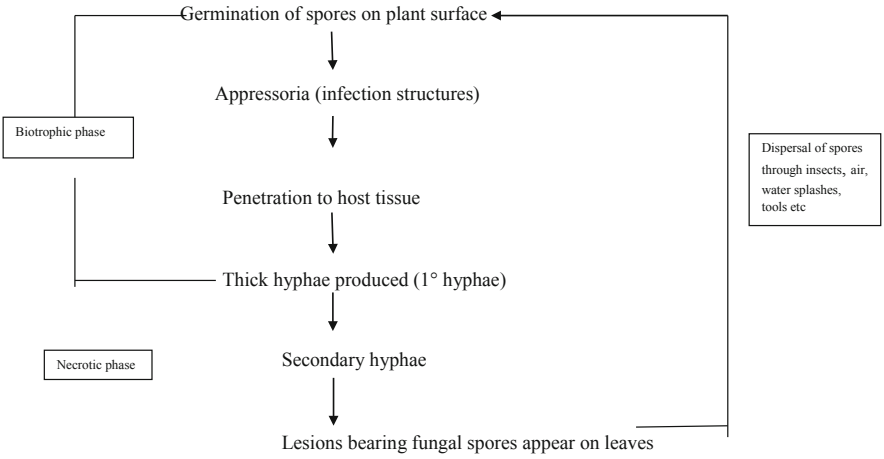

Fig. 11.5 Disease cycle of Colletotrichum gloeosporioides causing Cassava Anthracnose disease

the seedling stage to harvesting and even after that (Amusa et al. 2003; Okigbo 2005; Ezekiel et al. 2013). Fungal pathogens being the major destructive causal organism affect the growth and productivity of yam a lot. Collar rot has a devastating effect on the yam production and hence require utmost attention and curative measures to inhibit its occurrence and spread.

11.2.5.1 Collar Rot of Yam

Yam being designated as 'king of tubers' is prone to major fungal diseases known as 'collar rot'. This disease affects yam quantitatively as well as qualitatively hence need to be studied well (Palo 1932).

Causal Organism *Sclerotium rolfsii.*

11.2.5.2 Symptoms

- Deep cracks appear near collar region.
- White mycelium growth appears on the infected area.
- Roots get shredded.
- Brown to black colouration appear on the skin.
- Eventually if disease not controlled, plants rot.

11.2.5.3 Disease Cycle

Figure 11.6 depicts the disease cycle of *Sclerotium rolfsii,* where sporulation takes place when the fungus attains favourable conditions for its germination and produces thread like visible structures. The spores are transmitted through wind, water, insects, mechanical tools, etc. and infect the crop.

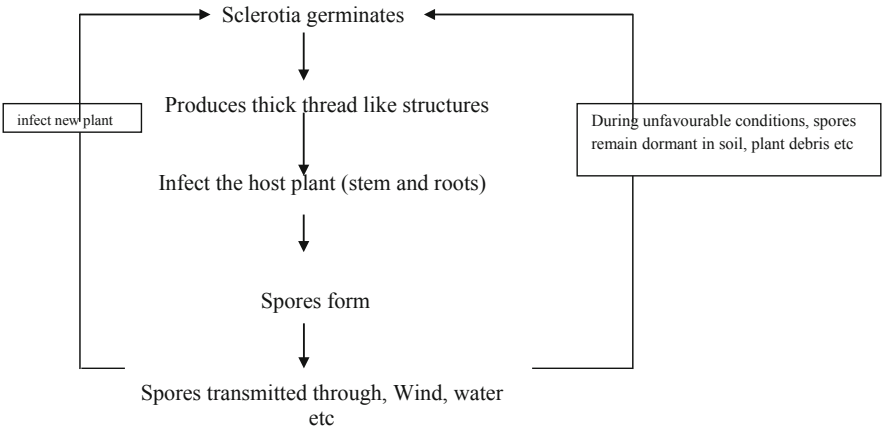

Fig. 11.6 Disease cycle of Sclerotia rolfsii causing Collar Rot of Yam

11.2.5.4 Management Practices

- Selection of disease free seed tuber for planting.
- Efficient cold storage is necessary.
- Chemical control.
- Fungicide control such as use of copper fungicides.
- Proper soil sanitation is mandatory.

11.3 General Management Practices to Control Soil Borne Fungal Pathogens

Although it is very difficult to completely eradicate the soil borne fungal pathogens but by employing these cultural, physical and chemical methods, one can prevent the epidemic to certain extent (Fig. 11.7).

11.3.1 Cultural Strategies

It is the integrated method for improving the quality and quantity of the crop by efficient farming techniques along with reducing the occurrence of harmful pathogens. It deals with altering the environmental conditions, making it unfavourable for the growth of pathogens (Islam 2001). Efficient execution of the cultural practices leads to good soil health as well as lowers the disease incidence rate. Practices like crop rotation, mulching and fallowing helps to reduce the amount of pathogens prevailing in the soil. Similarly proper sanitation control plays a crucial role in controlling the spread of fungal pathogen. Safe disposal of diseased plant by either removing the diseased parts or burning them is necessary (Neshev 2008). Also fungal pathogens spread rapidly in moist environment hence proper care should be

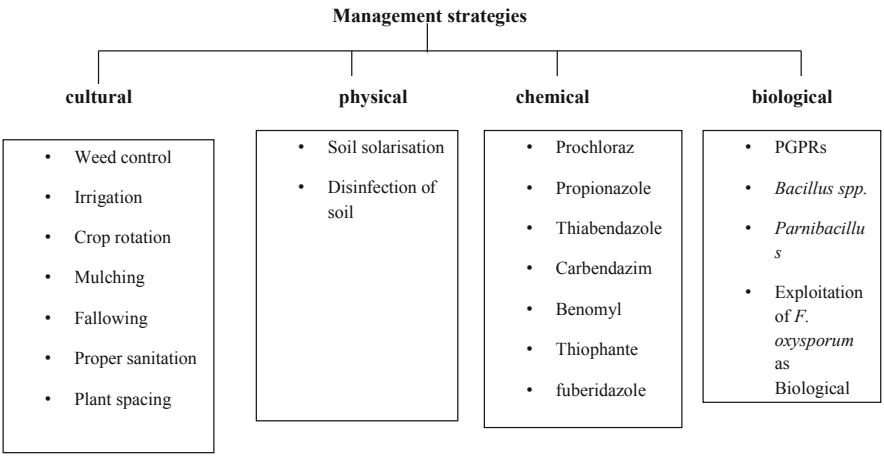

Fig. 11.7 Traditional management strategies for controlling fungal pathogens

taken while irrigation. One must avoid overhead irrigation and drainage facilities should be proper.

11.3.2 Physical Control

It deals with the soil solarization as well as disinfecting the soil using steam and raised temperature. In soil solarization process, plastic sheet is wrapped over soil surface for few weeks to entrap the solar energy that lad to increase in soil temperature and create dry environment which is unfavourable for the growth of fungal pathogens. Similarly disinfecting soil using hot water or passing steam through it creates unsuitable conditions for the pathogens to sustain in the soil (Gullino et al. 2003).

11.3.3 Chemical Control

Fungal pathogens can also be controlled using chemicals or fungicides. Fungicides such as Prochloraz, Propiconazole, Thiabendazole, Carbendazim, Benomyl, Thiophante, fuberidazole, etc. are mostly used for their eradication. Although exploiting these chemicals for fungal control is comparatively fast process but are not reliable as spraying these fungicides can harm the beneficial population of microorganisms as well and can also deteriorate the soil quality as well (Weller et al. 2002).

11.3.4 Biological Control

Biological control strategy has emerged as an efficient tool nowadays as compared to other methods for the control and eradication of the soil borne fungal pathogens. This method employs the use of biological organisms to control the spread of pathogens. Organisms such as PGPRs (Plant growth promoting rhizobacterium), *Bacillus spp. Paenibacillus,* non-pathogenic strain of *F. oxysporum,* etc. can be used for the management of the spread of fungal pathogens. These biological agents can either act as competitors for nutrient uptake, can secrete antifungal compounds, suppressors, harmful peptides, etc. thereby resisting the growth of fungal pathogens in the soil (Choudhary and Johri 2009; Jetiyanon and Kloepper 2002; Lugtenberg and Kamilova 2009).

11.4 Strategies for Development of Fungal Resistant Crops

Fungal pathogen affects the yield and quality of tuber crops to a greater extent. Their management is very important as to get rid of the considerable amount of loss they do to the crop plants (MacLean et al. 1993). Apart from the physical, chemical, cultural and biological management strategies (discussed above) which are temporary control, breeding techniques have led to the generation of fungal resistant crops as well. But the major constraint in applying breeding strategies is that it is suitable only in sexually compatible species and secondly is time consuming also (Islam 2006). It requires years of trials to develop a fungal resistant variety. To overcome these hurdles, genetic engineering comes into play where the disease resistant genes can be incorporated from one species to another within a limited time span.

11.5 Approaches for Fungal Resistant Transgenic Plant Production

Following transgenic approaches are being exploited for the generation of fungal resistant transgenic crops (Islam 2006):

- Expression of proteins that enhance the structural defence mechanism of plants naturally, e.g. Lignin and peroxidises.
- R gene (resistant gene) expression in response to interaction with the avirulence (*Avr*) gene.
- Overexpression of pathogenesis-related (PR proteins) and phytoalexins which resist the growth of fungal pathogens.
- Managing the plant defence signal transduction by expression of the signalling molecules such as elicitors, salicylic acid, hydrogen peroxide, etc.
- Oxalic acid, lipase and polygalacturonase expression for the neutralization of the components of fungal pathogen.

Table 11.2 Fungal resistant transgenic tuber crops

Gene incorporated	Donor crop	Recipient crop	references
Glucanase and chitinase	Pea	Potato	Chang et al. (2002)
Cationic peptide (mSrA3)	Synthetic	Potato	Osusky et al. (2004)
Chitinase Glucanase	Cucumber Tobacco	Potato	Moravcıkova et al. (2004)
Chitinase (BjCHI) Glucanase (HbGLU)	Mustard Rubber tree	Potato	Chye et al. (2005)
Chitinase (ricchi11)	Rice	Taro	He et al. (2008)
Chitinase (chi c)	*Streptomyces griseus*	Potato	Raham et al. (2008)
Thionin αHT	Barley	Sweet potato	Muramoto et al. (2012)
Ostlp	Cassava	Cassava (overexpression)	Ojola et al. (2018)

- Strategies such as RNAi, lysosome and RNase are also exploited for the production of fungal resistant crops (Table 11.2).

11.6 Conclusions

Soil borne fungal pathogens possess the ability to sustain in soil for longer duration due to which underground stem and tuber crops are more prone to the diseases as compared to other crop plants. Their initial attack and symptoms are difficult to analyse until the above ground parts show signs like wilting, chlorosis, rot, etc. Both quality and quantity of the tuber crops are affected by the fungal attack also accumulation of the mycotoxin into edible parts can affect human health as well on consumption. Although traditional management practices such as physical, chemical, biological and cultural strategies can help in reducing the onset and prevalence of fungal pathogens but still face many lacunas. In recent years, studies based on plant–pathogen interaction have led to exploitation of various breeding and genetic engineering strategies to develop fungal resistant transgenic crops but progress with respect to tuber crops is still lacking. Transgenic approaches should be utilized more in tuber crops so as to enhance the global tuber crop production by lowering the fungal disease incidence and their complete eradication.

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Plant Growth Promoting Rhizobacteria for Crop Health in Wheat-Maize Cropping Systems in Northwest Himalayas

12

Gaurav Sood and Rajesh Kaushal

Abstract

Advent of the green revolution initially raised the production in agriculture sector but the non-judicial and indiscriminate use of synthetic inputs lead to deterioration of soil health due to imbalance of nutrients coupled with decreased use efficiency is a matter of great concern which compelled the scientists to look for alternate with low cost, non-bulky renewable inputs for sustainable crop production without deterioration of soil health. Plant growth promoting rhizobacteria (PGPR) are group of rhizobacteria, i.e. *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter* and *Serratia,* etc. that produces metabolites those can promote growth and produce induced systemic resistance against various phytopathogens. The possible mechanisms of plant growth promotion and disease resistance have been well documented in other crops but still needed to be explored in North Western Himalayan region, especially for cereal crops. Indigenous PGPR isolated from different agro-climatic zones of Himachal Pradesh for wheat and maize and were molecularly identified as *Serratia* sp. and *Bacillus subtilis* for wheat and *Bacillus subtilis* for maize. The conjoint application of *Serratia* sp. and *Bacillus subtilis* in wheat and *Bacillus subtilis* in maize along with 80% recommended doses of fertilizers registered increase to the tune of about 9% increase in grain and 9% straw yield in wheat and 11% increase in corn and 17% straw yield in maize.

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Crop yield · Plant growth promoting rhizobacteria · Abiotic stress · Wheat and maize

12.1 Introduction

Incessant application of chemical fertilizers for achieving high crop yield in the last few years lead to high productivity of the crops with advent of green revolution in the initial few years. Accessibility and high prices of these chemical fertilizers limit crop production in India especially in the North West Himalayan region where maximum protein requirement of the people is met through cereal crops, chiefly wheat and maize, which requires large quantity of nitrogen and phosphorus in comparison to other essential nutrient elements. Chief macronutrients playing active function in different metabolic activities and phenology of crop plants are nitrogen and phosphorous (Khan et al. 2013), however, non-judicial and indiscriminate use of synthetic inputs lead to deterioration of soil health due to imbalance of nutrients coupled with decreased use efficiency is of great concern which compelled the scientists to look for alternate with low cost, non-bulky renewable inputs for sustainable crop production.

Biofertilizers/PGPR are well documented as a vital module of INM (integrated plant nutrient management) for sustenance of agriculture and hold a great assurance not only for improving crop yield but also maintain soil health for present and coming generations (Giri et al. 2019). Plant growth promoting rhizobacteria (PGPR), which colonizes the rhizospheric region of the crops in response to various plant flavonoids, thereby in return rhizobacteria produce metabolites those can enhance plant growth and produce ISR (induced systemic resistance) combating different phytopathogens (Kour et al. 2020). PGPR generally consist strains of genera such as *Paenibacillus*, *Azospirillum*, *Acetobacter, Actinoplanes*, *Azotobacter*, *Alcaligenes*, *Enterobacter*, *Serratia*, *Bacillus*, *Rhizobium*, *Erwinia, Pseudomonas*, *Burkholderia* and *Flavobacterium,* etc. (Prasad et al. 2015).

The plant growth promoting rhizobacteria (PGPR) augment plant growth either directly by release of plant growth regulators such as auxin, ethylene, cytokinins and by increasing the availability and plant uptake of some nutrients, especially fixed nitrogen, phosphate and iron in the rhizospheric region or indirectly, through induction of host defense mechanisms against various phytopathogens infecting the crops (Glick 1995). PGPR releases a variety of antifungal secondary metabolites e.g. siderophore, 2, 4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, ammonia, hydrogen cyanide, phenazines and lytic enzymes (proteases and chitinases), etc. to check phytopathogens poliferation (Loon et al. 1998).

Availability of the major macronutrients at soil–root interface considerably affects the productivity and growth of crops, which is affected by a wide range of factors including soil physico-chemical properties, species and genotype of plant and various interactions at soil–root interface which include soil macro and

microorganism communities and environmental conditions, i.e. biotic and abiotic factors (Marschner et al. 2011). Among these, drought is a major abiotic factor that is hampering crop growth and productivity in today's world. On the one hand, plants possess natural protection systems that protect them from a variety of stresses, but they also interact with a variety of soil microorganisms that can alleviate the stress symptoms (Hammad and Ali 2014; Manoj et al. 2020). Microbial communities are able to build up a variety of actions that are most vital in maintaining biological equilibrium and sustainability in soil particularly under stress conditions (Kavamura et al. 2013).

PGPR are bio-resources which perhaps considered as a novel and potential means for providing considerable profit to the agriculture. The application of PGPR is progressively escalating in agriculture and offers an efficient way to supplement chemical inputs.

12.2 PGPR as Root Colonizers

The rhizosphere term was first given by Hiltner in 1904 and is considered as a thin region of soil contiguous with the root that is under the influence of root system and is dominated by microorganisms. As compared to the bulk soil, rhizospheric region is affluent in nutrients, attributable to the accretion of different organic compounds as a result of root exudation, secretion and rhizodeposition. These organic compounds are used as a source of energy and carbon due to which microbial action is chiefly acute in the rhizosphere. The rhizosphere is therefore habitat to a group of bacteria associated with roots, generally referred to as rhizobacteria. Such beneficial bacteria that have positive effect on the plant growth by direct/indirect mechanisms (Fig. 12.1) are referred as plant growth promoting rhizobacteria (PGPR). Bacterial diversity associated with the rhizosphere of wheat and maize was studied during 2013–2016 from different agro-climatic zones of Himachal Pradesh falling in North Western Himalayan region (Sood et al. 2018a, b). A total of 127 isolates from wheat and 65 from maize rhizosphere were isolated and screened for various PGP (Plant growth promoting), viz. phosphate solubilization, siderophore production, growth on N-free medium, auxin production, ACC-deaminase production and antagonism against *Fusarium graminearum, Claviceps purpurea* and *Alternaria triticina* in case of wheat and *Fusarium oxysporum* and *Rhizoctonia solani* in maize (Fig. 12.2). On the basis of prominent PGP activities ten isolates from wheat and ten isolates from maize rhizosphere were studied for their efficacy to act as biofertilizer, biostimulant and bioprotectant (Table 12.1).

The extent of propinquity among the PGPR and host plant can differ based on where and how they colonizes the crop plant. The complexity of associations is at two levels, i.e. (1) Epiphytic and (2) Endophytic.

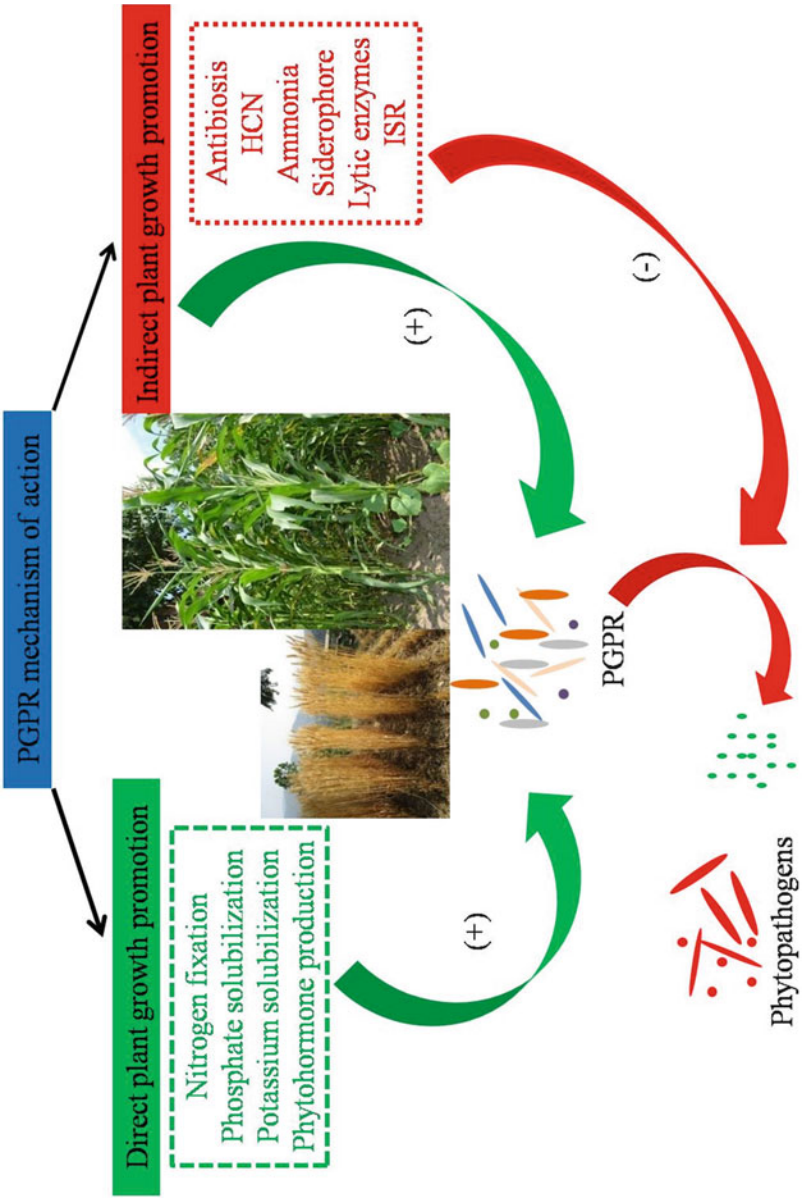

Fig. 12.1 Direct and indirect mechanisms adopted by PGPR to enhance plant growth and productivity

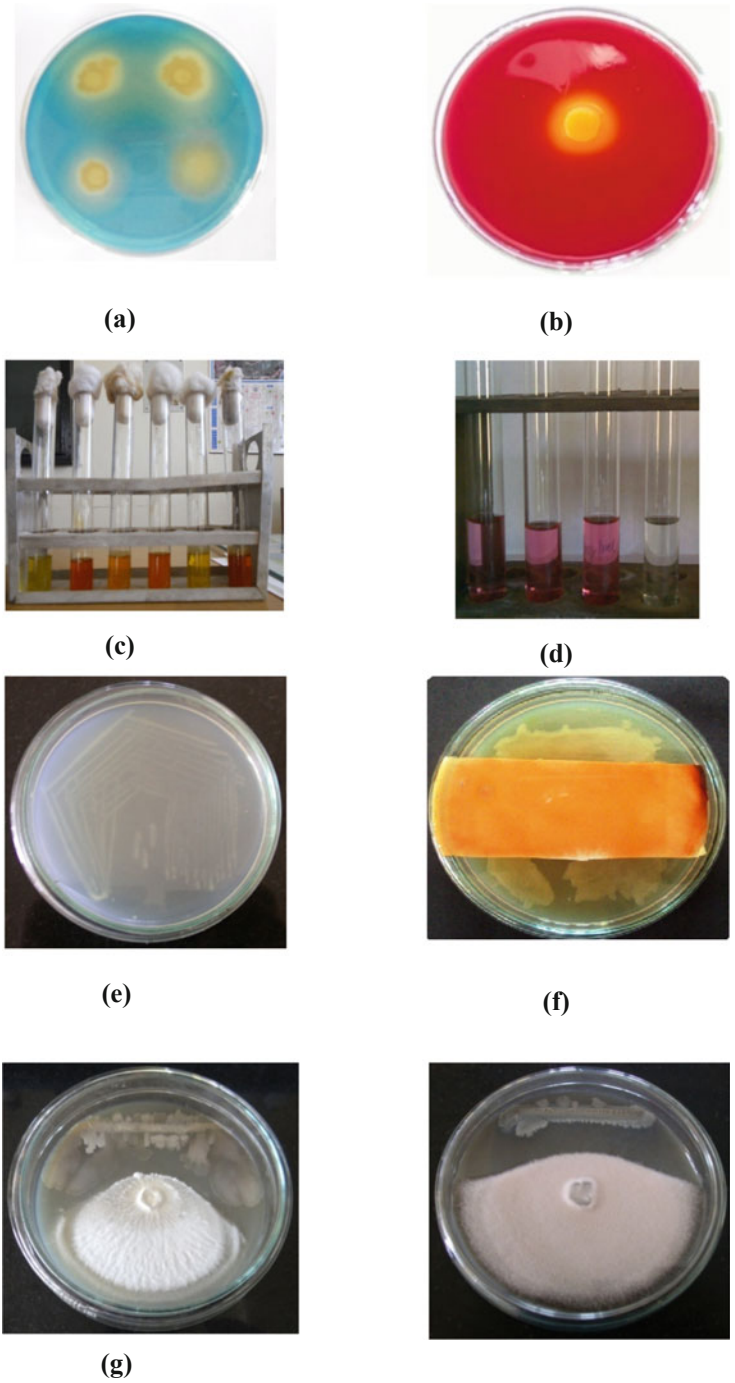

Fig. 12.2 Multifarious plant growth promoting activities of bacterial isolates: (**a**) Siderophore production (**b**) P-Solubilization (**c**) Ammonia production (**d**) IAA production (**e**) Growth on Nitrogen Free Medium (**f**) HCN Production (**g**) Antifungal activities against *C. purpurea* and *A. triticina*

12.2.1 Epiphytic Region

The rhizosphere/epiphytic region of soil is the region of soil that is under the influence of pant roots in association with root exudates that act as nutrient source for the bacterial communities, owing to their high population in this region. In numbers, the rhizosphere microbes are ten to hundred times more than those of the soil. Their composition would, however, depend upon the amount and type of the nutrients in the rhizosphere. The overall metabolic activity of the microorganisms in the rhizosphere is several times more than in the soil farther away from the rhizospheric region. In turn, the microorganisms that colonize the rhizosphere profoundly affect root and plant biology in relation to nutrition, development, health. Root surface colonization is influenced by numerous factors, i.e. biotic (genetic potential of the host plant and bacteria that colonizes it) or abiotic (growth substrate, soil moisture, temperature and soil pH). A total of eight (B2, UNS3, HAR3, BIS2, MAS1, CHS1, KIS2 and LSR1) isolates out of ten from wheat rhizosphere were epiphytic in origin and all the ten (B1N1, J2, J4, M3, R6, NRG, DHK, MAT1, MAT2 and KAN) isolates from maize were epiphytic in origin (Sood et al. 2018a, b) (Table 12.1).

12.2.2 Endophytic Region

Endophytes are group of bacteria/fungi that are found inside the plant roots, forming close associations with them, without causing any perceptible symptoms of disease. They show complex associations with their hosts which may be mutualism or antagonism. Plants firmly limit the endophytic growth of microorganisms and use different mechanisms to slowly adapt to their living environment inside the host plant. To maintain a stable symbiotic, endophytes produce a number of compounds that alleviates plant growth and assist them to acclimatize better to different environmental conditions (Compant et al. 2005). Commonly found endophytes, generally belongs to genera *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium* and *Allorhizobium*, collectively called as rhizobia, family Rhizobiaceae invade plant roots system and form root nodules (Wang et al. 2006). Endophytic associations in microbial communities are supposed to be result of colonization activity which is initiated in the root zone but they may initiate from different sources, viz. phyllosphere, atmosphere and spermosphere (Sturz et al. 2000). Out of ten isolates from wheat rhizosphere two (SIR1 and SHR1) were endophytic in origin and none of the endophytic isolates showed prominent PGP activities, so not selected for further studies (Sood et al. 2018a, b) (Table 12.1).

Joshi and Bhatt (2011) studied the root colonized bacterial diversity in wheat and selected 133 different bacterial isolates based on phenotypic and physiological characteristics. They found that 44 per cent were *Bacillus* sp. and 24 per cent belong to *Pseudomonas* sp. They further reported that Shannon-Wiener Index of microbial diversity was ranged from 1.75 to 1.59.

Table 12.1 Plant growth promoting activities of wheat and maize bacterial isolates

Agro-climatic Zones	Wheat isolates										Maize isolates									
	Zone-I				Zone- II		Zone -III		Zone-IV		Zone-I				Zone- II				Zone -III	
Isolates	B2	UNS3	HAR3	BIS2	MAS1	SIR1*	CHS1	SHR1*	KIS2	LSR1	B1N1	J2	J4	M3	R6	NRG	DHK	MAT1	MAT2	KAN
Phosphate solubilization[a]	++ +	−	+++	+++	−	+++	++	+++	++	+	+++	+ + +	+ +	++	++	+	+++	+++	+++	++
Growth on nitrogen free medium[b]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Siderophore production[c]	+	+	+	+	+	+	+	+	+	+	+	+ + +	+ +	+	++ +	+	+++	+++	++	+++
ACC-deaminase activity[d]	+	−	+	+	+	+	−	−	−	−	+	+	−	−	−	+	+	+	+	−

[a]P-solubilization in vitro: **+**:<5 mm wide halo zone; **++**: 5–10 mm wide halo zone; **+++**: >10 wide halo zone.
[b]Growth on nitrogen free medium: **+**:< 3 mm colony diameter; **++**: 3–6 mm colony diameter; **+++**: >6 mm colony diameter.
[c]Siderophore activity: **+**:<5 mm yellowish orange zone; **++**: 5–10 mm yellowish orange zone; **+++**: >10 mm yellowish orange zone.
[d]ACC-deaminase activity: (+) indicates growth; (−) indicates growth absent.
[e]Isolates endophytic in origin.

Cavaglieri et al. (2009) studied the influence of plant growth stages on the population size of culturable bacteria and fungi associated with rhizoplane and endo-rhizosphere of maize grown in field and reported that plant development did not have influence on total culturable microflora density but it selectively influenced some bacterial and fungal groups present in the rhizosphere. However, the microbial community structure changed markedly over time. Overall, the endophytic and rhizosphere microbial community is of dynamic structure and is influenced by biotic and abiotic factors, with the plant itself constituting one of the major influencing factors (Hallmann et al. 1997).

12.3 PGPR as Biofertilizers

Biofertilizers are microbial inoculants containing live preparations of microbial cells, which can convert the unavailable form of major macronutrients (N, P& K) to available form by using different biological processes that are readily absorbed by the plants (Hegde et al. 1999; Vessey 2003; Gou et al. 2020; Pagnani et al. 2020). In recent few decades, they have emerged as a vital module of the INM system and hold a great promise to alleviate crop yields by augmenting soil with nutrients. Some PGPR possesses both biofertilizer and biocontrol properties, which make them better candidate to act as plant growth regulators and at the same time protecting them from different phytopathogens, e.g. strains of *Burkholderia cepacia* have been revealed to have biocontrol characteristics against *Fusarium* sp. and can also enhance maize yield by sequestering iron from soil (Bevivino et al. 1998).

Enormous amount of chemical fertilizers are now a days used to replenish soil N and P, resulting in high costs and posed the problems of environmental pollution. Most of P applied from chemical fertilizers turns into insoluble compounds and is not absorbed by plants. Therefore N_2-fixing and P-solubilizing bacteria can combat this problem by increasing the accessibility of N and P to the crop plants and are key players in sustaining agro-ecosystems health.

12.3.1 PGPR as Nitrogen Fixer

Nitrogen is the most important element on which growth and productivity of plants depends. Even it is so much abundant (78.09%) in atmosphere, yet it cannot be utilized by plants. Plants can utilize Nitrogen in its most oxidized form; the nitrates or the most reduced form, i.e. ammonia. The indiscriminate use of nitrogenous fertilizers amounts to depletion of non-renewable fossil fuels used in fertilizer production. Annually nitrogen that is fixed biologically accounts for about 175 million tones of which around 79% accounts the amount fixed by terrestrial plants. Fixation of nitrogen biologically/biological nitrogen fixation (BNF) is an economically sound way to limit the use of nitrogen fertilizers, thus improving soil health and fertility (Bagyaraj 2011).

In nature, diazotrophy is restricted to some prokaryotes and archaebacteria only. Under natural conditions nitrogen can be fixed as free living or in association with higher or lower organisms. Under free living conditions, organisms can fix nitrogen under aerobic, microaerophilic and anaerobic conditions. Free living nitrogen fixers grow in soil or in rhizosphere and never enter plant roots. Though there are a number of nitrogen fixing bacteria reported in literature, the most important genera includes *Azotobacter*, *Azospirillum*, *Burkholderia*, *Gluconacetobacter* and *Pseudomonas,* etc. (Bashan and De-Bashan 2010). Another important group being free living cyanobacteria, which can fix nitrogen in both under aerobic and anaerobic conditions. Cyanobacteria aerobically fix nitrogen in specialized cells known as heterocysts which have anaerobic environment or by temporal separation of nitrogenase from oxygen during dark as seen in unicellular cyanobacteria such as *Gloeothece*. Some cyanobacteria are symbiotic diazotrophs like *Anabaena azollae* and *A. cicadae*. Symbiotic N_2 fixation refers to the organisms that form symbiotic relationship with different parts (root, stem and leaf) of plants. However, the process of symbiotic N_2 fixation is limited only to legume plants and various trees and shrubs that form actinorrhizal roots with *Frankia*. The most familiar example of symbiotic nitrogen fixation is the close association between legumes and rhizobial bacteria (*Rhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Bradyrhizobium*) infecting three major botanical sub-families *Papilionodeae*, *Ceasalpinioideae* and *Mimosoideae*. Among leguminosae, the largest number of plants are in *Papilionoideae* sub-family. Apart from legumes, the roots of some plants belonging to different non-leguminous angiosperms like *Alnus*, *Myrica*, *Casuarina*, *Discaria* are nodulated by *Frankia* sp. All the ten isolates each from wheat and maize rhizosphere showed growth on Jensen's medium, there by fixing the atmospheric nitrogen (Fig. 12.2) and made it available for the crop plant (Sood et al. 2018a, b). Endophytic diazotrophs of rice, maize and sugarcane are *Azotobacter diazotrophicus*, *Herbaspirillum seropedicae*, *Azoarcus* sp., *Enterobacter asburiae* and some strains of *Burkholderia* sp. which serve as nitrogen fixers when other available sources of nitrogen are absent or at low levels (Hurek et al. 2002).

12.3.2 PGPR as P-Solubilizers

After nitrogen phosphorous is the second most commonly limiting macronutrient that is affecting the plant growth. It is a vital element of the cellular activities of the living organisms. Satirically, soils have big reserves of total P, but very little amount of this is available for plants growth (Khan et al. 2010). The availability of phosphate for biological processes will depend not only on the quantity of phosphorous in soil but also on its availability, which in turn is made available by series of chemical reactions and biological interactions present in the soil. The different forms of phosphorous can be usually classified as soil solution P, insoluble organic and inorganic P and plants can only absorb P in two soluble forms, the monobasic and diabasic ions (Pandey and Maheshwari 2007).

PSB (Phosphate solubilizing bacteria) are widespread in the rhizospheric region and production of organic acids (malic, glyoxylic, fumaric, tartaric, oxalic and citric acid, etc.) and phosphatases enzymes by them facilitates the transformation of unavailable forms of P to plant available ones. The organic acids produced by the microorganisms convert insoluble phosphate and amount of soluble phosphate produced depends upon the strength and kind of the acid released. In addition to organic acids, inorganic acids (nitric and sulphuric acids) are also formed by *Nitrosomonas* and *Thiobacillus* sp. as a result of oxidation of nitrogenous and inorganic sulphur complexes, which reacts with calcium phosphate present in soil and make them available. The conversion of P into available form by the activity of microorganisms in the rhizosphere is the key mode of action implicated by PGPR (Bhattacharyya and Jha 2012). Diverse genera like *Bacillus, Beijerinckia, Pseudomonas, Serratia, Azospirillum*, *Flavobacterium, Erwinia, Azotobacter*, *Burkholderia*, *Microbacterium, Enterobacter* and *Rhizobium* are well considered as major PSB (Mehnaz and Lazarovits 2006). All the ten isolates each from wheat and maize rhizosphere solubilized tricalcium phosphate (Fig. 12.2) in the Pikovskaya's agar medium, act as phosphate solubilizers in wheat and maize (Sood et al. 2018a, b).

12.3.3 PGPR as Biostimulater

Phytohormones also known as PGRs (plant growth regulators) are well recognized as they have regulatory role in development and growth of plants. Plant growth regulators are organic substances altering the physiological processes of plants at extremely low quantities. As the concentration of hormonal signals is decisive for the regulation of various physiological processes in plants, local changes of phytohormone levels can alter growth patterns in plants. The phytohormones formed by PGPR generally include indole acetic acid, gibberellins, cytokinins and abcissic acid, etc. which vest morphological alterations in the plants. Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement, usually produced by PGPR (Salisbury 1994). The, IAA producers morphologically alters the roots of plant by increasing its growth and length, as a result of which plants are able to access more mineral elements from soil because of improved surface area. Cytokinins enhance cell divisions, enlargement and tissue expansion in certain plant parts (Salisbury 1994). Gibberellin alters morphology of plant tissues, mainly stem tissue. Evidence of gibberellic acid (GA) production by PGPR is scarce, however, production of four alternate forms of GA by *Bacillus pumilus* and *Bacillus licheniformis* were reported by Esitken et al. (2006). Another plant hormone ethylene (ET) is gaseous in nature among the all produced by PGPR. It is also known as the ripening hormone in addition to its recognition as 'wounding hormone', as ethylene promotes adventitious root and root hair formation, stimulates germination and breaks dormancy of seeds, however, if its concentration remains high during germination root elongation is inhibited. It is proposed that many PGPR augment plant growth by lowering the concentration of ethylene by producing 1-

aminocyclopropane-1- carboxylate (ACC) deaminase, which hydrolysis ACC, the immediate precursor of ET and by products released as a result (ammonia & α-ketobutyrate) can act as nitrogen and carbon source (Glick et al. 1998). All the ten isolates from wheat rhizosphere produced Indole-3-acetic acid (IAA) ranging from 19.33 to 31.70 μg/mL, similarly in maize IAA was produced in range of 20.33–29.00 μg/ml by all the isolates (Fig. 12.2). ACC-deaminase production was shown by five isolates (B2, HAR3, BIS2, MAS1 and SIR1) in wheat and only four isolates (J4, M3, R6 and KAN) did not showed ACC production in maize (Sood et al. 2018a, b).

12.4 PGPR as Biocontrol Agents

Biocontrol/biological control is the reduction in the disease severity of a pathogen by using one or more living organisms. PGPR are native to the rhizospheric region and actively controls phytopathogens. Biocontrol efficacy of PGPR is shown against a variety of pathogens (bacterial, fungal and nematodes, etc.) (Reddy 2014). Although, significant control of phytopathogens has been verified by PGPR under laboratory and greenhouse conditions, results under field conditions still needed to be duly verified for further recommendations. The modes of action are struggle for substrates, niche exclusion production of inhibitory antibiotics, parasitism and induced systemic resistance (Bloemberg and Lugtenberg 2001). Only two isolates (CHS1and KIS2) did not show any biocontrol activity against *F. graminearum*, five (UNS3, MAS1, SHR1, KIS2 and LSR3), four (MAS1, CHS1, SHR1 and LSR1) isolates against *C. purpurea* and *A. triticina* in wheat. Whereas, in maize all the isolates exhibited biocontrol activity against *F. oxysporum* and *R. solani* except J2 isolate (Table 12.2 and Fig. 12.2).

Following are the antagonistic activities mediated by the microorganisms:

12.4.1 Antibiotics and Lytic Enzymes

Antibiotics are the suppressive substances released by the living organisms in very low concentrations that may interfere with the biological processes of the pathogens or may kill them. Most antibiotics belong to the class of nitrogen containing heterocycles such as phenazines and pyrrolnitrin. The most dominant example is agrocin 84 released by *A. radiobacter* to control infection caused by *A. tumefaciens*. Some biocontrol bacteria releases potent extracellular lytic enzymes like β-1, 3 glucanases, cellulases, lipases, chitinases and proteases capable of dissolving fungal cell walls of various fungal pathogens such as *Botrytis cinerea*, *Phytophthora* sp., *Pythium ultimum*, *Fusarium oxysporum* and *Sclerotium rolfsii* (Frankowski et al. 2001). Maximum lytic enzyme activity was exhibited by B2 and SIR1 isolates in case of wheat, however, B1N1 and DHK were found to show maximum activities in maize (Table 12.2).

Table 12.2 Biocontrol activities (ammonia, HCN production, Antifungal and lytic enzyme activity) of wheat and maize bacterial isolates

Agro-climatic zones	Wheat Isolates	Lytic enzyme activity[a]						Ammonia production[b]	HCN production[e]	Antifungal activity against phytopathogens[c]		
		Amylase activity	Pectinase activity	Protease activity	Cellulase activity	Lipase activity	Chitinase activity			*F. graminearum*	*C. purpurea*	*A. triticina*
Wheat												
Zone-I	B2	+++	+	++	++	−	+	++	+	+	++	++
	UNS3	+++	++	+++	++	−	+	−	−	+	−	+
	HAR3	−	+	−	−	−	+	+++	−	+	+	++
	BIS2	+++	+	+++	+	−	+	−	−	+	+	++
Zone- II	MAS1	+	+	+	+	−	−	++	−	+	−	−
	SIR1[b]	++	++	++	+	++	++	+++	++	++	++	+
Zone -III	CHS1	+	+	+	+	−	−	+	+	−	+	−
	SHR1[b]	+	−	+	+	−	−	−	−	+	−	−
Zone-IV	KIS2	+	−	−	−	+	−	++	−	−	−	+
	LSR1	+	−	+	+	−	−	−	−	+	−	−
Maize										Antifungal activity against phytopathogens[c]		
										F. Oxysporum		*R. Solani*
Zone-I	B1N1	+++	++	++	++	+	+	+	+++	+++		+++
	J2	++	++	+++	++	−	−	+	−	++		++
	J4	−	+	−	−	−	+	−	+	−		−
	M3	+++	+	+++	+	−	+	+	++	++		++
	KAN	++	−	−	+	−	++	+	−	+		++
	NRG	−	++	−	−	+	−	++	−	++		++
Zone- II	R6	+	+	+	+	−	−	+	−	+		+
	DHK	++	++	++	+	++	++	++	+++	+		+++
Zone -III	MAT1	+	+	+	+	−	+	++	+++	++		++
	MAT2	+	−	+	+	−	−	+	−	+		+

[a]Lytic enzyme activity: <3 mm zone; ++: 3–6 mm zone; +++:>6 mm zone.
[b]Isolates endophytic in origin.
[c]Antibiosis by agar streak assay: +:< 70% inhibition; ++:70–80% inhibition; +++: >80% inhibition.
[d]HCN production: (+) indicates light brown colour, (++) indicates dark brown colour, (+++) indicates orange brown colour, no activity (−).
[e]Ammonia production: ++ = indicates light brown colour; +++ = indicates dark brown colour; ++++ = indicates orange brown colour, no activity (−).

12.4.2 Siderophores

Availability of iron is extremely limiting in the rhizosphere and is considered as most important nutrient required for the growth of almost all living organisms. Siderophores are produced by the rhizospheric microorganisms to survive in such environments by secreting iron binding ligands having high affinity for the Fe (iron) from the microenvironment in the rhizosphere. Iron chelating molecules are hydroxymates (ferrioxamine B), catecholates (enterobactin) and carboxylates (rhizobactin) which are transported actively because of their high molecular weight (600–1500 Da) (Das et al. 2007). The siderophore molecules secreted by these organisms effectively bind most of the Fe^{3+}(ferric ion) and once inside the microbial cell it is reduced to ferrous (Fe^{2+}) form and thus sequestering iron and as a result preventing any fungal pathogens in the immediate vicinity from proliferating because of lack of Fe (Kloepper et al. 1980). PGPR possessing biocontrol activity effectively out-compete fungal pathogens for available iron and on the other hand, the plant growth is not generally compromised by the sequestration of iron in rhizosphere caused by the siderophores produced by PGPR, because mostly plants can grow at much lower concentrations of iron than most microorganisms (Sullivan and Gara 1992). All the bacterial isolates of wheat showed siderophore production ranging from 29 to 65% SPE (Siderophore production efficiency) in liquid CAS assay, whereas isolates of maize showed 5–14 mm zone on CAS (Chrome-Azurol-S) plate assay (Sood et al. 2018a, b).

12.4.3 Competition for Niche and Nutrients

Root and seeds of plants release exudates for which beneficial rhizobacteria and pathogens compete and are responsible for small degree of biocontrol activity introduced by bacteria. Majority of bacteria colonized in rhizosphere act as a partial sink for nutrient elements and thus reduced amount of carbon and nitrogen is accessible to stimulate germination of fungal spores. Fluorescent pseudomonas are extensively studied for rapid scavenging of nutrients, are nutritionally versatile and commonly found in the rhizosphere. Niche exclusion is an important mechanism shown by PGPR to antagonize the harmful rhizomicrobes by secreting the products inhibiting their growth.

12.4.4 Induced Systemic Resistance

Interaction of PGPR with plant roots can result in providing resistance to plants against some pathogenic bacteria, viruses and fungi, phenomenon is known as induced systemic resistance (ISR). ISR is a type of hypersensitive response, whereas SAR is very much similar to the inherent immunity of the host plants. ISR is systemic resistance that is naturally present in the plant but gets enhanced when come in vicinity of PGPR. It depends on ethylene and jasmonate signaling and is independent of salicylic acid therefore no pathogenesis related proteins are released.

ISR is plant specific, i.e. host specificity shown by rhizobacteria in activating resistance response and dependent on the genotype of the plant, thus conferring resistance against phytopathogens infecting them.

12.5 PGPR as Modulator of Abiotic Stress

In today's climate change scenarios, crops are exposed more frequently to episodes of abiotic stresses such as drought, salinity, elevated temperature, submergence and nutrient deficiencies, limiting crop production. Drought is considered as the one of the main obstacle hampering the crop growth and productivity in today's world, which is the result of global climate change events and is estimated to have reduced cereal productivity by 9–10% (Lesk et al. 2016). Over 50% of the cultivable lands by 2050 are going to face devastating drought consequences on plant growth (Vinocur and Altman 2005). Plant and water relationships were affected by drought stress at both cellular and whole plant levels, resulting in various physiological complex processes and phenotypical responses in plant. Oxidative stress is generated within sub-cellular compartments due to alleviated levels of reactive oxygen species (ROS). ROS consist of superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH), all of these affect building components of the cell (lipids, proteins, carbohydrates, nucleic acids, etc.) and causes cell demise (Mittler 2002). Therefore, there is an increased interest among the scientists in finding solutions to drought associated problems and its impacts on food security. Particularly, there is an utmost need to redress different solutions, which will improve drought tolerance in crop plants, so as to satisfy the food requirement with the limited water resources in today's world (Mancosu et al. 2015).

Crop productivity can be increased by inoculating plants with PGPR facing drought stress (Ngumbi and Kloepper 2016). PGPR mitigates drought stress effects by altering some processes at both physiological and biochemical (alteration in phytohormone levels, antioxidant enzyme activities (peroxidase, catalase and superoxide dismutase) and increase of several organic solutes like amino acids, sugars, etc.) levels. Moreover, heat-shock proteins, dehydrins and volatile organic compounds which are produced under extreme conditions also plays key role in attaining drought tolerance (Kaushal and Wani 2016; Mishra et al. 2017).

The endogenous bacterial endophyte SIR2 (*Bacillus subtilis*) of wheat and rhizospheric isolate B1N1 (*Bacillus subtilis*) of maize possessing ACC (1-aminocyclopropanre-1-carboxylate) deaminase activity was evaluated at four water regimes: (1) Uninoculated control (100% field capacity (FC)), (2) 80% field capacity (FC), (3) 60% field capacity (FC), (4) 40% field capacity (FC) in combination for 45 days, starting 15 days after sowing to the maturity by Sood (2016). The results revealed maximum significant increase in root length (14.72%), shoot length (1.23%), root biomass (66.67%), shoot biomass (33.04%), relative water content (1.85%), total chlorophyll content (19.35%) and total amino acid content (20.00%) in the treatments receiving SIR2 inoculation as compared to uninoculated control (100% field capacity) in wheat and similar increase in root length (15.41%), shoot length (21.8%), root biomass (60.9%), shoot biomass (65.45%), relative water content (3.0%), total chlorophyll content (18.51%) and total amino acid content

(9.52%) was observed in the treatments receiving B1N1 inoculation in maize as compared to uninoculated control (100% field capacity) in maize. Furthermore, wheat plants receiving SIR2 (*Bacillus subtilis*) inoculation and inoculation of B1N1 (*Bacillus subtilis*) in maize, subjected to 40% FC soil moisture showed significant increase in the antioxidant enzyme activity, i.e. superoxide dismutase (32.7 U/g fresh weight), peroxidase (2.20 U/g fresh weight) and catalase (13.60 U/g fresh weight) in wheat and in maize, i.e. superoxide dismutase (25.6 U/g fresh weight), peroxidase (1.70 U/g fresh weight) and catalase (26.4 U/g fresh weight) as compared to uninoculated ones.

12.6 PGPR and Chemical Fertilizers for Enhancing Crop Yield, Quantity, Soil Health and Microbiological Properties

Microbial inoculants/Biofertilizers are carrier based ready to use live microbial formulations, which on application to compost pits, soil and plants helps in mobilization of various nutrients by using direct or indirect mechanisms. Although role of plant growth promoting rhizobacteria for enhancing the plant growth already been discussed in this chapter, however, alone use of bacterial formulations may not meet the full nutrient requirements of the plants, so conjoint application of biofertilizers along with the chemical fertilizers can be used for enhancing crop yield and sustaining soil health besides saving vast amount of chemical fertilizers. A 2-year study was conducted by Sood et al. (2018a, b) to test the effects of combined application of indigenous plant growth promoting rhizobacteria (PGPR) and chemical fertilizers on productivity of wheat and maize and soil properties. Ten morphologically distinct indigenous PGPR isolates from wheat and maize rhizosphere were evaluated at Solan, Himachal Pradesh, India. Three PGPR isolates (B2, SIR1 and BIS2) of wheat and B1N1, MAT1 and DHK isolates of maize, showing maximum PGP traits were screened at different doses of nitrogen (N) and phosphorus (P) (80%, 60% and 40% of recommended fertilizer dose, RFD) under net-house conditions during first year. Two isolates, B2 (*Serratia* sp.), SIR1 (*Bacillus subtilis*) of wheat and B1N1 (*Bacillus subtilis*), MAT1 (*Bacillus amyloliquefaciens*) isolates of maize along with the optimum NP dose (i.e. 80% RFD) were selected for field experimentation, which was performed over two consecutive years. Combined application of 80% RDF of NP with PGPR (B2) significantly increased wheat yield by 9.4%, number of tillers per plant by 28.03%, grain number per spike by 19.61%, 1000-grain weight by 10.5% and biomass by 9.2% relative to the uninoculated control with 100% RFD. Similarly, in maize conjoint application of 80% recommended doses of NP with PGPR (B1N1) recorded an increase of 12.9% for plant height, (21.4%) number of cobs per plant, (16.61%) cob length, (16.51%) 1000 seed weight, (11.7%) grain yield and (17.9%) straw yield over uninoculated control. Soil properties in the terms of available N, P and potassium, microbial biomass carbon, soil enzyme activities and population of phosphate-solubilizing bacteria in the wheat and maize crop were significantly increased by the combined application of bacterial inoculants (B2 and B1N1) with 80% RFD of NP in both years over the uninoculated control (Fig. 12.3).

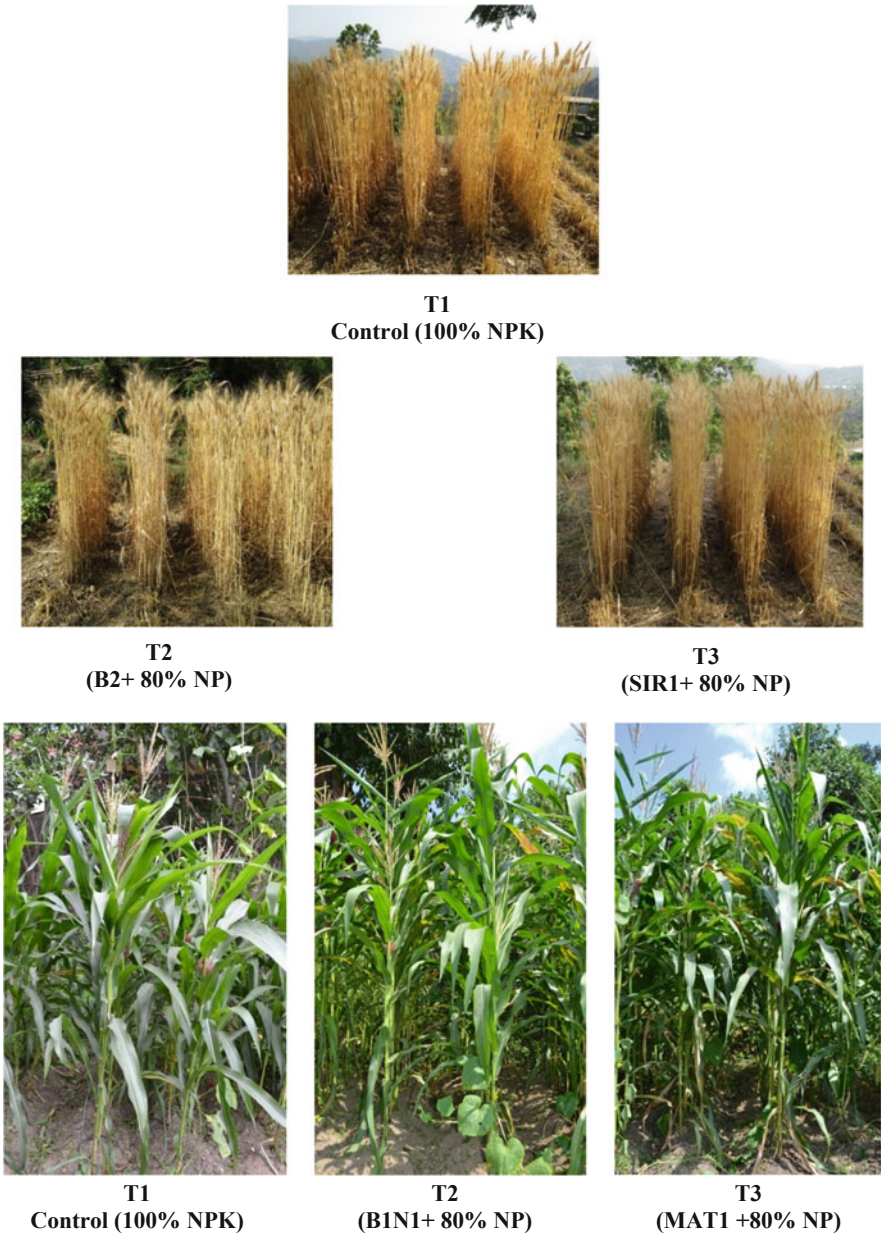

Fig. 12.3 Effect of PGPR isolates and chemical fertilizers on growth and yield of wheat and maize under field conditions

12.7 Conclusion

This chapter highlights the role of PGPR's (plant growth promoting rhizobacteria) isolated from different agro-climatic zones of Himachal Pradesh in enhancing the productivity of cereal crops (wheat-maize cropping system) in northwest Himalayas. The isolated bacteria showed significant increase in crop productivity, nutrient uptake and improved soil physico-chemical properties in wheat-maize cropping system and thus, may act as a source of biofertilizers, biostimulants and bioprotectants. Knowledge of such interactions can give direction as to which microbes might be selected for sustainably increasing the crop yield without hampering the soil health and environment. PGPR offers an attractive way to reduce the use of chemical inputs, pesticides and other supplements for cultivation of cereal crops. It is essential to keep on exploring the rhizosphere of different agricultural, horticultural and forestry plants for environmentally friendly and sustainable production.

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Plant–Microbe Interactions: Promoting Biocontrol of Phytopathogens of Cereal Grains

13

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Abstract

Cereal grains were the first agricultural attempts by early man, and are particularly important to humans because of their role as staple food crops worldwide. Given the nutritional and economic importance of grains, microbial diseases are a real danger to global food security. Several methods are implied to control diseases of cereal crops such as cultural practices, chemical control, using resistant varieties and biological control. Due to negative impact of chemical management of phytopathogens on soil ecosystems emerging interest on nontoxic microbial formulations has shown some promise, and despite being relatively recent approach there are some bacterial biocontrol products that are available against such diseases. In order to develop sustainable farming approaches such as biofertilizers and biopesticides, the study of host plants and associated microbial interactions in the rhizosphere plays an important role. Growth promotion and productivity of crop plants being important globally, it is central to know what type of microorganisms are present and what functions they are performing in the rhizosphere. In this chapter, we have discussed soil borne fungal and bacterial pathogens of cereal crops along with management of various phytopathogens via plant–microbe interactions.

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Keywords

Biocontrol · Biofertilizers · Biopesticides · Cereal grains · Phytopathogens · Plant–microbe interactions

13.1 Introduction

The active release of organic compounds from plant roots serves as nutrient and energy source for colonization and growth of active microbial population in the rhizosphere. Thus, rhizosphere is a warfield for many microflora including saprotrophs, biotrophs, and several other plant pathogenic microorganisms that may cause disease in the susceptible host plants. These beneficial and deleterious microbial populations continuously interact with each other as well as with host plant for their survival in the rhizosphere (Fig. 13.1). Study of these plant–microbe and microbe–microbe interactions is crucial since plant health is majorly dependent on the outcome of these interactions (Prasad et al. 2015; Sharma and Minakshi 2017; Rana et al. 2020). Plants get diseased when attacked by pathogenic microorganisms and flourish well when colonized by beneficial growth-promoting microorganisms.

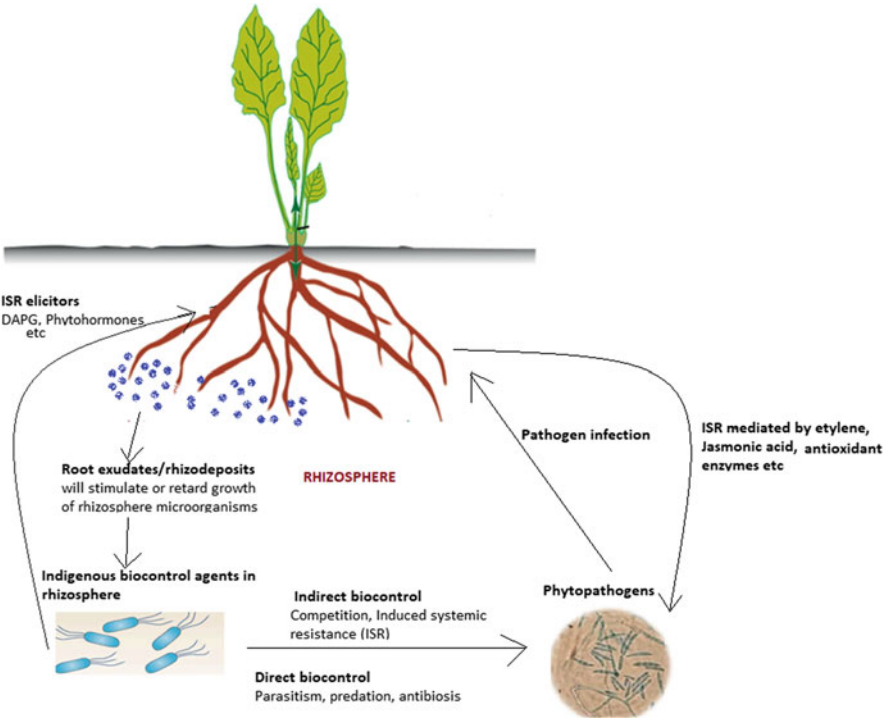

Fig. 13.1 Biocontrol mediated suppression of plant pathogens

In last few decades practices for manipulation of rhizosphere microflora are evident where population of beneficial growth-promoting microorganisms is increased those specially target disease causing microorganisms and suppress their growth. This approach to inhibit plant pathogens by employing antagonistic microflora is known as biological control, is being actively harnessed in several plant disease management practices (Sharma et al. 2015).

Interest in biocontrol of plant diseases has increased in past few decades due to high cost and negative environmental concerns associated with chemical protectants. The key to achieve successful biocontrol is the apt understanding of host–microbe interaction, environment, and screening effective antagonists with reproducible biocontrol (Deacon 1994). Successful biocontrol of many plant diseases under in vitro and *in planta* conditions has led to the commercialization of many biocontrol agents in the market-place (Whipps and Davies 2000). The commercialization of biocontrol agent requires the effective and reproducible biocontrol potential under variable environmental conditions and study of interactions between biocontrol agent, host plant with its indigenous microbial population and environment (Agrios 1997).

Several positive and negative interactions exist between plants and microorganisms in the rhizosphere; these include mutualism, commensalism, neutralism, competition, antagonism, parasitism, and predation (Chisholm et al. 2006). Mutualism is a type of interaction where both the interacting partners get benefitted. A mycorrhizal fungus is a type of obligatory mutualistic interaction between host plant and fungi. This interaction involves both physical and biochemical contact between partners and also contribute to biocontrol by improving the plant nutrition and by stimulating host defense response. Protocooperation is also a form of mutualistic interaction which is non-obligatory. Commensalism is a type of positive interaction where one organism gets benefit from other and other is neither benefitted or harmed (Hulme-Beaman et al. 2016). The interaction where one biological community has no effect on the other is called neutralism (Berg et al. 2006). However, antagonism, on the other hand, is a negative interaction between two organisms in which either one or both the partners are negatively inhibited. Competition often results in poor growth or complete eradication of the susceptible and weak population (Ryder and Talbot 2015). Competition is regarded as one of the important mechanisms of biocontrol where non-pathogenic biocontrol agents compete with plant pathogens for nutrients and space. Parasitism is also a negative biological interaction in which two organisms co-exist in such a way that the smaller partner (parasite) gets benefited and larger partner (the host) is harmed (Tzortzakakis et al. 2003). However, the parasite does not kill the host as its survival is dependent upon host survival. One more interesting interaction that leads to biological control of plant diseases is the interaction between host plant and avirulent pathogen. The exposure to avirulent strain of pathogen stimulates host defense response even against the virulent strain of pathogen (Agrios 1997). A varying degree of biological control is achieved under these plant–microbe interactions and an effective biocontrol is achieved by manipulating positive interactions between microbes and plant host or by manipulating negative interactions between antagonistic microbes,

pathogens, and host plant. To that end, the objectives of this chapter are to present an advanced survey of the nature and practice of biological control as it is applied to the suppression of plant diseases. In this chapter, different aspects of biological control of cereal pathogens including interactions among biocontrol agents and cereal pathogens, modes of action, genetically modified cereals, and future development will be discussed.

13.2 Root Exudates-Chemical Mediators of Plant–Microbe Interactions

Roots support plant growth by absorbing mineral nutrients and making them available to the plants. Besides this, roots also maintain rhizosphere structure and dynamics by secreting certain organic compounds in the rhizosphere zone called root exudates. Rhizosphere is thus a physiologically active zone under the influence of plant roots where these exudates secreted are utilized as nutrients by microorganisms those in turn affect the plants either positively or negatively (Kamilova et al. 2006; Kumar et al. 2007a, b; Naik et al. 2020; Shrivastava et al. 2014). Root exudates are chiefly photosynthetically derived carbon compounds the product of and act as secondary metabolites (Chaparro et al. 2013). A wide range of distinct organic compounds are exuded by plant roots those determine the microbial diversity in the rhizosphere, they include water, sugars, nitrogen compounds, mucilage, waxes, and other secondary metabolites (Nardi et al. 2000; Kour et al. 2020). It is a well-known fact that root exudates serve as nutrient source for soil microbes especially those mineralizing organic matter and providing mineral elements to the plants. So, if diverse plant community is present above ground, diverse microbial population will be present in the rhizosphere.

Plant root exudates can be grouped into two categories; first are the low molecular weight compounds such as monosaccharides, phenolics, amino acids, hormones, and other miscellaneous secondary metabolites. Exudates falling in the second group are high molecular weight compounds such as proteins and polysaccharides (Sharma and Minakshi 2017). The composition and concentration of exudates depends upon host plant, age, plant variety, soil physical and chemical properties, and environmental conditions (Uren 2000). These differences create a specific rhizobacterial community structure around specific host plant.

Many of the root exudate compounds have been known as the mediators of plant–microbe interactions such as release of flavonoids by legume roots starts nodulation process by activating nod genes of *Rhizobium meliloti*. These exudates are also responsible for root colonization by arbuscular mycorrhizal fungi (Becard et al. 1995). Roots are also known to exude certain compounds such as phytoalexins and other phenolics that are involved in plant defense against pathogens (Flores et al. 1999). Phytoalexins are released in response to the pathogen attack. However, a vast array of root exudates compounds are known with their functions still, a large chemodiversity of rhizodeposits is unexplored which can be exploited for searching new compounds and antimicrobials. A few years ago, a root exudate of hairy roots of

sweet basil called rosmarinic acid was identified that was elicited by cell wall extract of *Phytophthora cinnamomi* (Bais et al. 2006). Doornbos et al. (2012) have also reported the role of root exudates compounds in plant defenses; these findings have suggested the importance of root exudates in defending the plants against pathogenic microbes.

13.3 Diseases of Cereal Crops and Interactions Involved in Biological Disease Control

Cereal grains are grown in large quantities and are primary source of food and energy worldwide than any other type of crops and therefore known as staple crops. Green revolution has lead to increase in the cereal crop productivity worldwide to feed ever-growing human population which has led to more and more dependence on chemical based inputs in crop cultivation. But cereal crops are attacked by a vast range of pathogens leading to severe crop losses worldwide which was estimated to be ranging between 20 and 40% of global agricultural productivity (Oerke 2006). Root infecting pathogens of cereals are either soilborne or seed-borne. The sign and severity of these diseases caused by phytopathogens greatly depends upon climatic conditions. Research findings show that *Gaeumannomyces graminis* var. *tritici* causing take-all disease of wheat, *Rhizoctonia* spp. causing root rot of wheat, *Fusarium culmorum* and *Bipolaris sorokiniana* causing seedling disease in barley and wheat, *Ustilago tritici* and *U. nuda* causing loose smut in wheat and barley, *Exserohilum turcicum* causing leaf blight and *Puccinia* spp. causing rusts in cereal crops are most common and widespread (Knudsen et al. 1995; Johnsson et al. 1998; Yang et al. 2014; Sartori et al. 2017). Besides this mycotoxin contamination is another big challenge during post-harvest practices, which makes the cereal grains unsuitable as human and animal feed. A large amount (approximately 30–40%) of cereal grains get contaminated with mycotoxins producing fungi worldwide (Kumar et al. 2007a, b). Aflatoxins, Fumonisins, Patulins, and Ochratoxin are the most common and important mycotoxins (Suleiman and Kurt 2015). The fungi associated with cereal contamination and mycotoxin production are *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus, Penicillium*, *Fusarium moniliforme*, etc. (Suleiman and Kurt 2015).

Significant amount of chemical protectants are used worldwide to control diseases of cereal crops. One of the alternatives for controlling pathogens of cereal crops is microbial biocontrol. Reports on search for microbes that could control cereal diseases are numerous and have highlighted the role of various antagonist–pathogen interactions in combating these pathogens. The interactions may involve production of inhibitory compounds such as antibiotics those suppress the pathogen growth. In maize, growth-promoting and antifungal compounds-producing bacteria have been shown to have inhibitory effects on southern leaf blight disease caused by the fungus *Cochliobolus heterostrophus* (Huang et al. 2010; Ye et al. 2012). In another study by Yang et al. (2014), an antagonistic strain of *Pseudomonas fluorescens* HC1-07 isolated from the wheat phyllosphere inhibited the soilborne

pathogen *Gaeumannomyces graminis* var. *tritici* causing take-all disease in wheat. The strain HC1-07 also suppressed root rot of wheat caused by *Rhizoctonia solani*. Production of a cyclic lipopeptide (CLP) antibiotic in the rhizosphere was reported to be its mechanism of biocontrol. Although bicontrol abilities of antagonistic fungi against various cereal pathogens have been well recognized, commercial fungal biocontrol agents to replace chemical fungicides are yet limited. Research on biocontrol of plant pathogens by antagonistic fungi has been primarily associated with *Trichoderma* so far. This has led to the use of *T. harzianum* C82-93 strain as a biocontrol agent against diseases caused by *Fusarium*, *Botrytis cinerea*, *Sclerotinia*, *Cladosporium*, and *Alternaria*. A fungal biocontrol agent "Trichoderminas" was produced on commercial scale that contained the strain *T. viride* M10. In contrast to fungi, research on bacteria as biocontrol agents for cereal pathogens has proved to be more promising, and there are some bacterial biocontrol agents available for use against these pathogens. The examples include Mycostop, Cedomon, and Cerall currently registered as biopesticides in agriculture market. While Mycostop contains antagonistic *Streptomyces* and is used on vegetable crops, ornamental plants, and rarely on cereals, Cedomon and Cerall, on the other hand, are designed for seed dressing of cereals and contain *Pseudomonas chlororaphis* MA342 for combating seed-borne diseases. Despite the fact that commercial biocontrol agents are available for cereal crop protection, indigenous microflora are still considered more suitable for local application. This is because, the native microorganisms are well adapted to the crop rhizosphere in that area and their antagonistic potential will not get hindered by unfavorable climatic conditions (Khan et al. 2010; Khan 2013).

13.4 Interactions Between Biocontrol Agents and Fungal Pathogens

Fungi that invade cereals are categorized into two groups (1) field fungi which incorporate species of *Alternaria*, *Cladosporium*, *Fusarium*, and *Helminthosporium*; and (2) storage fungi which include species of *Aspergillus* and *Penicillium*. Natural/biological control of such kind of parasitic pathogens has been viewed as a reasonable elective technique to chemical control (Burge 1988). All through their lifecycle, plants and pathogens interact with a wide assortment of organisms and these interactions can fundamentally influence plant wellbeing in different ways. Diverse methods of activities of biocontrol-active microorganisms in controlling fungal phytopathogen infections include direct (mycoparasitism), blended way (anti-toxin production, secretion of lytic enzymes), and indirect (induction of host defense system against pathogen) (Ownley et al. 2008). Some of most generally discovered biocontrol soil microorganisms incorporate different types of fungal species (*Trichoderm*a, *Coniothyrium*, *Pythium spp.*) and bacteria (*Pseudomonas* and *Bacillus*) consequently securing plants and lessening disease incidence occurrence in a wide range of soil types. These highly interacting microorganisms have been broadly studied and live cell formulations of these are economically showcased fundamentally as biopesticides/biofertilizers and soil alterations.

Cases of economically accessible biocontrol organisms and their mode of activities:

13.4.1 Interactions Between Biocontrol Fungi and Mycophytopathogens

Interactions between biocontrol fungi and fungal plant pathogens keep on being the focal point of numerous scientists. Several interactions between biocontrol fungi and fungal pathogens have been enlisted in Table 13.1. There is an assortment of biocontrol fungal species, but *Trichoderma* species plainly dominate, simply because of its ease of cultivation and wide host range (Whipps and Lumsden 2001). Non-pathogenic species of *Pythium*, *Rhizoctonia*, *Phialophora, Gaeumannomyces graminis* var. graminis have likewise been accounted for as biocontrol of cereal pathogens. *Trichoderma* species have been examined widely in the previous years and are marketed worldwide as biofungicides, phytostimulants, and natural soil alterations for number of horticultural, ornamental, and other crops (Lorito et al. 2010).

Some examples of the methods of activity found in the rhizosphere during interactions amongst mycophytopathogens and fungal biocontrol agents are given beneath:

13.4.1.1 Competition

Competition for supplements and space are imperative elements for biocontrol of soilborne pathogens. This is entrenched for non-pathogenic strains of *Fusarium oxysporum* controlling pathogenic *F. oxysporum* on an assortment of crops (Eparvier and Alabouvette 1994), hypovirulent or non-pathogenic strains of *Rhizoctonia* species to control pathogenic species of *R. solani* (Herr 1995) and various other fungal species of *Phialophora*, *Gaeumannomyces graminis* var. graminis (Kirk and Deacon 1987; Shivanna et al. 1996). Production of lytic enzymes could be ascribed to rhizosphere competence in *Trichoderma harzianum* that prompts enhanced biocontrol efficiency against immense soil fungal pathogens (Ahmad and Baker 1987). *T. harzianum* can possibly diminish levels of fusarium head blight of wheat by lessening perithecial and ascospore generation of *Gibberella zeae* on wheat straw (Inch and Gilbert 2007). In another example, sterile red fungus competes for thiamine could be a significant mechanism of controlling *Gaeumannomyces graminis* var. *tritici* in wheat rhizosphere (Shankar et al. 1994).

13.4.1.2 Parasitism

The fundamental biocontrol system that numerous fungal biocontrol species uses indirect confrontation with phytopathogenic fungi is parasitism (Vinale et al. 2008). Vujanovic and Goh (2009) reported mycoparasite (*Sphaerodes mycoparasitica*) of *Fusarium graminearum*, the causative agent of cereals head blight. Mechanism of parasitism depends on recognition followed by binding and lastly enzymatic disruption of cell wall of host fungi or its propagules. Among fungi, the species of

Table 13.1 Examples of interactions between biocontrol agents and fungal diseases of cereals

Host plant	Pathogen	Biocontrol agent	Reference
Fungal-fungal pathogen interactions			
Barley	*Bipolaris sorokiniana*	*Idriella bolleyi*	Duczek (1997)
	Fusarium culmorum	*Pythium mycoparasiticum* *P. Acanthophoron* *P. oligandrum* *P. Periplocum*	Davanlou et al. (1999)
Maize	*Fusarium graminearum* *Pythium arrhenomanes* *P. ultimum*	*Trichoderma virens* GL-3	Mao et al. (1997)
	Macrophomina phaseolina	*T. viride*	Bagyaraj (2011)
Wheat	*Ustilago segetum* *Gaeumannomyces gramminis* var. *tritici*	*T. Koningii* *Phialophora* sp. I-52	Bagyaraj (2011)
	Pyrenophora tritici-repentis *Pseudocercosporella herpotrichoides*	*T. harzianum* T-22 *T. Harzianum,* *F. culmorum*	Mathre et al. (1998) Da luz et al. (1998) Hinton and Parry (2008)
Bacterial-fungal pathogen interactions			
Wheat	*Alternaria tritici*	*Bacillus subtilis,* *Serratia marcescens*	Sood et al. (2018a) Yang et al. (2014)
	Gaeumannomyces graminis var. *tritici., Tilletia caries*	*Pseudomonas fluorescens*	Reiss and Jorgensen (2017); Jorgensen and Matzen (2017)
	Rhizoctonia solani *Puccinia striiformis*, *Blumeria graminis*, *Rhizoctonia solani*; *G. graminis* var. *tritici*	*Bacillus subtilis*	Huang and Wong (1998), Sood et al. (2018a)
	Fusarium graminearum, *Cleviceps purpurea*	*Burkholderia cepacia* A3R *B. subtilis* *Serratia marcescens*	
Maize	*Fusarium* spp. *Fusarium oxysporum* *Rhizoctonia solani*	*B. cepacia* PHQM 100 *Bacillus subtilis*	Hebber et al. (1998) Sood et al. (2018b)
Barley	*Drechslera graminea, D. teres, U. hordei*	*Pseudomonas chlororaphis* MA342	Johnsson et al. (1998)
Oats	*D. avenae* *Ustilago avenae*		
Rice	*R. solani*, *Pyricularia grisea*	*P. fluorescens* VO61	Vidhyasekaran and Muthamilan (1999)
	Bipolaris oryzae, Sclerotium oryzae Cattaneo	*Pseudomonas* sp., *P. aeruginosa,* *Bacillus* sp., *B. subtilis*	Vasudevan et al. (2002)

Trichoderma, *Gliocladium* and *Pythium* are known mycoparasites (Elad 1995). Chitinolytic and glucanolytic enzymes are especially valuable for biocontrol applications in light of their capacity to efficiently dissolve fungal hyphae. *Trichoderma* composed of set of genes encoding for variety of lytic enzymes and hence showed antifungal activity towards expansive fungal pathogens of grains (i.e., species of *Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Venturia*, and *Colletotrichum*, as well as lower fungus species of *Pythium* and *Phytophthora* whose cell wall lacks chitin).

13.4.1.3 Antibiosis

Secondary metabolites production by certain fungal species for biocontrol is one of the chief phenomenons. Antibiotics, volatile compounds, isocyanide derivatives, water soluble compounds (heptelidic acid or koningic acid) are the examples of metabolites having antagonistic potential against phytopathogenic fungi. The fungal antibiotics, gliovirin and gliotoxin produced by different strains of *Trichoderma virens* (Bisset 1991) as well as chaetomin and gliotoxin by the soil-inhabiting biocontrol fungi, *Chaetomium globosum* and *Gliocladium virens*, respectively, are important for biocontrol of cereal pathogens. Along with antibiotics, production of some volatile compounds such as 6-n-pentyl-2H-pyran-2-one (6-PAP) by *Trichoderma* species has inhibitory potential against *Botrytis cinerea*, *Fusarium* sp., *Phytophthora megasperma*, *Rhizoctonia solani*, and *Armillaria mellea* (Tarus et al. 2003).

13.4.1.4 Induced Resistance

Induced resistance is perceived as an imperative method of biocontrol in vegetative tissues (Kloepper et al. 1992). Resistance may be induced locally or may be systemic. Induction of ISR (induced systemic resistance) by certain rhizospheric microorganisms prevents plant from foliar and soil borne pathogens infection (Paulitz and Matta 2002). ISR is attributed to an assortment of non-pathogenic microorganisms that can control multiple pathogens such as saprophytes, plant growth-promoting and avirulent races of pathogens. The root-colonizing fungus *Piriformospora indica* (phylum Basidiomycota) was discovered in the Indian Thar desert (Verma et al. 1998; Prasad et al. 2005, 2013, 2015). This non-pathogenic fungus protects barley from infections by root and leaf pathogens, thereby offering a model system for "systemic" resistance in cereals. The elicitors responsible for inducing resistance are not known in detail. Xylanase from *Trichoderma* sp. inoculated in plant tissues induced K^+, H^+, and Ca^{2+}channeling, PR protein synthesis, ethylene biosynthesis, and glycosylation and fatty acylation of phytosterols hence, provided ISR (Bailey and Lumsden 1998). In another study, *T. harzianum* acts as an effective antagonist against Septoria leaf blotch by causing a biochemical induced response in wheat plants (Cordo et al. 2007).

13.4.2 Interactions Between Biocontrol Bacteria and Mycophtyopathogens

Other than parasitic biocontrol agents, bacterial species have additionally assumed an imperative part in suppressing growth of mycophytopathogens and the volume of writing around there keeps on expanding at a fast rate, encouraged by their growth, easy handling, and aggressive colonization of the rhizosphere. A few cases of the distinctive sorts of bacteria and fungal pathogen interactions analyzed in the rhizosphere in recent years are given in Table 13.1. In spite of the fact that a range of various bacterial genera and species have been contemplated, but the mind-boggling number of papers have included the utilization of *Pseudomonas* and *Bacillus* species as biocontrol agents (Kushwaha et al. 2020). Obviously, *Pseudomonas* and *Bacillus* species must have activity, yet it makes one wonder with regard to the highlights that make these genera so powerful and the choice of many workers. The key features of *Pseudomonas* and *Bacillus* are their rapid growth, easy to modify genetically, and can metabolize variety of organic compounds hence culture easily in complex medium, therefore, making them amenable to experimentation. But, in addition, they are common rhizosphere organisms and must be adapted to life in the rhizosphere to a large extent (De Weger et al. 1995; Marilley and Aragno 1999). Having appropriate ecological rhizosphere competence may be a key feature for reproducible biological control activity in the rhizosphere. A couple of cases of the modes of activity required for bacterial biocontrol of contagious pathogens in the rhizosphere are given below.

13.4.2.1 Production of Inhibitory Allelochemicals

A primary mechanism of pathogen inhibition is the production of inhibitory secondary metabolites such as antibiotics and lytic enzymes as well as other factors such as siderophore production and microbial cyanide (Fravel 1988; O'Sullivan and O'Gara 1992; Kumar et al. 2020). Fluorescent pseudomonads are known to produce number of metabolites, such as phenazine-1-carboxylicacid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin, and oomycin A. Among these, DAPG has been implicated as the mechanism involved in the biological control of some of the most important crop diseases, such as the root rot of wheat caused by *Fusarium oxysporum* f. sp. graminis (Garagulya et al. 1974) and the "take-all" of wheat caused by *Gaeumannomyces graminis* tritici (Keel et al. 1992).

Different strains of *Bacillus* and *Pseudomonas* can synthesize lipopeptide-type compounds, which have been considered for their biocontrol activity against phytopathogens (Leclere et al. 2005; Chen et al. 2009). Lipopeptides (fengycin, surfactin, and iturin) belong to a group of microbial-based peptides that enable the plant activation of defense mechanism (Ongena et al. 2005; Chen et al. 2009). Gond et al. (2015) reported antifungal lipopeptide production by *Bacillus subtilis* that upregulated the expression of pathogenesis related proteins thereby induced plant defense system against fungal pathogens. In another study, cyclic lipopeptide producing strain *Pseudomonas fluorescens* HC1-07 was able to control wheat root

diseases of *Gaeumannomyces graminis* var. *tritici*. and *Rhizoctonia solani* (Yang et al. 2014).

Lytic enzymes such as Chitinase and β-1, 3-glucanase suppress mycophytopathogens by degrading chitin and β-1, 3-glucan, major constituents of many fungal cell walls. Chitinase produced by *S. plymuthica*, *Serratia marcescens*, *Paenibacillus* sp., and *Streptomyces* sp. as found to be inhibitory against *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. cucumerinum (Barka et al. 2002). Similarly, expression of β-1, 3-glucanase from *Trichoderma atroviride* reduced disease incidence of *Sclerospora graminicola* causing downy mildew in pearl millet (O'Kennedy et al. 2011).

13.4.2.2 Induced Resistance

Certain biocontrol agents show indirect mode of antagonism by increasing level of basal resistance to several pathogens simultaneously, which is of advantage to survive in pathogen prone ecosystem of rhizosphere (Van Loon and Glick 2004). Induction of disease resistance is regulated by a network of signaling channels. The initial components of this network involve various plant signal metabolites—ethylene (ET), nitric oxide (NO), jasmonic acid (JA), and salicylic acid. Most work has concentrated on the induced systemic resistance provided by rhizosphere colonizing non-pathogenic strains of *Bacillus* and *Pseudomonas*. Specific ISR induction depends upon type of pathogen, host plant, and strain for biocontrol used. Rais et al. (2017) accessed *Bacillus* spp., for ISR in rice against *Pyricularia oryzae*, the outcomes demonstrated that treatment with *Bacillus* sp. upgraded antioxidant defense activities in infected rice that leads to oxidative damage to *P. oryzae* hence, suppressing disease incidence in crop. Whereas, in another study induction of systemic resistance by *Pseudomonas fluorescens* WCS374r was based upon pseudobactin mediated priming for salicylic acid against rice blast pathogen *Magnaporthe oryzae* (De Vleesschauwer et al. 2008).

13.5 Interactions Between Biocontrol Agents and Bacterial Pathogens

The utilization of biocontrol organisms against bacterial pathogens of cereals remains to be investigated in detail. However, earlier work has been documented for suppression of bacterial blight using antagonistic strains of *Bacillus* and *Pseudomonas* (Vasudevan et al. 2002). *Xanthomonas* sp. causing bacterial blight and bacterial leaf streak/brown stripe caused by pathogenic species of *Pseudomonas* are the most severe disease of cereals. Kumar et al. (2017) reported *Bacillus subtilis* for its biocontrol efficiency against rice blast (*Magnaporthe oryzae*), sheath blight (*Rhizoctonia solani*), and bacterial leaf blight (*Xanthomonas oryzae*). Moreover, the phylloplane microorganisms, namely *Erwinia herbicola*, *Aspergillus sp*, *Streptomyces* sp., *Pseudomonas fluorescens* and *Trichoderma harzianum*, etc., suppressed bacterial growth and reduced bacterial blight incidence significantly (Manmeet and

Table 13.2 Examples of biocontrol agents for nematodes and bacterial pathogens of cereals

	Pathogen	Biocontrol agent	Reference
Bacteria	*Xanthomonas oryzae*	*Bacillus sp., Pseudomonas aeruginosa BRp3*	Bagyaraj (2011) Yasmin et al. (2017) Kakar et al. (2013)
	Pseudomonas fuscovaginae Erwinia carotovora	*B. amyloliquefaciens Bk7 Pseudomonas fluorescens*	Bagyaraj (2011)
Nematodes	Root knot nematodes (*Meloidogyne* spp. *M. incognita M. javanica M. hapla*)	*Trichoderma harzianum, Verticillium chlamydosporium*, *Bacillus* sp., *Azotobacter* sp., *Pasteuria* sp.	Sahebani and Hadavi (2008); Bagyaraj (2011)
	Cereal root eelworm (*Heterodera avenae*) (*Heterodera filipjevi*)	*Trichoderma longibrachiatum T6, Achromobacter xylosoxidans, Bacillus cereus*	Zhang et al. (2016, 2017)

Thind 2002). Some examples of biocontrol of bacterial diseases examined in rhizosphere of cereal grains are given in Table 13.2.

As described earlier, antibiosis or competition for nutrients and space are the main mechanisms for biocontrol in the rhizosphere. Native strains of *Pseudomonas fluorescens* and *Erwinia herbicola* colonizing roots of rice, pearl millet, and citrus reduced development of bacterial blight hence, proved inhibitory to bacterial blight pathogen (Gnanamanickam et al. 1999). While, inhibitory secondary metabolite production by *Pseudomonas aeruginosa* controlled the growth of *Xanthomonas oryzae* causing leaf blight of rice. Hence, the bacterial antagonists proved to have double advantage of faster growth rate and higher competence in rhizosphere. Several other metabolites or factors are also responsible for antagonism rather than competition. Unnamalai and Gnanamanickam (1984) reported siderophore mediated inhibition of *X. Campestris* pv. citri by *Pseudomonas* rather than competition for space or nutrients. Similarly, antibiotic (DAPG) production by *P. fluorescens* suppressed growth of bacterial blight pathogen *Xanthomonas oryzae* pv. oryzae (XOO) in rice (Velysamy et al. 2005). Biological control has more potential to suppress growth of phytopathogen than chemical treatment which was proved in a study conducted by Jayalakshmi et al. (2010) who found combination of seed treatment and soil application with *P. fluorescens* gives the minimum disease incidence of bacterial leaf blight with maximum yield in comparison with the chemical treatment.

13.6 Interactions Between Biocontrol Agents and Nematodes

Plant parasitic nematodes persisting in the soil attack several crop plants and reduce their growth and yield. Nematode control measures may either be corrective or preventive but the biological control has both these possibilities. Biological nematode control in relation to crop production system is a subject of considerable current interest, because of a perceived urgency to develop and adopt safe, economic, and efficient method for managing nematode pests.

Cereal crops are central food source globally and hence nematodes parasitizing cereals including cyst (*Heterodera avenae*) and root knot (*Meloidogyne graminicola*) are of worldwide concern. Certain biocontrol agents applied to nematicidal infections of cereal crops are listed in Table 13.2. *Heterodera avenae* can easily parasitize wheat, barley, and oats (Al-Hazmi et al. 1994) while wheat-rice crop rotation areas are more prone to *Meloidogyne graminicola* infection. Although several methods have been used like chemical control, use of pest resistant transgenic varieties, but crop rotation has been the most effective method to control nematodes but if limited choice of crop rotation and occurrence of diverse pathogens then, other methodologies are required (Riley and Qi 2015). Use of chemicals is also considered economically as well as environmentally unsatisfactory (Bontempo et al. 2014), so advancement of microbial antagonists for nematodes may be one of only a handful few remaining choices (Riley et al. 2010).

Biological control of nematodes includes the use of predaceous or parasitic organisms and the dominating are bacteria and fungi. Variety of microorganisms has been explored as potential biocontrol agents for cereal cyst and root knot nematodes (Siddiqui and Mahmood 1996, 1999). Some fungal species, including *Verticillium chlamydosporium*, *Paecilomyces lilacinus* (Khan et al. 2006), and *Trichoderma longibrachiatum* (Zhang et al. 2014), *Fusarium* sp. and *Gliocladium virens* have been found to have strong parasitic and lethal effects on cereal nematodes. Similarly, some bacteria have been shown to offer potential as biocontrol agents. *Pasteuria* spp. was shown to prevent juveniles from invading wheat roots by parasitizing *H. avenae* (Davies et al. 1990). Application of *Bacillus subtilis and Bacillus megaterium* caused significant mortality of *H. avenae* juveniles (Gokte and Swarup 1988) and *M. graminicola* (Padgham and Sikora 2007), respectively.

13.7 Mycorrhizae as Biocontrol Agent

Enhanced growth of plants because of AM fungal inoculation is well documented. Most of the studies on AM fungi–root pathogens interaction suggest that AM fungi decreased or mitigated the disease severity (Brimmer and Boland 2003; Mukerji et al. 2002). AM fungi have been well documented for disease reduction of various fungal (*Phytophthora parasitica*, *Gaeumannomyces graminis* var. tritici, *Fusarium oxysporum*); bacterial pathogens (*Pseudomonas syringae* and *Ralstonia solanacearum*) as well as nematodes (*Tylenchulus semipenetrans*, *T. vulgaris*, *Meloidogyne arenaria*, *Radopholus similis*). Direct and indirect mechanisms are

involved in AMF mediated biological control. Direct mechanisms involve mainly competition for space and nutrients, on contrary plant mediated effects come under indirect mechanisms including ISR, alteration in root exudates that results in altered rhizosphere interactions. Khaosaad et al. (2007) reported systemic reduction of root damage caused by *Gaeumannomyces graminis* var. *tritici* in mycorrhizal colonized roots of barley and disease reduction was correlated with complex interactions among AMF, pathogens, and plant (Harrier and Watson 2004). The interaction between AMF and diverse rhizobacteria has also been well documented (Nemec 1994). Such interactions may have detrimental, neutral, or beneficial effects on bacterial pathogens (Filion et al. 1999). Soybean roots having mutualistic symbiosis with *Glomus mosseae* suppressed the growth of *P. syringae* in soybean rhizosphere (Shalaby and Hanna 1998), thereby preventing pathogen infection in plant. The role of AMF in lessening detrimental effects of root infection by numerous parasitic nematodes in plants is likewise all around perceived (Jothi and Sundarababu 2002; Shreenivasa et al. 2007). Most of the AM fungi and nematode interaction studies have been made with sedentary endoparasites, especially with the root knot nematode belonging to genus *Meloidogyne* in multiple horticultural crops.

13.8 Genetically Modified Cereals

As explained earlier, certain rhizo-microorganisms inhibit the growth of phytopathogens via production of antimicrobial compounds. With the emergence of genetic engineering, genes encoding to such products can be isolated and transferred to desired plant hence, forming GMOs. A GMO that uses a gene from a microbial antagonist is a bio-based method for disease and pest control. There is minimal success data of genetically modified cereal crops in comparison to other economic crop. Because, this required large investment of money as well as time to form a stable transgenic due to requirement of techniques and issues with respect to worthiness of GM grains. Certain examples of pathogen resistant verities of cereal crop have been explained below:

13.8.1 Pest Resistant Varieties of GM Cereals

Cereals and various other crops have been engineered genetically to become resistant against lepidopteran and coleopteran species by forming toxic proteins from *Bacillus thuringiensis* (*Bt*). *Bt* toxins are of interest of several researchers because of its specificity for insects and no effect on predators or beneficial insects (Mendelsohn et al. 2003; Christou 2005). Among cereals, the most well-known transgene for pest control available today includes transgenic maize that expresses *Bt* genes (CryIA(b), CryIa(c) or Cry9C.

Rice is one of the important cereal crops that serve as staple food for more than half of the world's population. Stem borers infesting rice crop results in yield losses (Pathak and Khan 1994). *Bt* genes *Cry1A*, *Cry1Ab* and *Cry1Ac* and *Cry1Ab/Ac*

fusion gene that have been successfully transferred and expressed in different rice varieties, viz., KMD1, KMD2 (Shu et al. 2000; Ye et al. 2001), Minghui 63, IR64, Pusa Basmati-1, Karnal Local (Khanna and Raina 2002) showed resistance against striped stem borer, yellow stem borer, and leaf folder under field conditions, resulted in yield advantage.

13.8.2 Disease Resistant Varieties of GM Cereals

Fungal, bacterial, and viral diseases of cereals are well acknowledged. Bio-based transgenic plants development is one of the alternative ecofriendly approaches to develop resistance and increasing crop yield of cereals.

Fusarium graminearum is one of the major pathogens of wheat causing Fusarium Head Blight (FHB). Polygalacturonases (PGs) secretion during early fungal infection is the cause of various fungal diseases, and plants should have evolved to restrict pectin degradation during fungal infection. Polygalacturonase-inhibiting proteins (PGIPs) production could restrict pectin degradation by mycopathogens. Keeping this in view, expression of *PvPGIP2* in transgenic wheat significantly reduced the symptoms of *F. graminearum* (Ferrari et al. 2012). The bovine lactoferrin gene is known to have a wide spectrum of antimicrobials gene. In a study, expression of bovine lactoferrin cDNA induced in wheat and Barley plants increased the resistance against head blight (Han et al. 2012).

Blast (*Magnaporthe grisea*), bacterial leaf blight (*Xanthomonas oryzae pv. oryzae*), and sheath blight (*Rhizoctonia solani*) are serious constraints for high productivity of rice. Chitinase or 1, 3-glucanase lytic enzymes of fungal cell wall are of great consideration to suppress fungal pathogens. Genes encoding these enzymes have been broadly used in creation of transgenic rice (Fujikawa et al. 2012). Transgenic rice plants that expressed chitinase gene, *ChiC from Streptomyces* griseus showed significant resistance against *Magnaporthe grisea* over non-transgenic rice. In other study, puroindoline genes *PinA* and *PinB* from wheat when expressed in rice, the disease incidence of *M. grisea* and *R. solani* was significantly reduced (Krishnamurthy et al. 2001). Induction of disease resistance in its broadest sense implies biological control of pathogens and pests by earlier activation of genetically modified plant defense systems. In example, insertion of AtNPR1 in transgenic rice induced the expression of *PR1b, PR5, PR10,* and *PBZ1* genes encoding salicylic acid thus responsible for acquired systemic resistance against *M. grisea* and *Xanthomonas oryzae pv. oryzae* (Quilis et al. 2008). Furthermore, manipulation in jasmonic acid biosynthesis pathway via expression of *OsAOS2* (pathogen inducible gene) in rice that encodes enzyme allene oxide synthase which is involved in JA biosynthetic pathway results in accumulation of JA leading to higher expression of pathogenesis related genes thus increased resistance to *M. grisea* infection (Mei et al. 2006).

Despite of huge efforts devoted and achievement accomplished in rising disease and pest resistant transgenics in cereal crops, no commercial discharge has been

reported until this point. This technology is disputable, has varying perceptions of acceptance, and is limited by government controls around the world.

13.9 Conclusions and Future Prospects

For growth of agricultural production has led several new challenges, making further growth possible only if these challenges are met appropriately and timely. Increase in crop productivity using the modern farming techniques is possible but the environmental issues arising due to excessive use of chemical fertilizers and pesticides are required to be addressed. So, the biological control can be alternate system, which may play an important role in achieving the goal of agriculture. BCAs introduced as inoculants or amendments, as well as active ingredients directly derived from natural origins and having a low impact on the environment and non-target organisms. With the growing interest in reducing chemical inputs, companies involved in the manufacturing and marketing of BCAs should experience continued growth. New, more effective, and stable formulations also will need to be developed.

Much has been learned from the biological control research conducted over the past years. But, in addition to learning the lessons of the past, biocontrol researchers need to look forward to define new and different questions, the answers to which will help facilitate new biocontrol technologies and applications. Currently, fundamental advances in computing, molecular biology, analytical chemistry, and statistics have led to new research aimed at characterizing the structure and functions of biocontrol agents, pathogens, and host plants at the molecular, cellular, organismal, and ecological levels. Growers are interested in reducing dependence on chemical inputs, so biological controls (defined in the narrow sense) can be expected to play an important role in Integrated Pest Management (IPM) systems.

Most pathogens will be susceptible to one or more biocontrol strategies, but practical implementation on a commercial scale has been constrained by a number of factors. Cost, convenience, efficacy, and reliability of biological controls are important considerations, but only in relation to the alternative disease control strategies. Cultural practices (e.g., good sanitation, soil preparation, and water management) and host resistance can go a long way towards controlling many diseases, so biocontrol should be applied only when such agronomic practices are insufficient for effective disease control. In general, though, regulatory and cultural concerns about the health and safety of specific classes of pesticides are the primary economic drivers promoting the adoption of biological control strategies in urban and rural landscapes. Soil borne and post-harvest diseases have been controlled effectively by biological control agents that act as bioprotectants (i.e., preventing infections).

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Role of Indigenous Microbes for the Control of Major Fungal Pathogens of Turmeric

14

Meenakshi Dhiman, Vibha Singh, and Rajesh Kaushal

Abstract

Curcuma longa commonly known as turmeric is a rhizotomous herb of family Zingiberaceae common to Indian subcontinent and Middle Eastern countries. The rhizome is commonly used as spices and traditional medicine in Indian households even since the ancient times. Turmeric is a rich source of phenolic compounds, curcuminoids, and sesquiterpenoids. Leaf spot incited by *Colletotrichum capsici* is one of the most serious foliar diseases of turmeric and becoming a major limiting factor for production and quality of turmeric which causes 15–60% losses in India. Use of antagonistic microbes to manage the diseases replaces the chemicals and protects the environment from toxic hazards. Plant growth promoting rhizobacteria from organic sources of nutrients, viz. Panchagavya, Jeevamarit, and organic soil are known to show their antagonistic activity on the mycelial growth of fungal pathogens. Biofertilization and bioprotectant characters of PGPR have paved the way for their use at commercial level to supplement chemical fertilizers for enhanced production. This chapter summarizes the sustainable farming approaches such as biofertilizers and biopesticides where the study of host plants and associated microbial interactions in the rhizosphere plays an important role.

Keywords

Turmeric · PGPR · Fungal pathogen · Biocontrol agent

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14.1 Introduction

The prosperity of life in any form mainly depends upon the soil quality and fertility because healthy soil leads to a healthy agriculture system and food security. The microorganism present in the soil plays an important role in the plant growth promotion and maintains health and fertility of soil by natural means. There is a positive correlation between the beneficial microorganism and higher quality of soil, lower disease incidence, better plant growth, higher nutrient contents, soil pH, and enzyme activities (Wang et al. 2017). With the sudden rise in world's population, the requirement of quality food with good agricultural yield has increased accordingly and therefor to meet the required use of chemical fertilizers has also increased. The continuous use of chemical fertilizers and pesticides in the agricultural fields leads to the degradation of soil quality and fertility, thus the development of sustainable agriculture decreasing day by day (Bhardwaj et al. 2018). Most of the crop loss occurs due to disease incidence caused by the soilborne pathogen. The pathogen which leads to crop destruction may be bacterial or fungal. To prevent the disease incident the chemical fungicidal and bactericidal spray are given to the plant to overcome the loss. The chemical spray ultimately leads to the degradation of human health as well as soil health and their continuous use may degrade the fertility of soil and also disturb the ecosystem.

Turmeric being a high value crop and to overcome their losses due to disease incident by fungal and bacterial pathogens, scientists have shifted their attention toward preparation of efficient biocontrol agents which maintain the plant health and fertility of soil for sustainable agriculture. Turmeric (*Curcuma longa* L) is a herbaceous rhizomatous perennial plant belonging to ginger family (Zingiberaceae). It is native to the Indian Subcontinent and Southeast Asia. It requires the temperature ranges between 20 and 30 °C (68 and 86 °F) and a significant amount of annual rainfall in the range of 1200–1500 to thrive. The yellow color of turmeric is due to the presence of three main curcuminoids, namely curcumin, demethoxycurcumin, and *bis*-demethoxycurcumin (Chainani-Wu 2003). The curcumin melts at a temperature of about 184.2 °C. Curcumin is soluble only in ethanol, acetone, and insoluble in water (Joe et al. 2004). Turmeric powder contains pepper-like flavor, warm, bitter, and earthy like aroma. The rhizomes of turmeric are boiled in water for about 30–45 min and then dried in hot ovens, after which they are ground into a deep-orange-yellow powder commonly used as a coloring and flavoring agent in many Asian cuisines, especially for curries, as well as for dyeing, potential as medicine and beauty aid (Luthra et al. 2001; Selvan and Manojkumar 2003). Curcumin is an efficient antioxidant and most bioactive component of turmeric laden with anti-platelet, lowering cholesterol, anti-inflammatory, antifungal, and antibacterial properties (Peter 2000).

14.2 Nutritional Composition of Turmeric (*Curcuma longa*) and Its Antimicrobial Properties

Turmeric being a rhizomatous medicinal plant belongs to Zingiberaceae family (Chattopadhyan et al. 2004). The antimicrobial properties of turmeric are due to the presence of curcumin fraction. The curcumin possesses vitamins or vitamin precursor which produces beta-carotene, vitamin C, and essential oil. Turmeric (*Curcuma longa* L.) is one of the most important and ancient spices of India, contains about 69.49 carbohydrate, 6.30 protein, 5.10 oil, and 3.50% mineral and other important elements in dry turmeric (Swain et al. 2007). The essential oil and curcumin are important components of turmeric whose content ranges up to 5%. The leaves of turmeric also act as great source of vitamins and minerals (Chattopadhyan et al. 2004). Turmeric is a good source of minerals like iron, calcium, potassium, manganese, copper, zinc, and magnesium. Nutritive value of turmeric (*Curcuma longa*) has been presented in Table 14.1.

14.3 Health Benefits and Medicinal Use of Turmeric

- It is used for its anti-inflammatory (painkiller), anti-tumor, carminative, anti-flatulent, and antimicrobial.
- It possesses essential oils such as turmerone, zingiberene, cineole, and p-cymene which have health benefits.
- Being a richest source of antioxidant, dietary fiber, and does not contain any cholesterol leads to control on "bad cholesterol" or LDL levels.

Table 14.1: Nutritive value of turmeric (*Curcuma longa*), per 100 g

Principle	Nutrient value	Percentage of RDA	Vitamins	Nutrient value	Percentage of RDA
Energy	354 kcal	17%	Folates	39 µg	10%
Carbohydrates	64.9 g	50%	Niacin	5.140 mg	32%
Protein	7.83 g	14%	Pyridoxine	1.80 mg	138%
Total fat	9.88 g	33%	Riboflavin	0.233 mg	18%
Cholesterol	0 mg	0%	Vitamin A	0 IU	0%
Dietary fiber	21 g	52.5%	Vitamin C	25.9 mg	43%
Minerals			Vitamin E	3.10 mg	21%
Calcium	183 mg	18%	Vitamin K	13.4 µg	11%
Copper	603 µg	67%	Electrolytes		
Iron	41.42 mg	517%	Sodium	38 mg	2.5%
Magnesium	193 mg	48%	Potassium	2525 mg	54%
Manganese	7.83 mg	340%			
Phosphorus	268 mg	38%			
Zinc	4.35 mg	39.5%			

- It contains essential vitamins like pyridoxine (vitamin B6), choline, niacin, riboflavin, etc. Pyridoxine is generally used to cure the sideroblastic anemia, homocystinuria, and radiation sickness. Niacin which acts as essential vitamin prevents "pellagra" or dermatitis.
- The good level of vitamin C is present in the fresh root which helps to increase immunity against infectious agents in the body and eradicate harmful free oxygen radicals.

According to Recommended Daily Allowance (RDA) percent, 100 g of turmeric gives 138% of pyridoxine (vitamin B-6), niacin 32%, dietary fiber 53%, vitamin C 43%, vitamin E is 21%, potassium is 54%, iron is 517%, manganese about 340%, and 40% of zinc and contains no cholesterol.

14.4 Culinary Uses

The turmeric powder is traditionally documented as "Indian saffron" because of its deep yellow-orange color and plays an important role in food preservation by natural means, food colorant, and flavor base since the earliest period. It is used to maintain the shelf life of raw materials such as fish, chicken, and meat by application of its paste.

- It is used in the preparation of masala curry powder for kitchen use by mixing the dried roots with other spices such as curry leaves, peppers, etc. to enhance the taste of food.
- It plays a significant role in the preparations of salad dressings, soups, canned beverages, dairy products, baked products, yellow cakes, ice cream, yogurt, orange juice, biscuits, popcorn color, sweets, etc.
- It is used as popular drink in Okinawan population and many Asian countries by the name of Turmeric-tea.

14.5 Major Fungal Pathogens and Deficiencies Symptoms of Turmeric

Turmeric is severely affected by soilborne and as foliar diseases. Rhizome rot takes a heavy toll in majority of turmeric growing areas. Among foliar diseases, leaf blotch and leaf spot caused by *Colletotrichum gloeosporioides* and *Colletotrichum capsici* are important, respectively. Numerous insignificant diseases have also been reported on the crop. Future studies on crop loss assessment due to these diseases, their epidemiology under different cropping systems and the role of biocontrol agents as well as organic amendments in disease suppression need a thorough investigation to develop appropriate disease management strategies for enhanced productivity.

The major disease as under the following subheads:

14.5.1 Rhizome Rot

The golden spice (Turmeric) is majorly affected by diseases like rhizome rot caused by *Pythium aphanidermatum* (Rathaiah 1982). Rhizome rot is soilborne and occurs during June to September, during the storage losses are about to the extent of 50–80%. The crop is cultivated in all the regions of India. Due to the regular incident of disease many farmers gave up its cultivation. *Pythium* is a severe pathogen of several vegetables, fruits, grasses, rhizomes, and ornamental crops in several parts of the world (Hendrix and Campbell 1973; Plants-Niterink and Vander 1981; Rathiah 1987; Nageshwar Rao 1994). Chenniappan et al. (2020) identify fungi associated with rhizome rot disease. They isolated a total of 51 fungal isolates from symptomatic rhizomes out of which 11 fungal isolates could cause disease symptoms with ≥30% disease severity. *Fusarium solani* was the major pathogen followed by *Rhizoctonia solani*, *Schizophyllum commune*, *Macrophomina phaseolina*, *Fusarium graminearum,* and *Fusarium verticillioides* identified by molecular analysis.

14.5.1.1 Disease Symptoms

Source from: (Rakesh, 2010)

1. Disease begins at the collar region of the pseudostem and increases upwards and downwards.
2. In the initial stage, the center part of the leaves remains green, while the borders turn yellow and later, the yellowing spreads to all leaves and followed by drooping, withering, and drying of pseudostems.

14.5.1.2 Survival and Spread

- The fungus survives in two ways: (a) in diseased rhizomes kept for sowing and (b) the resting structures like chlamydospores and oospores.

14.5.1.3 Favorable Conditions for Proliferation of Fungus

- Younger sprouts are the most vulnerable to the pathogen. Nematode infestation aggravates rhizome rot disease.
- The temperature higher than 30 ° C, high soil moisture, and waterlogged conditions in the field favoring the disease.

14.5.2 Leaf Spot

Leaf spot caused by *Colletotrichum capsici* in rainy season under humid condition. The symptoms are usually seen in the kharif and pre-Rabi season and cause major damage by reducing rhizome size and weight, the losses may extent up to 52% (Ramakrishnan 1954).

Disease is soilborne and occurs on the leaves from July to October months.

Source from: agritech.tnau.ac.in

14.5.2.1 Disease Symptoms

- The irregular spot of various sizes with white, brown, or gray in the center appears on the upper surface of the young leaves.

14.5.2.2 Survival and Spread

- Disease spreads through rain splashes during irregular showers. The incidence is severe in turmeric under exposed conditions.

14.5.2.3 Favorable Conditions

- High soil moisture, temperature (25 C), and leaf wetness are congenial for the spread of pathogen.

14.5.3 Leaf Blotch

Leaf blotch in turmeric is caused by *Colletotrichum gloeosporioides*. The disease leads to great loss of rhizome yield. It is very difficult to control the pathogen once appears as pathogen spreads very fast for disease development. Therefore, it is precarious to wait for the first disease symptom to initiate spray applications. Colletotrichum leaf spot (CLS) can be managed by prophylactic sprays of efficient fungicides. Nowadays various biocontrol agents are available which can control the disease by natural means.

14.5.3.1 Disease Symptoms

Source from: (Gourav potdar, 2006)

- A small, oval, rectangular, or irregular brown spots appear on either side of the leaves which turns dirty yellow or dark brown.

14.5.3.2 Survival and Spread

- Pathogen is soil as well as seedborne and survives in the infected plant debris.

14.5.3.3 Favorable Conditions

- High soil moisture, temperature (25 °C), and leaf wetness are congenial conditions for its spread.

14.6 Major Bacterial Diseases in Turmeric

14.6.1 Bacterial Wilt

Disease caused by *Ralstonia solanacearum* and is a major pathogen found all over India. It causes major damage to crop and leads to decrease in the yield of crop in the country.

14.6.1.1 Disease Symptoms

- Characteristic symptoms are rapid wilting and death of the entire plant without any yellowing or spotting of leaves.
- Grayish slimy ooze comes out on pressing the stem and in later stages extensive hollowing of the stem.

14.6.1.2 Favorable Conditions

- The pathogen is destructive in moist soils at high soil temperatures above 24 ° C.

14.7 Management of Diseases by Biocontrol Agents

Since the use of chemical fungicide causes an adverse effect on the health of human, environment, and soil there is an urgent need to develop biocontrol agents for protection of plant with good quality food, feed, and fiber. Numerous eco-friendly approaches are available to control or eradicate the plant disease caused by various pathogens. For good quality yield and prevention of crop from disease, the growers and farmer mainly depend upon the chemical fertilizers and pesticides which reduce the fertility of soil. The continuous use of chemical fertilizer and pesticide in the agricultural fields decreases the fertility of soil and causes ill effect on the human as well as environment health so strict regulation should be implemented on the use of chemical fertilizer and pesticides. Therefore, researchers of pest management have focused their attention to develop an effective biocontrol agent alternative to the synthetic fertilizers and pesticides. Since numerous biocontrol agents are available in the market but their adoption by the people and farmer will require some interactions with scientist or entrepreneur.

14.7.1 Definitions

The term biocontrol has been defined accordingly in different fields of biology like entomology and plant pathology. According to entomologist, biocontrol is defined as the use of live predatory insects, nematodes, or microbial agents to suppress the growth of pathogens. According to plant pathologist, it may be defined as the use of efficient microbial agents to suppress diseases. The microorganism which suppresses the growth of harmful pest or pathogen is referred as biocontrol agent (BCA).

14.8 Mechanism of Action of Biological Control

In nature a variety of interactions exist for biological control of pathogens in different ways. Microorganism has some direct or indirect interactions which help to control the growth of pathogens (Odum 1953), Fig. 14.1. Throughout their lifecycle, plants and pathogens interact with a wide variety of organisms which

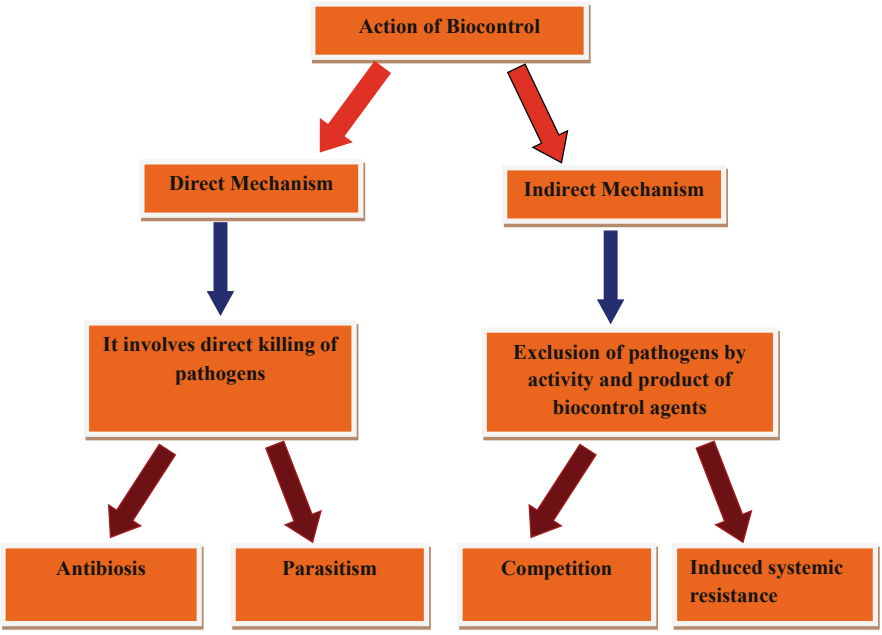

Fig. 14.1 Mechanism of action of biological control agents

significantly affect plant health in various ways. The types of interactions are given under the following:

1. Mutualism
2. Protocooperation
3. Commensalism
4. Neutralism
5. Competition
6. Amensalism
7. Parasitism
8. Predation

14.8.1 Mutualism

It is an association between two or more species where both species receive benefits. Sometimes, it is an obligatory lifelong interaction involving close physical and biochemical contact. For example, bacteria in the genus *Rhizobium* can reproduce either in the soil or, to a much greater degree, through their mutualistic association

with legume plants. These types of mutualism can contribute to biological control, by fortifying the plant with improved nutrition and/or by stimulating host defenses.

14.8.2 Protocooperation

It is a form of mutualism, but the organisms involved do not depend exclusively on each other for survival, BCAs can be considered facultative mutualists involved in protocooperation, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions.

14.8.3 Commensalism

It is a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited, most plant-associated microbes are assumed to be commensals with regard to the host plant, because their presence, individually or in total, rarely results in negative consequences to the plant.

14.8.4 Neutralism

It describes the biological interactions when the population density of one species has absolutely no effect whatsoever on the other biological control, an inability to associate the population dynamics of pathogen with that of another organism would indicate neutralism, In contrast, **antagonism** between organisms results in a negative outcome for one or both.

14.8.5 Competition

It occurs within and between species, results in decreased growth, activity of the interacting organisms. Biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant.

14.8.6 Parasitism

It is a type of association, one organism, usually the physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable extent. The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol.

The *hyperparasitism* is an interaction in which specific biocontrol agent directly attacks and kills the pathogen or its propagules. The biocontrol agents (BCAs)

Table 14.2 Types of interspecies antagonisms leading to biological control of plant pathogens

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism/ predation	*Ampelomyces quisqualisLysobacter enzymogenesPasteuria penetransTrichoderma virens*
Mixed-path antagonism	Antibiotics and lytic enzymes	2,4-diacetylphloroglucinolPhenazinescyclic lipopeptides, chitinasesGlucanases, proteases
	By-products produced by microorganism	Ammoniahydrogen cyanide Carbon dioxide
Indirect antagonism	Competition	Exudates/leachates consumptionSiderophore scavengingphysical niche occupation
	Induction of host resistance	Contact with fungal cell wallsdetection of pathogen-associated

express various mechanisms which results from direct antagonism results from physical contact or by an elevated-level of selectivity for the pathogen to exert suppressive effect Table 14.2.

14.9 Antibiotic-Mediated Suppression

Antibiotics are the substance produced by microorganism that can be toxic and kill other microorganisms at low concentrations. The majorities of microorganisms secrete and produce one or more antibiotic compounds. In some case, antibiotics compound produced by various microorganisms suppress the targeted pathogen which causes disease in vitro and/or in situ. For effective biocontrol agents, sufficient quantity of antibiotics should be produced in adequate amount to control the pathogens. In situ antibiotics formed by numerous biocontrol agents have been deliberate (Thomashow et al. 2002); however, it is difficult to measure the quantities because of very small amount produced by microorganisms. In some cases, the production of antibiotic by biocontrol bacteria has been conformed and one or more genes responsible for antibiotic production have been altered. For example, strain of *Bacillus cereus* UW85 is recognized to produce zwittermycin (Silo-Suh et al. 1994) and kanosamine (Milner et al. 1996). The aptitude of biocontrol agent to produce multiple antibiotics helps to suppress various microbial competitors such as plant pathogens. In recent times, genetically engineered strain *Pseudomonas putida* WCS358r is able to produce phenazine and DAPG showed superior capacities to inhibit plant diseases of wheat grown in field (Glandorf et al. 2001; Bakker et al. 2003). Some of the antibiotics produced by the biocontrol agents are given in Table 14.3.

Table 14.3 Antibiotics produced by BCAs

Antibiotic	Source	Target pathogen	Disease	References
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomycescochlioides*	Damping off	Islam et al. (2005)
Gliotoxin	*Trichodermavirens*	*Rhizoctonia solani*	Root rots	Wilhite et al. (2001)
Iturin A	*B. subtilis* QST713	*Botrytis cinerea* and *R. solani*	Damping off	Paulitz and Belanger (2001), Kloepper et al. (2004)
Mycosubtilin	*B. subtilis* BBG100	*Pythiumaphanidermatum*	Damping off	Leclere et al. (2005)
Pyoluteorin, pyrrolnitrin	*P. fluorescens* Pf-5	*Pythium ultimum* and *R. solani*	Damping off	Howell and Stipanovic (1980)
Zwittermicin A	*Bacillus cereus* UW85	*Phytophthora medicaginis* and *P. aphanidermatum*	Damping off	Smith et al. (1993)

14.10 Lytic Enzymes and Other Byproducts of Microbial Life

Numerous microorganisms produce a wide variety of metabolites which inhibit the growth and activities of plant pathogen. Lytic enzymes are proteinous identity and have capacity to hydrolyze various polymeric compounds such as cellulose, hemicellulose, chitin, proteins, and DNA produced by various microorganisms. Various microorganisms secrete these enzymes directly and suppress the activities of plant pathogen. For example, chitinase expression controls the *Sclerotium rolfsii* caused by *Serratia marcescens* (Ordentlich et al. 1988) and α β 1,3-glucanase showed biocontrol behavior of *Lysobacter enzymogenes* strain C3 (Palumbo et al. 2005). However, microorganism which illustrates the effect of colonizing and inhibits plant pathogens can be classified as biocontrol agents. Some efficient microorganisms such as *Lysobacter* and *Myxobacteria* are recognized to produce abundant quantity of lytic enzymes and exhibit positive response for suppression of fungal plant pathogens (Kobayashi and Yuen 2005; Bull et al. 2002). Moreover, several products of lytic enzyme activity help in disease suppression by indirect way, e.g. oligosaccharides produced from cell wall of fungal are recognized to be effective inducers of plant host defenses system.

The contribution of the above compounds for disease suppression mainly depends upon fertility status of soil because healthy soil serves as a good source for diverse microorganisms and colonizes rhizosphere. Conversely, the activities can be altered for better suppression of disease. For example, disease control in post-harvest practices, the addition of chitosan can excite microbial deprivation of pathogens, similar in case of hyperparasite (Benhamou 2004). Chitosan is a polymer of beta-1,4-glucosamine generally non-toxic and biodegradable in nature produced

by alkaline deacylation from chitin. The application of chitosan can inhibit the growth of *Fusarium oxysporum* cause root rot disease in various plants (Lafontaine and Benhamou 1996). Chitosan exhibited antifungal, antibacterial and antiviral properties and have been extensively used in agriculture system. It is mainly used to control the disease or to reduce their spread, to chelate minerals and nutrient and enhance plant innate defense system. It induce host defense response which includes lignification, ion flux varaition, cytoplasmic acidification, memebrane depolymerization, chitinase and glucanase activation, phytoalaxin biosynthesis, jasmonic acid biosynthesis and expression of defense related genes (Nishizawa et al. 1999).

14.10.1 HCN Production

Other byproducts such as HCN produced by microorganism also may involve in pathogen suppression. Efficient microorganisms produce hydrogen cyanide (HCN) which efficiently acts on cytochrome oxidase pathway and blocks the pathway and even picomolar concentration is highly toxic to all aerobic microorganisms. Certain fluorescent pseudomonads produce HCN which greatly involved in the inhibition of root pathogens. Howell et al. (1980) concluded that *an Enterobacter cloaca* produces volatile substances such as ammonia which were involved in the suppression of *Pythium ultimum*. So it is clear that microorganism produces various compounds which inhibit the plant pathogens and helps in disease management with eco-friendly way.

14.11 Commercial Biocontrol Agents

On the basis of different fungal and bacterial antagonists a large number of commercial products have been registered both at national and international levels. These commercial products such as Bioguard, Ecofit, Biocon, F-Stop contain *Trichoderma* sp. as active ingredient and other products such as Mycostop and *Rhizoplus* subilex, etc. involve *Bacillus* species as active ingredient. Disease suppression by biological control involves competition, mycoparasitism, antibiosis, cell wall degradation and induced resistance, plant growth promotion, and capacity of rhizospheric colonization. Till date most efficient biocontrol agent *Pseudomonas putida* strain WCS358r is studied and genetically engineered to produce Phenazine and 2, 4-diacetylphloroglucinol (DAPG) to suppress diseases in field crops. *Agrobacterium radiobacter* strain K 84 was the initial bacteria registered with the United States Environmental Protection Agency (EPA) for the crown gall disease control in 1979. After that *Trichoderma harzianum* ATCC 20476 fungus was registered with the EPA to inhibit the pathogen which causes disease in plants. At present, EPA is registered with 14 bacteria and 12 fungi to control the plant diseases caused by various pathogens (Fravel 2005).

14.12 Induction of Host Resistance

Plants respond to a diversity of chemical stimuli produced by soil- and plant-associated microbes. Two pathways are generally involved in the host resistance, first pathway termed as systemic acquired resistance (SAR) is mainly regulated by salicylic acid (SA), produced during the pathogen infection and leads to the release of pathogenesis-related (PR) proteins, Table 14.4. These PR proteins contain diverse enzymes which may act directly to degrade invading cells and support cell wall boundaries to defend against infections. A second pathway involves the induced systemic resistance (ISR), which is regulated by jasmonic acid (JA) and ethylene, which are produced during applications of some nonpathogenic rhizobacteria. The defense pathway of SA and JA dependent can mutually act as antagonistic and pathogens may take advantage of this to defeat the SAR. For example, pathogenic strains of *Pseudomonas syringae* produce coronatine, which is similar to JA, to overcome the SA-mediated pathway (He et al. 2004).

Numerous microbial strains colonizing the roots have been recognized as potential agents of host plant defense mechanism. The effective *Pseudomonas* and *Trichoderma* strains are biocontrol agents that positively encourage host plant defenses (Haasa and Defago 2005; Harman et al. 2004). Nowadays applications of plant growth promoting rhizobacteria (PGPR) are useful in controlling different pathogens which cause plant diseases such as angular leaf spot (*Pseudomonas syringae*), anthracnose (*Colletotrichum lagenarium*), and bacterial wilt (*Erwinia tracheiphila*). On inoculation PGPR may produce number of chemical substances, like siderophore, salicylic acid, lipopolysaccharides, and other volatile substances (Van Loon et al. 1998; Ongena et al. 2004; Ryu et al. 2003).

Table 14.4 Bacterial determinants and types of host resistance induced by biocontrol agents

Bacterial strain	Plant species	Bacterial determinant	Type	References
CHA0	Tobacco	Siderophore	SAR	Maurhofer et al. (1994)
WCS374	Radish	Lipopolysaccharide	ISR	Leeman et al. (1995)
Pseudomonas putida strains	*Arabidopsis*	Lipopolysaccharide	ISR	Meziane et al. (2005)
WCS 358	*Arabidopsis*	Lipopolysaccharide	ISR	Meziane et al. (2005)
BTP1	Bean	Z,3-hexenal	ISR	Ongena et al. (2004)
Serratia marcescens 90–166	Cucumber	Siderophore	ISR	Press et al. (2001)

14.13 PGPR as Biocontrol Agent in Turmeric

Plant growth promoting rhizobacteria may be defined as the bacteria that inhabit roots or rhizospheric soil and enhance the crops yield by various direct and indirect mechanisms. The efficient PGPR inoculants used for commercialization should have at least one mechanism such as improved nutrient acquisition (termed biofertilizers), suppression of plant disease (termed bioprotectants), and phytohormone production (termed biostimulants) (Kumar et al. 2014).

Plant growth promoting rhizobacteria (PGPR) grant promise for establishing eco-friendly environment with sustainable agriculture systems and is a well-known substitute against the harsh chemicals because of their broad effect on the plant growth promotion by direct and indirect way (Prasad et al. 2015). Indirect mechanism involves the plant growth promotion through the suppression of disease which involves antibiosis, induced systemic resistance (ISR), high affinity siderophore production, and production of lytic enzymes. In addition, the present day bio-products used for commercial formulations should have increased shelf life and broad spectrum of action with reliable performance under field conditions at a faster rate and could pave the way for commercialization of the technology (Lucy et al. 2004). Actinomycetes as biocontrol agent were screened by Laid et al. (2016) against *Fusarium culmorum* responsible for several cereal diseases. Four isolates, namely D2, D5, D8, and AST1 were tested to determine PGPR effect and biocontrol characters of bread wheat (*Triticum aestivum* L.). They showed that these isolates had a significant effect on seed germination and seedling growth. Islam et al. (2016) studied the ability of PGPR to suppress Phytophthora crown rot in cucumber. A total of 66 isolates were isolated, out of which 10 were selected on the basis of their plant growth promoting attributes and antagonism of phytopathogens. Treated cucumber seeds with these isolates significantly suppressed Phytophthora crown rot caused by *Phytophthora capsici*. Paramanandham et al. (2017) isolated *Pseudomonas aeruginosa* isolates from the rhizosphere soil and evaluated for the growth promotion traits, germination percentage, shoot and root length, and disease resistance in tomato (*Solanum lycopersicum* L.). The selected isolates showed significant improvement in growth of plants and successfully suppressed disease severity of *Fusarium oxysporum* and *Alternaria solani* in pot experiments. Biocontrol of rhizome rot caused by *Pythium aphanidermatum* is one of the major destructing pathogen in India studied by Nandini et al. (2018), they isolated microorganism from rhizomes of healthy turmeric plants. Among 154 endophytic isolates 12 bacterial isolates, 16 fungal isolates, and four actinomycetes showed inhibition to *Pythium aphanidermatum* in vitro. The result showed that among the isolated endophytes, *Pseudomonas* exhibited maximum inhibition against the test pathogen. Vinayarani and Prakash (2018) isolated plant growth promoting rhizobacteria from turmeric (*Curcuma longa* L.). They selected a total of 50 bacterial isolates and further screened for antagonistic activity against *Pythium aphanidermatum* and *Rhizoctonia solani* causing rhizome rot and leaf blight diseases in turmeric. Results revealed that only five isolates of PGPR showed more than 70% suppression of test pathogens. The rhizome inoculation followed by soil application of *B. cereus* showed lowest

percent disease incidence of rhizome rot and leaf blight, 16.4% and 15.5%, respectively. Similarly, *P. aeruginosa* recorded 17.5% of rhizome rot and 17.7% of leaf blight. Thus, these isolates can be exploited as efficient biocontrol agent for suppressing rhizome rot and leaf blight diseases in turmeric. Vitorino et al. (2020) studied the biocontrol effect of BA48R strain of *Enterobacter* sp. and BA88R strain of *Bacillus cereus* against the *Sclerotinia sclerotiorum* and they found that plants treated with the BA48R strain of Enterobacter sp., and in particular, those treated with the BA88R strain of *Bacillus cereus* presented the best results in terms of systemic resistance induction and suppression of S. sclerotiorum. The BA48R bacterial strain and BA88R strain have enormous prospective for development of more sustainable agricultural processes.

14.14 Management of Turmeric Disease Using Eco-Friendly Biocontrol Consortia

Consortium is a culture which contains two or more effective microorganisms commonly occurring in nature. Important group of microorganism selected for biocontrol consortia are: N2-fixers, P solubilizers, photosynthetic microorganisms, lactic acid bacteria, yeasts, plant growth promoting rhizobacteria, various fungi and actinomycetes. Within the consortium, every microorganism has its individual positive function into nutrient cycling which occurs in nature, plant protection against various fungal and bacterial pathogens, enrichment of soil fertility and health.

The consortial application of *Trichoderma viride* and *Pseudomonas fluorescens* @ 4 g kg^{-1} of seed and 2.5 kg ha^{-1} on the soil as basal and top dressing at 150 days after planting was found effective against rhizome rot caused by *Pythium aphanidermatum* in turmeric. It is the destructive disease causing huge loss and reduces yield and quality of rhizomes. Microorganisms which show antagonistic activity against various pathogens are alternative of the chemicals and protect the environment from toxic hazards (Muthulakshmi and Saveetha 2009).

14.14.1 Biofertilizers as Biocontrol in Turmeric

The term biofertilizer may be defined as the preparation that possesses beneficial living microorganisms, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and increase the growth by supplying the accessibility of primary nutrients to the host plant (Vessey 2003; Giri et al. 2019). Primary nutrients added in the soil by biofertilizers through the natural mechanism of nitrogen fixation, phosphate solubilization enhance plant growth by synthesis of growth-promoting substances. Biofertilizers can be likely to reduce the use of synthetic fertilizers and pesticides. The microorganisms present in biofertilizers sustain the soil's normal nutrient cycle and help in manufacturing of soil organic matter from decomposition of organic waste. Biofertilizers promote the plants

Table 14.5 Groups of biofertilizers

S. no.	Groups	Examples
N2 fixing biofertilizers		
1.	Free-living	*Azotobacter, Beijerinckia, clostridium, Klebsiella, anabaena, Nostoc*
2.	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3.	Associative symbiotic	*Azospirillum*
P solubilizing biofertilizers		
1.	Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus subtilis, Bacillus circulans, Pseudomonas striata*
2.	Fungi	*Penicillium sp, aspergillus awamori*
P mobilizing biofertilizers		
1.	Arbuscular mycorrhiza	*Glomus sp., Gigaspora sp., Acaulospora sp.*, *Scutellospora sp.,* and *Sclerocystis sp.*
2.	Ericoid mycorrhizae	*Pezizella ericae*
3.	Orchid mycorrhiza	*Rhizoctonia solani*
Biofertilizers for micro-nutrients		
1.	Silicate and zinc solubilizers	*Bacillus* sp.
Plant growth promoting Rhizobacteria		
1.	Pseudomonas	*Pseudomonas fluorescens*

growth as well as enhance the sustainability and the health of the soil. Thus, the use of microorganism and their byproducts are enormously beneficial in elevating soil fertility and enhancing plant growth by providing the organic nutrients. Hence, the use of biofertilizers maintains the fertility of soil in eco-friendly manner for longer period and acts as alternative way of chemicals in the field which are harmful to the living soil. Since a long time *Rhizobium*, *Azotobacter*, *Azospirillum,* and blue green algae (BGA) as a biofertilizer are available and widely used to enhance the yield and control the disease in various crops, Table 14.5. For leguminous crops *Rhizobium* is used as biofertilizer. The biofertilizers are eco-friendly, cost–effective, and easily accessible organic agro-input. Biofertilizer like *Azotobacter* can be used in crops like wheat, maize, mustard, cotton, potato, and other vegetable crops. *Azospirillum* biofertilizers are used mainly for sorghum, millets, maize, sugarcane, and wheat. For paddy crop blue green algae inoculations are used which generally belongs to a cyanobacteria genus, *Nostoc*, *Anabaena*, *Tolypothrix,* or *Aulosira*, fix atmospheric nitrogen both under upland and low-land conditions. *Anabaena* associated with water fern *Azolla* helps in nitrogen fixation in paddy field up to 60 kg/ha/season and also boosts the soil by means of organic matter (Malboobi et al. 2009).

Trichoderma act as biocontrol agent in natural way to control the soilborne disease in plant. It is a free-living fungus, highly interactive in root and soil. It reduces pathogen development, survival or infections by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion.

Recommended dose of Trichoderma application is 6–10 g of Trichoderma powder/Kg for management of disease before the seed sowing. For efficient biocontrol preparation 1 kg of Trichoderma formulation is mixed with 100 kg of farmyard manure and covers it by polythene for 7 days. It is commonly used for the turmeric crop to control disease (Rakesh 2010).

Mycorrhizal fungi protect the plants from disease by covering the roots of plant, forming a fungal mat, provides physical barrier to pathogen, releases antagonistic chemicals, escalating plants nutrient-uptake ability, and creates a direct competition with pathogen (Panth et al. 2020; Prasad et al. 2017).

14.14.2 Disease Management by Organic Inputs

Nowadays organic agriculture is finding position in the majority of development and these organic inputs contain efficient microorganism which act as biofertilizers and biocontrol agent for disease management in various crops. Liquid formulations that are used in organic agriculture like panchagavya, beejamrit, and jeevamrit are the fermented products which are obtained from the cow and used as plant growth promoter by the farmers. These organic inputs are the richest sources of valuable microflora which stimulate the plant growth and leads to high-quality yield of agricultural crops (Devakumar et al. 2011). Although interest has been increased in the organic farming by using the organic inputs such as panchagavya, beejamrit, jeevamrit, and other liquid organic formations in the field because these are cost-effective and eco-friendly in nature and increase the fertility of soil. Devakumar et al. (2008) and Sreenivasa et al. (2010) also reported the occurrence of numerous useful microorganisms, viz. nitrogen fixers, phosphorus solubilizers, actinomycetes, and fungi in jeevamrit and beejamrit. The microorganism present in panchagavya acts as biofertilizer and biopesticides which played significant role in providing resistance to pests and diseases, resulting in increased overall yields (Tharmaraj et al. 2011; Sireesha 2013). Panchagavya has resulted in positive impact on development and productivity of crops as reported by Somasundaram et al. (1997). There is an urgent need to build up a feasible and well-suited package of nutrient management through efficient microorganism in organic resources for various crops to overcome the problem of degrading fertility of soil by chemical fertilizer and pesticide (Kannaiyan 2000). Punitha et al. (2010) studied that Panchagavya, the blend of cow's five products, prepared by aerobic fermentation method showed great diversity of micro-organism such as *Bacillus*, *Pseudomonas*, *Lactobacillus,* and *Azotobacter* and fungi *Aspergillus* and Yeast (*Saccharomyces cerevisiae*). The microbes present in Panchagavya secrete enzymes and plant growth hormones which favor plant growth and control the disease incidence in various crops. Organic manures prepared from compost, organic wastes, and peats have been anticipated to manage soilborne diseases. Various fungal pathogens like *Rhizoctonia solani*, *Thielaviopsis basicola*, *Fusarium*, *Phytophthora*, *Pythium,* and *Sclerotium* can be effectively managed by the organic inputs. Besides improving the soil structure these organic inputs also increase water holding capacity and also nourish other beneficial microorganisms

which lead to suppression in harmful soilborne pathogens. The activity of these beneficial microorganisms in the soil leads the competition, which control the destructive soilborne pathogens. The utilization of biofertilizers is one of the important components of integrated nutrient management, as they are cost-effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Numerous microorganisms and their involvement with crop plants are being exploited in the manufacturing of biofertilizers. These microorganisms can be classified in diverse traditions on the basis of their nature and function.

14.15 Conclusion

Soilborne pathogen generally causes huge loss of crop up to 70% in India during cropping season. Numerous fungicides have significant effect in disease management but their continuous use and adverse side effects have shifted the attention toward biological control which acts as efficient alternative method of disease management. Recently, in the agriculture system application with biological control agents (BCAs) as biofertilizers has gained recognition as a means to minimize or eradicate the utilization of chemical pesticides. Biological control by using the PGPR and biofertilizers represents the best approach for extended sustainability and efficient executive of soilborne diseases against various soilborne pathogens.

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Microbiome Role in Control of Sustenance of Rice Health and Production

15

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Abstract

Plant pathogens are an emerging threat to global food security leading to severe losses of economically important food crops. Rice, catering 40% of global population, faces tremendous yield and economic losses due to pathogen incidences. Today, agriculture practices are more bound to the use of chemical pesticides and fertilizers for disease management and improved crop yields. Thus, the crop protection product sector is becoming a fast developing industry in order to compel with the growing population and need to minimize crop damage. This entails the usage of chemicals for crop protection in a judicious manner within the national, confined regulatory framework. However, the application of high doses of chemical pesticides and fertilisers in intensive farming practices negatively affects both human health and natural ecosystems. Hence, there is the urgent demand for the use of safer, environmentally sound and sustainable alternative technologies for profitable crop production. Therefore, the present chapter fosters majorly on the different biological and molecular approaches for disease management.

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15.1 Introduction

The human population, over the past 50 years, has almost doubled and it is estimated to increase across 9 billion by 2050. Henceforth, to feed this increasing population, the global food security has raised many challenges for increased agricultural productions, particularly of cereals like wheat, rice and maize (FAO 2017). There are many factors which contribute to the unequal distribution of food around the world, including socio-economic factors, widening urbanization and agricultural marginalization.

Crop losses due to various biotic and abiotic factors have become a serious issue in the present agricultural scenario. They include primary and secondary losses occurring both during the production cycle (pre-harvest) and/or during the storage (post-harvest). Whether pests or diseases, the crop losses are devastatingly increasing with short/long-term consequences on agricultural productivity (Savary et al. 2012). There are approx. 10–30% crop losses due to plant diseases in the field, directly affecting farmers' livelihood and global food production (Cerda et al. 2017).

Biotic stresses (pests and diseases), comprise viruses, fungi, bacteria, weeds, insects and other pests, raise a major issue of concern from fields to markets in the developing world (Boyd et al. 2013). This focuses attention on the urgent need to understand plant–pathogen interactions more distinctly and how this finds application in moulding the agricultural strategies. Losses due to pathogenic incidence also affect the population dynamics and ecosystem nutrient cycling. Plant pathogens are either necrotrophs (kill their hosts), biotrophs (require live hosts for nutrients) or hemibiotrophs (initially biotrophs with necrotrophic mode at later stages of disease) on the basis of their mode of nutrition (Laluk and Mengiste 2010). Various fungal and bacterial diseases such as downy and powdery mildew by *Puccinia striiformis*, wilt disease by *Fusarium* species, bacterial blight and canker by *Xanthomonas* spp., bacterial wilts by *Pseudomonas* spp. in crops like rice, wheat, cotton, cucurbits, grape, chickpea, tomato, potato, etc. are among the most common incidences. There are also losses due to viruses (local lesions, stunting, chlorosis), soil insect pests (feeding damage, oviposition damage, vectors of plant diseases) and nematodes (nutrient deficiency), which adversely affect shallow rooted crops and vegetables (Thind 2015; Gimenez et al. 2018). These biotic factors, often termed as microbiome constituted of plant associated different organisms like viruses, fungi, oomycetes, bacteria, protozoa and archaea may be of phyllosphere, endosphere and rhizosphere types (del Carmen Orozco-Mosqueda et al. 2018; Kim and Lee 2020). These plant microbiomes being the integral part of plant are valuable for plants as they are involved in the alteration of plant physiology, defense, growth and development

(Prasad et al. 2015; Berg et al. 2016; Santoyo et al. 2017; del Carmen Orozco-Mosqueda et al. 2018; Singh et al. 2019).

Different abiotic stresses such as salinity and temperature affect the growth rate and reproduction of bacterial pathogens like *Ralstonia solanacearum* (tomato), *Acidovorax avenae* (cucurbits) and *Burkholderia glumae* (rice) (Pandey et al. 2017). Abiotic stress also leads to susceptibility of host plants to pathogenic organisms and insects. Therefore, it has been estimated that multiple stresses together can cause 65–87% approximate reduction to plant productivity depending on the crop (Pandey et al. 2017).

Rice, cultivated throughout the world, is the staple food crop serving about 70% of the population in India, China, East-Asia, South East Asia, Africa and Latin America. Worldwide, it occupies ~161 million ha arable land, with the annual production of about 678.7 million tons (FAO 2009). Almost 90% of world's rice is grown (143 million ha) and produced (612 million tons of paddy) in Asia (FAO 2009). India is the second largest producer of rice in the world after China, as rice occupies the central position in Indian agriculture with 24% of gross cropped area. But its production is constrained by many factors, including pests and diseases, in the agro-ecological zones of tropical and subtropical areas, especially in Asia. About 52% of the global rice production is hampered due to damage caused by rice disease epidemics which not only affect crop yield but also cause menace to global food security; also the disease management becomes unaffordable (Shrivastava et al. 2010; Yang et al. 2020). Losses to rice production thus open new prospects to elucidate the role of plant microbiome in rice during the onset and development of plant diseases through the interaction between microbiome and invading pathogens. This projects the aim of this chapter to gather and discuss different approaches including traditional (cultural), biological and molecular techniques for management of plant diseases in *Oryza sativa,* i.e. rice. The chapter will focus on different fungal and bacterial rice diseases and their severity, hampering its growth and production. It will provide a brief overview of important perspectives for management of emerging rice diseases, resulting in improved crop resistance and productivity.

15.2 Major Rice Diseases

The worldwide annual losses led by various rice diseases have been estimated to be about 10–15%, thus alarming the nation for its judicious management. Plant pathogens may be fungal, bacterial and viral or nematodes and diseases caused by them damage plant parts above or below the ground. In particular, fungal diseases such as rice blast (*Magnaporthe grisea*), brown spot (*Bipolaris oryzae*), stem rot (*Sclerotium oryzae*), sheath blight (*Rhizoctonia solani*), sheath rot (*Sarocladium oryzae*); bacterial diseases such as bacterial blight (*Xanthomonas oryzae* pv. *oryzae*); and viral disease (rice tungro virus) are the major rice limiting factors hampering its production (Kumar et al. 2017). Among them, rice blast is the most widespread in Uttar Pradesh, India; with many epidemics which have occurred in various parts of the world, resulting in yield losses of 50 to 90% of the expected

harvest (Agrios 2005). *Xanthomonas oryzae* devastatingly affects all the varieties of basmati rice in tropical Asia and therefore its production is severely affected in India (Joseph et al. 2004). Among various rice diseases, major yield losses are caused by brown spot, leaf blast, bacterial blight, sheath blight and tungro virus. The most devastating rice diseases which often cause huge economic losses are enlisted below.

15.2.1 Sheath Rot

Sheath rot, a seed-borne disease is among the major fungal disease, which causes havoc to rice production in India. Causal pathogens include fungi and bacteria both but fungi are the major pathogens causing sheath rot, mainly *Sarocladium oryzae* and *Fusarium* sp. (*Fusarium fujikuroi*) complex. Other fungal pathogens include *Gibberella fujikuroi complex, Fusarium graminearum, Fusarium oxysporum complex, Cochliobolus lunatus, Gaeumannomyces graminis, Sclerotium oryzae, Rhizoctonia oryzae*. Bacterial pathogen is *Pseudomonas fuscovaginae, Pseudomonas syringae, Pseudomonas sp., Pantoea ananatis, Burkholderia glumae, Acidovorax oryzae* (Bigirimana et al. 2015*)*. The pathogen enters through stomata or wounds, caused by mites and insects or other pathogens (Pearce et al. 2001). The disease symptoms appear as sheath discoloration, greyish brown lesions resulting in partial emergence of young panicles, reduced tillering and yield. Disease severity prevails in warm and humid climate mainly during rainy season with temperature 20–30 °C and relative humidity 65–85% favourable for pathogen proliferation (Velásquez et al. 2018). The disease spans over all the rice-growing countries, viz. India, Sri Lanka, Bangladesh, China, Indonesia, Malaysia, Nepal, Pakistan, Japan, Saudi Arabia, Thailand, Vietnam, Gambia, Kenya, Madagascar, Nigeria, Tanzania, Mexico, USA, Argentina, Brazil and Australia.

15.2.2 Brown Spot

Among various rice diseases caused by fungal pathogens, brown spot is also a major production constraint in all rice-growing areas especially under semi-dry conditions. Causal pathogen is both the asexual stage (*Bipolaris oryzae,* Breda de Haan) and the sexual stage that is *Cochliobolus miyabeanus* Drechsler ex Dastur. It is known since twentieth century from the occurrence of 'Great Bengal Famine' (1942–1943) by the fungus *Bipolaris oryzae* (Sunder et al. 2014). The initiation of disease symptoms appear as brown spots on leaves and glumes resulting in fungal growth and sporulation, which are carried to the grains and when spores germinate the seeds get shrivelled and discoloured (called as 'pecky grain' or 'kernel spotting'), the plants become stunted. The diseased nursery and the affected field appear to be burnt or scorched due to seedling death (Harish et al. 2008). The disease has worldwide distribution in the rice-growing countries (Asia, America and Africa). In India, brown spot of rice is endemic to all the southern (Andhra Pradesh, Kerala, Tamil Nadu and Karnataka), and eastern states of India; most prevalent in rainfed lowlands

and uplands, and in abnormal or poor soil conditions. Almost 6–90% of yield losses have been found during disease spread of brown spot in Asia (Mew and Gonzales 2002).

15.2.3 Blast

Rice blast, being a devastating fungal disease, is deterrent to increased production of rice and leads to huge yield losses to the farmers. It is caused by fungal pathogen *Pyricularia oryzae* with the asexual state *Magnaporthe oryzae* (Asibi et al. 2019). It affects the plant at every growth stage and disease appears as white to grey-green lesions on leaf; panicles and nodes followed by collar region necrosis. Leaves and panicles are the major plant parts affected by pathogen attack, causing leaf and neck blast thereby reducing the photosynthetic rate and yield (Agbowuro et al. 2020). There are reports of about 5–70% grain yield losses by rice blast in Kashmir (Bhat et al. 2013); about 25–45% in Rajasthan (Maheshwari and Sharma 2013).

15.2.4 False Smut

False smut, earlier considered as minor disease, has now shown its increased incidences in rice-growing areas of world like India, China and the USA (Jecmen and TeBeest 2015; Fan et al. 2016). Fungal pathogen *Villosiclava virens* (anamorph: *Ustilaginoidea virens*) is the causal organism of rice false smut. The pathogen infects rice flowers and transforms them into balls called 'smut ball' which appears orange while older ones are olive-green to brown to greenish-black. Due to increased growth of pathogen, these balls burst and release chlamydospores. The smut balls produce two mycotoxins: ustiloxin and ustilaginoidin which contaminate rice grains and become highly toxic to humans and animals (Wang et al. 2019).

15.2.5 Kernel Smut

Kernel smut or black smut in rice is caused by fungal pathogen *Tilletia horrida* (Synonym: *Tilletia barclayana*) and was first reported from Japan in 1896. Its black powdery spores can survive for more than 1 year in soil and up to 3 years in seed after infection. (Carris et al. 2006; Chen et al. 2016). Presently, its occurrence is in Asia, Australia, America (North, South, Central), Europe and Africa. In China and Pakistan, about 80–100% of disease incidences majorly occur in rice fields of hybrid seed production (Biswas 2003; Carris et al. 2006). In India, the disease is endemic in Gujarat, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, West Bengal, Andhra Pradesh, Orissa, Tamil Nadu and Assam.

15.2.6 Sheath Blight

Sheath blight, one of the lethal diseases of rice, leads to huge quality and production losses (25–50%) worldwide. Symptoms appear as elliptical or oval water-soaked lesions in the infected sheaths caused by *Rhizoctonia solani* (fungus). The disease advances during hot and humid climate, from milking to tillering stage, even at stages of panicle emergence resulting in partially filled discoloured grains with brown to black spots leading to yield losses (Singh et al. 2016; Datta and Vurukonda 2017; Srivastava et al. 2016). Being a necrotroph, the pathogen is calamitous because it forms 'sclerotia' (infecting bodies) which remain quiescent in soil for almost 1–3 years; has high genetic diversity and wide host range (Tsiboe et al. 2017). The disease spreads from plant to plant and field to field through floating sclerotia and mycelia disseminated by irrigation water (Singh et al. 2016). Disease spans over the temperate and tropical rice-growing areas and is prevalent in China, Germany, Formosa, India, Indonesia, Iran, Korea, Malaysia, Africa, Bangladesh, Brazil, Burma, Colombia, Nigeria, Philippines, Russia, Sri Lanka, Thailand and the USA (Singh et al. 2016). In India, it occurs in Punjab, Bihar, Haryana, Chhattisgarh, Uttar Pradesh, Uttarakhand, West Bengal, Andhra Pradesh, Jammu and Kashmir, Tamil Nadu, Karnataka, Kerala, Madhya Pradesh, Assam.

15.2.7 Sheath Spot

Rhizoctonia oryzae Ryker & Gooch causes sheath spot of rice. The pathogen was first identified by Ryker and Gooch (1938) as the causal agent of sheath spot. The pathogen dispersal was first reported by Hashioka and Makino (1969) in Taiwan, West Africa, Japan, Cambodia, Brazil, Thailand and the USA. The pathogen has also been reported in subtropical and tropical rice-growing regions due to favourable low temperature climates.

Disease symptoms appear as oval, spot-type lesions which are bleached or straw coloured in the centre surrounded by a reddish-brown border. The spot initiates from the lower leaf sheath above the waterline and reaches the upper stem as a result of secondary infection by basidiospores (Lanoiselet et al. 2007).

15.2.8 Stem Rot

Among various diseases, stem rot of rice has also raised innumerable difficulties for rice growers in India. It is caused by *Sclerotium oryzae* firstly reported from Italy and named as *S. oryzae* Catt. (Cattaneo 1876). Sclerotia initiate primary infection in rice stem at the waterline and symptom appears as black lesions on the leaf sheath (Singh et al. 2002; Pramesh et al. 2017). The culm of infected plants becomes discoloured to black and rotten resulting in the dehydration, wilting and drooping of leaves (Gopika et al. 2016).

Evidences of rice stem rot in India have been reported from Manipur, Karnataka and Andhra Pradesh. There are reports of 10–80% grain yield losses due to stem rot in various parts of the world (Gopika et al. 2016).

15.2.9 Bacterial Blight

Bacterial blight is among the important and oldest rice diseases known to cause yield losses. It is caused by the bacterial pathogen *Xanthomonas oryzae* and its incidences were first reported from Japan in 1884 (Saha et al. 2015). The pathogen mainly infects the vascular tissue—xylem; where it proliferates and propagates in the plant. The infection results in tannish-grey to white or yellow lesions on leaf blades at tillering stage of crop growth resulting in plant death on progression of disease. The disease flourishes during warm and humid temperatures of 25–30 °C especially in wetlands with these prevailing conditions (Sharma et al. 2017). Worldwide, the disease is reported to occur in parts of Africa, Asia, the USA, whereas in India the disease causes crop loss in Bihar, Haryana, Kerala, Punjab and Uttar Pradesh.

15.3 Rice Pathogen Interaction

The major difference between plants and animals is that plants are immovable from its location. When attacked by a pathogen they rely on the immune system to identify the pathogen and respond against the same (Dangl and Jones 2001; Ausubel 2005; Chisholm et al. 2006).

15.3.1 Attack

The usual mechanism by which pathogen enters into plant is adhering of spores on plant surface and entry through stomatal pore. Their attacks on plant are based on their life cycle in host plants, viz. Biotrophs, necrotrophs and hemibiotrophs (Zhou 2016; Doehlemann and Hemetsberger 2013). The spore germinates by appropriate chemical signalling and forms a germ tube that migrates towards the favourable sites for causing infection through formation of an appressorium, which penetrate the cuticle and cell wall layers. Later on, the penetration peg forms a specialized structure known as haustoria, which is responsible for nutrient uptake and also is a prime site for the effector secretion (Horbach et al. 2011). So, the foremost step for any pathogen attack on the host plant is to release apoplastic effectors like toxins, cell wall-degrading enzymes (CWDEs), and various cysteine-rich proteins that makes plant cell/tissue suitable for pathogen invasion (Gupta et al. 2015). Apoplastic effectors like CWDEs and toxins are more important for necrotrophs and less important for biotrophs and hemibiotrophs (Cantu et al. 2008).

15.3.2 Early Response and Defense

The very first level of defense provided by cuticle and cell wall that acts as a physical barrier is often not enough to prevent pathogen entry into the plant system. The entry of pathogen leads to activation of next level of defense response, i.e. innate immunity which provides resistance against the invading pathogen (Boller and Felix 2009). Plant innate immunity is stimulated by root microbiota with the induction of systemic resistance against potential pathogens. It is triggered via microbe-associated molecular patterns (MAMPs), which are the general elicitors recognized by pattern recognition receptors (PRRs). This recognition leads to MAMP-triggered immunity (MTI) (Jones and Dangl 2006; Boller and Felix 2009; Pel and Pieterse 2013). The common MAMPs include bacterial flagellin derived small peptide (*flg22*), elongation-factor Tu peptide (*elf18*) (Felix and Boller 2003; Zipfel 2009; Trdá et al. 2014, eicosapolyenoic acids (Savchenko et al. 2010), rhamnolipids (Varnier et al. 2009; Sanchez et al. 2012), lipopolysaccharides (Newman et al. 2002; Erbs and Newman 2012), β-glucans (Klarzynski et al. 2000), peptidoglycans (Willmann et al. 2011), etc. When MTI is supressed by pathogen then effector-triggered immunity (ETI) comes in action which is activated by perception of specific effectors [avirulence (*Avr*) proteins] within the infected tissues, which weaken the host cellular processes, damage cytoskeletal machinery, block translation and suppress the immune response (Pel and Pieterse 2013; Liu et al. 2013). The ETI is stronger than MTI due to generation of oxidative burst like ROS (Reactive oxygen species), NO (Nitric Oxide) that can trigger the hypersensitive response (HR), which is the major element of plant disease resistance leading to cell death. The HR usually deprives the pathogen from food supply and restricts them to the initial infection site showing antimicrobial effects (Delaunois et al. 2014). ROS and NO also have role in signalling and cell wall strengthening by oxidative cross-linking polymers (Delaunois et al. 2014). However, HR does not have the ability to protect the plant from necrotrophic pathogen. Phytohormones, chiefly salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play role in signal transduction during plant defense responses (Robert-Seilaniantz et al. 2011). Different defense enzymes which use ascorbate and glutathione as electron donors are well known for their role in H_2O_2 detoxification in plants (Chandrashekar and Umesha 2012). Interaction of plant with the necrotrophs and biotrophs results in the modulation of jasmonic acid (JA)/ethylene and salicylic acid (SA) dependent response, respectively (Zhou 2016; Glazebrook 2005). SA induced plant resistance to a wide spectrum of pathogens results in activation of systemic acquired resistance (SAR). Some artificial chemicals, viz. probenazole and benzothiadiazole are also known to induce SAR (Iwai et al. 2007). After exogenous SA applications, different defense responses (ROS production, PR genes expression and disease resistance) get induced against a wide range of biotrophic and hemibiotrophic fungal, bacterial, viral pathogens as well as phloem-feeding insects (Andersen et al. 2018; Park et al. 2007).

15.3.3 Effect of Environment and Co-Infection on Host–Pathogen Interaction

Usually, plant involves multiple microbes in a host pathogen system that may be pathogenic as well as non-pathogenic. The environment where the interaction occurs plays an important role in the pathogenicity as the environment–host–pathogen is always in tripartite interaction. Environment can have profound effects on host's growth, physiological state, immune signalling and abiotic stress response as well as pathogen's survival, germination and virulence property (Kaushal and Wani 2016). The variable environment can render the host being fully susceptible to fully resistant as well as pathogen fully virulent to non-virulent or weakly pathogenic (Velásquez et al. 2018). These environmental factors include both abiotic and biotic factors.

Among biotic factors, microorganisms from the co-infection systems affect the pathogenicity in three ways, viz. competition, cooperation and coexistence. In competition, pathogens devise strategies like toxin production and development of physical barriers in order to remove competitors from resource dense site (Al-Naimi et al. 2005). In cooperation pathogens beneficially interact each other by the exchange of materials in form of biochemical signals essential for survival (Mordecai et al. 2016), while, in coexistence the pathogens reside through niche specialization site. These interactions may be detrimental as well as beneficial for the plant defense system depending on its microbiome (Abdullah et al. 2017).

In the era of climate change, three factors, viz. CO_2 concentration, temperature and water availability are predicted to affect the crops most likely. The effect of increased CO_2 concentration increases the disease severity of rice blast and sheath blight in rice plants (Kobayashi et al. 2006). The most favourable temperature for *Xanthomonas oryzae* in daytime is 35 °C and night-time 27 °C to colonize rice, thus increase in temperature below and above optimum level alters the pathogenicity potential. Xa7, a rice disease resistance protein against *Xanthomonas oryzae*, is more effective at higher temperatures than at lower temperatures, contrary to most R proteins (Webb et al. 2010). The devastating pathogen of rice *Magnaporthe oryzae* infection requires water availability, i.e. at least 5 h of leaf wetness (the duration in which the leaf has water on its surface) for the infection to occur (Magarey et al. 2005). Environmental conditions like drought worsen the condition even more by causing aggressive infections and more visible symptoms in rice. Plants respond to attacks by activating different defense responses which involve accumulation of defense enzymes, inhibitors and different antibiotics which help in preventing infection. Plant–pathogen interactions are governed by complex network of molecular and cytological processes determining the final outcome ranging between susceptibility and resistance. In *M. oryzae,* successful colonization and further pathogen reproduction in the host plants have been demonstrated to be governed by a novel pathogenicity gene *DES,1* which regulates counter-defense against host basal resistance (Chi et al. 2009).

Rice endophytic microbiome shows varied diversity and composition depending on growth stage, environmental factors and genotype (Walitang et al. 2018; Qin et al.

2019). The tremendous potential of its microbial communities is known to raise protection against pathogens. Different microbes like *Bacillus, Pantoea, Achromobacter, Trichoderma,* and *Streptomyces* have been reported for their antagonistic behaviour against *Rhizoctonia solani*, *Xanthomonas oryzae*, *Magnaporthe oryzae* (De Costa et al. 2006; Harsonowati et al. 2017; Kim and Lee 2020). Studies using multi-omics and DGGE techniques showed dominance of archaea and bacteria in rhizosphere of rice when compared to bulk soil of similar diversity (Breidenbach et al. 2016). Spence et al. (2014) have reported that the rhizospheric abundance of beneficial microbes like *Pseudomonas* spp. limits the pathogens through their hormonal signalling.

15.3.4 Plant Defense Response

The delicate relationship between plant and pathogen is governed by the apoplastic interaction of the secreted proteins and other metabolites, derived from both organisms (Gupta et al. 2015). Plants have developed a complex and multi-layered immune system while co-evolving with pathogens, resulting in a plant–pathogen interaction which is either incompatible (disease resistance/tolerance) or compatible (pathogen infection and disease), governed by their genetic makeups. Protein–protein interaction map of *Rhizoctonia solani* has been constructed to provide insights into the potential pathogenic mechanisms of the fungus (Lei et al. 2014). Through cytological evidences, Araujo et al. (2016) reported higher number of necrotic epidermal cells in the compatible interactions due to unlimited fungal growth within the leaf tissues in contrast to the limited growth in the incompatible interaction, probably due to a defense response (Araujo et al. 2016). During compatible and incompatible interactions of rice blast, the fungus modulation of metabolites, viz. alanine, malate, glutamine, proline, cinnamate and an unknown sugar has also been reported, with a potential of biochemical changes during plant–pathogen interactions (Jones et al. 2011). Plant associated microbes (bacteria or fungi) also regulate hormonal balance involving the secretion of secondary metabolites (Manganiello et al. 2018; Pascale et al. 2020) thus modulating the plant hormonal signalling as defense mechanism. Phytohormones like indole acetic acid (IAA), salicylic acid (SA), cytokinins, gibberellins, jasmonic acid (JA) generally effectuate the microbial aggregation thus altering the plant immunity (Patkar and Naqvi 2017; Stringlis et al. 2018; Pascale et al. 2020). Involvement of some rare sugars like turanose as defense inducer has also been reported (Srivastava et al. 2016). The ability of a pathogen to invade and infect plants depends upon the effectors playing role in the suppression of plant immune responses. In reaction, the plant resistance against a pathogen depends upon its potential to recognize it as non-self and induce an immune response to limit its growth (Kumar and Verma 2013). After detection of microorganism, the complex interactions involve activation of a set of genes via recognition of elicitor molecules released which include carbohydrate polymers, lipids, glycopeptides and glycoproteins (Thakur and Sohal 2013). It also results in biochemical and physiological changes in plants, such as cell

wall lignification, suberization and callose deposition via production of phenolic compounds, phytoalexins and pathogenesis-related (PR) proteins, which subsequently prevent invasions. Plant diseases also put detrimental effects to endophytic microbial diversity as reported in case of clubroot disease (causal agent, *Plasmodiophora brassicae*) in cruciferous plants (Breidenbach et al. 2016). Many findings report the pathogenic resistance of endophytes such as *Bacillus, Streptomyces* and *Azospirillum* against *Rhizoctonia solani, Fusarium oxysporum, Magnaporthe grisea*, etc. via production of nutrients, allelochemicals and phytohormones thus invigorating induced systemic resistance in rice. Yang et al. (2020) have revealed the increased diversity of endophytic microbial community during bacterial blast disease in rice signifying the disease resistance related to shift in diversity and structural composition.

Protection of rice against *Xanthomonas oryzae* (*Xoo*) by *Xa21* gene induces downstream defense mechanisms, viz. expression of PR (pathogen related) genes and hypersensitive reaction (programmed cell death) (Peng et al. 2015). A variety of plant defense mechanisms are known to manage the oxidative burst in both compatible and incompatible interactions. Understanding molecular mechanisms of disease resistance with different approaches led to the development of new tools for breeding of improved resistance against different rice pathogens. Assays with marker assisted breeding techniques signify the near-isogenic resistant line (NIL) Pusa Basmati-1 (PB1), carrying rice blast resistance gene *Pi9,* thus inducing resistance mediated by activation of kinases, WRKY, MYB, and ERF transcription factors, JA-ET hormones, chitinases, glycosyl hydrolases and lipid biosynthesis (Jain et al. 2017). Though plants have developed a complex defense system to fight with the pathogens, however their co-evolution or incapable potency of the defense system leads to disease development in plants.

15.4 Urge and Approaches for Disease Management

Plant health is of utmost global importance to achieve agricultural sustainability. Therefore, the major concern in the coming years is to increase rice productivity in order to sustain the hiking population. Currently, the emerging crop protection strategies involve genetic improvement of plants to impart resistance against pests and pathogens. Still in areas of high disease pressure, new crop varieties with single sources of genetic resistance cannot combat pathogen attack. Indiscriminate use of herbicides and pesticides has also been found as ineffective and unsafe for use (Jallow et al. 2017). The demand is urgent for safer and more sustainable methods of crop management.

Primarily, the overall strategy for crop disease management might involve three components: reduction of the initial inocula, alleviation of the infection rate and minimisation of the duration of the epidemic. Each component employs the traditional principles of disease control: (1) *Avoidance*: a method which prevents the disease by selecting a time of the year or a site where the disease causing inoculum is less/absent or where the environment is unfavourable for its spread, (2) *Exclusion*—a

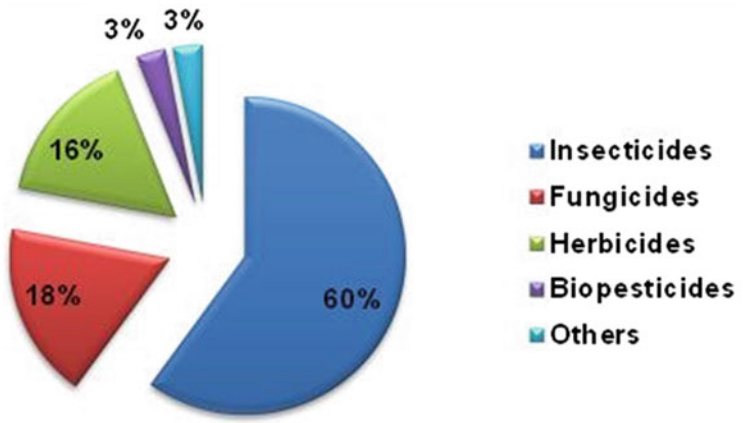

Fig. 15.1 Different classes of pesticides used in India (Source: Industry reports, Analysis by Tata Strategic)

method which prevents the introduction of inoculums, (3) *Eradication*—a method which destroys or makes the inoculum (or its source) inactive, (4) *Protection*—a method which prevent infection using a toxicant to infection, (5) *Resistance*—a method which use the resistant/tolerant cultivars of infection and (6) *Therapy*—a method of curing plants which are already infected. *These principles signify the goal of zero disease; however, they work to manage the disease rather than achieving its elimination or control*. Therefore, rather than neglecting these principles, they must be made appropriate to be used as different strategies based on epidemiological principles (Nutter 2007).

Plant pathogens, since many years, have been controlled mainly by the use of chemical methods (pesticides; Fig. 15.1). However, the insurgence of resistance in the pathogen or pest populations towards these chemicals has raised many concerns in the agriculture system.

Moreover, the application of chemical pesticides has caused serious hazards to human health and surrounding environment. In view of the above circumstances and problems there is an urgent need/demand to switch towards the use of environment friendly approaches for plant disease control. In the following paragraphs some non-chemical approaches, and how they can be applied for management of many diseases affecting rice crops, are discussed.

15.4.1 Soil Solarisation

Soil solarisation, a non-chemical technique has also been introduced in agriculture to reduce the use of agro-chemicals (pesticides) and to manage soil-borne inocula of pathogenic fungi, bacteria, nematodes, soil-borne pathogens, certain insects and weeds (Chandrakumar et al. 2002). It does not release any harmful chemicals to

the soil and is a safe, effective and eco-friendly technique resulting in physical, chemical and biological changes, specifically in the top 10 cm soil (Bacha et al. 2007). Solarisation causes many biological changes in treated soil such as the destruction of several mesophilic microorganisms. Following solarisation the surviving microorganisms remain higher in number than pathogens. They include bacteria belonging to *Bacillus* and *Pseudomonas* spp., fungi such as *Trichoderma* and some free-living nematodes. The treatment effectively maintains the biological equilibrium in soil and thus prevents recolonization of pests and pathogens (Stapleton and DeVay 1995). Some of the successfully managed soil-borne fungal and bacterial pathogens by solarisation include: *Rhizoctonia* spp., *Fusarium* spp., *Sclerotinia* spp., *Macrophomina* spp., *Phytophthora* spp., *Verticillium* spp., *Agrobacterium tumefaciens*, *Clavibacter michiganensis*, *Pythium* spp. and *Streptomyces scabies* (Chellemi and Mirusso 2006; Gelsomino et al. 2006). Devi and Chhetry (2013) have employed soil solarization in Manipur, and their findings showed its efficiency in managing pigeon pea wilt (caused by *Fusarium udum* Butler), when compared other treatments like organic manures (FYM or poultry), or intercropping with maize.

15.4.2 Nutrients Management

Plant nutrients are the chemical elements essential for plant health and play critical role in plant growth and development. Based on their requirement, they are categorized as: macro (required in large quantities) and micronutrients (required in small quantities). These nutrients not only affect the growth and development of crop plants but also influence microbial growth and play important role in disease control (Agrios 2005). There are many factors, which affect the nutrient availability to plants, therefore, it is important to manage through fertilizers in different amounts and forms or via change in the soil environment, as immobilization of nutrients in the rhizosphere and their translocation from root to shoot may cause nutrient deficiency and increasing its susceptibility towards disease like *Fusarium oxysporum f. vasinfectum* can increase P content in leaves, but decreases N, K, Ca and Mg. Some most common examples of interaction between nutrients and plant diseases include *Streptomyces* scab (potato), *Verticillium* wilt, take-all (wheat), stalk rot (corn), clubroot (crucifers). Ahmad et al. (2012) reported the application of NPK against urdbean leaf crinkle virus (ULCV) to be most effective showing 65% of reduced disease severity. Other examples to elucidate the role of nitrogen, phosphorous and potassium include:

15.4.2.1 Nitrogen (N)

Increased application of N elevates the severity of disease caused by obligate pathogens *Puccinia graminis* and *Erysiphe graminis,* whereas it reduces the disease severity of facultative pathogens due to higher application of N, e.g. *Alternaria*, *Fusarium* and *Xanthomonas* spp. (Hoffland et al. 2000).

Besides the rate, type of N source and $NH4^+$:$NO3^-$ ratio is also a key factor to develop disease in plants (Celar 2003; Harrison and Shaw 2001). Higher $NO3^-$ concentration decreases the disease in case of *Fusarium oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani* and *Pythium* spp. Likewise, higher concentration of $NH4^+$ decreases the disease in *Pyricularia*, *Thielaviopsis basicola*, *Sclerotium rolfsii* and *Gibberella zeae* (Agrios 2005; Vidhyasekaran 2004).

15.4.2.2 Phosphorous (P)

Phosphate application aid in reduction of bacterial leaf blight (rice), downy mildew, blue mould, leaf curl virus disease (tobacco), pod and stem blight (soybean), yellow dwarf virus disease (barley), brown stripe disease (sugarcane) and blast disease (rice) (Reuveni et al. 2000; Dordas 2008). However, other studies showed adverse effect of application of P causing disease severity.

15.4.2.3 Potassium (K)

Potassium finds its application in various cellular processes and acts as enzyme activator, aid in protein synthesis, stomatal opening and exchange of CO_2 in leaves (Kumar et al. 2020). Optimum application has been found to be effective in obstructing disease occurrence of bacterial leaf blight, sheath blight, stem rot and seedling rot caused by *Rhizoctonia solani* (Sharma and Duveiller 2004; Sharma et al. 2005). Application of K also aid in decreased severity of helminthosporium leaf blight and consequently improves the grain yield of wheat (Sharma and Duveiller 2004; Sharma et al. 2005).

Other macronutrients, viz. calcium, sulphur and manganese are secondary source of macronutrients that have least information about their role to provide resistant against pathogen.

Like macronutrients, micronutrients also regulate plant metabolism by affecting their phenolics and lignin content. Deficient plants are more susceptible by acting as feeding substrate due to leakage of reducing sugars and amino acids outside the plant cell. For example, *Oidium* spp. infects the zinc deficient plants as mentioned by Li et al. (2016); boron deficient wheat plants are more susceptible to fungal attack than boron sufficient plants as mentioned by Dordas (2008).

15.4.3 Biological Approaches

Worldwide awareness on the hazards associated with the synthesis and use of agrochemicals and their wide scale application on crops have imposed strict regulations which preclude large scale application of pesticides. However, the recurring episodes of pests and pathogens attacks on crops compromise cereal yields. Therefore, the more reliable eco-friendly and sustainable methods are being exploited for management and control of crop diseases. Biological approaches are better than chemical pesticides as they offer a wider range of activity with less incidence of resistance development in pathogens. Biological methods rely on the use of biopesticides that suppress pathogen attack and invasion on host plants.

Biopesticides or biological pesticides are mass-produced, biologically active natural compounds derived from living microorganisms and plants useful to control plant pests and pathogens. These include (1) bio-control agents, (2) plant and microbe based natural substances (biochemical pesticides) and (3) plant-incorporated protectants; PIPs (genetically modified plants) as discussed subsequently under different headings:

15.4.3.1 Bio-Control Agents and their Importance in Plant Disease Management

Bio-control agents (BCAs) are live microorganisms which are antagonistic to pathogens without causing any adverse effects on host plants. Bio-control relies on the use of BCA and/or their formulations to suppress target pathogens in soil and on plant surface. Bio-control agents establish themselves in soil, and plant phytosphere to continuously produce bioactive compounds. These active molecules are in direct contact (close proximity) to the target pathogens, therefore, needed in very limited quantities and often have better efficacy. Microbial interactions with rhizospheric micro-flora and plant roots promote plant growth and improve plant nutrition by releasing insoluble nutrients in an available form. Increased plant nutrition enhances their overall resistance against pathogens and various stress factors (Paulitz and Bélanger 2001).

Plant microbiome has also been the critical factor during plant defense against pathogens. It is the diversity level which determines the pathogenic resistance by microbial community which undergoes shift in microbiome (Trivedi et al. 2012; van Elsas et al. 2012; Podolich et al. 2015; Singh et al. 2019). Mosses own distinctive microbial diversity and their ecology make them potent antagonists (Opelt et al. 2007; Bragina et al. 2015); medicinal plants are another unique example of biodiversity being the rich source of secondary metabolites altering plant microbiome (Köberl et al. 2013); endemic plants have been reported for their antagonistic nature and biodiversity by Zachow et al. (2014); and seed endophytes have been reported among the novel bio-control agents by Berg et al. (2017). These microbiome shifts, although unexplored completely, have been presumed to have direct and indirect interactions with invading pathogen and plant, respectively, thus priming the plant immunity (Berg 2009; Berg et al. 2017).

Microorganisms produce plant hormone-like compounds including auxin, gibberellins, cytokinins, etc. thereby stimulating plant growth; and enzymes involved in degradation of ethylene precursors leading to increased plant growth especially under stress conditions (Lugtenberg and Kamilova 2009; Prasad et al. 2015; Kaushal and Wani 2016). Bio-control agents are less specific towards non-target species and their mode of action is usually different from those of conventional pesticides which suppress resistance development in the pathogens (Table 15.2). They have a wide action spectrum to provide protection and reduce disease incidence in crops. Bio-control agents with antagonistic effects on major rice pathogens are well studied and some BCAs useful in management of rice diseases are given in Table 15.1.

Table 15.1 List of commercially available bio-control agents in the management of plant pathogens

Commercial formulations of bacterial and fungal antagonists for pathogen control and disease management				
Trade name	Bio-control agent	Manufacturer	Target disease/ organism	Method of application
Bacterial Antagonists				
Gallex and Galtrol	*Agrobacterium radiobacter* K84	AgBioChem, Inc.	*A. tumefaciens*	Emulsion spray
Norbac 84C		New BioProducts, Corvalis, OR	*A. tumefaciens*	Root and stem cutting dip or slurry
Nagol	*A. radiobacter* K1026	Bio-Care	*A. tumefaciens*	Emulsion spray
Nogall, Diegall	*A. radiobacter*	Bio-Care Technology Pvt. Ltd., Australia	*A. tumefaciens*	Root dip
Epic	*Bacillus subtilis*	Gustafson, USA Dallas, TX	*Rhizoctonia solani, Fusarium spp., Alternaria, Aspergillus spp.*	Added to slurry, mix with chemical fungicides for seed treatment
GB34	*B. subtilis* GB34	Gustafson, USA	*Rhizoctonia*, *Fusarium*, *Pythium*, *Phytophthora*	Drenching during sowing and transplanting
Kodiac, companion	*B. subtilis* GB03	Growth Products, USA	Rhizoctonia, Aspergillus	Drenching during sowing and transplanting
System 3		Helena Chemicals Co., Memphis TN	Seedling pathogens	Seed treatment
Rhizo-Plus	*B. subtilis* FZB24	KFZB Biotechnik GmBH Germany	*R. solani*, *Fusarium* spp. *Alternaria* spp., *Sclerotinia*, *Streptomyces scabies*	Seed treatment, soil drenching, root dip application
Deny	*Burkholderia cepacia*	Stine Microbial Products	*Rhizoctonia*, *Fusarium* and *Pythium*	Seed treatment, aqueous suspension for drip irrigation
Intercept	*Pseudomonas cepacia*	Soil Technologies Fairfield, IA	*R. solani*, *Fusarium spp*. *Pythium spp*.	Seed treatment, foliar spray, soil application
Biosave-100 Biosave-	*P. syringae* ESC-10	EcoScience Corp, Orlando Florida	*Botrytis cinerea, Penicillium*	Pellets post-harvest to fruits as drench dip or spray

(continued)

Table 15.1 (continued)

Commercial formulations of bacterial and fungal antagonists for pathogen control and disease management				
Trade name	Bio-control agent	Manufacturer	Target disease/ organism	Method of application
1000 Biosave-110	*P. syringae* ESC-11		*spp., Mucor piriformis, Geotrichum candidum*	
Frostban, Blightban A506	*P. fluorescens* strain A506	Plant Health Technologies	Fire blight, frost damage, bunch rot	Spray at flowering and fruiting
Bio-jet, spot less	*P. aureofaciens* strain TX-1	Eco Soil Systems	*Pythium*, *R. solani*	Overhead irrigation
Fungal Antagonists				
AQ 10	*Ampelomyces quisqualis* M-10	Ecogen, USA	Powdery mildew	Spray
Contans	*Coniothyrium minitans*	Prophyta Biologischer Pflanzenschultz	*Sclerotinia sclerotiorum* and *S. minitans*	Spray
Biofox C	*Fusarium oxysporum* (non-pathogenic)	SIAPA, Bologna, Italy	*F. oxysporum, F. moniliforme*	Seed treatment or soil incorporation
GiloGard more recent SoilGard	*Gliocladium virens* GL-21	Thermo Trilogy, Columbia, MD	Damping off, root rot pathogens, *R. Solani*, *Pythium spp.*	Granules incorporated in soil
Prima stop soil guard	*G. catenulatum* JI446	Kemira Agro Oy, Finland	Soil-borne pathogens	Seed treatment, foliar spray, soil application
Bioact or Paecil	*Paecilomyces lilacinus*	Technological Innovation Corporation Pvt Ltd	Various nematodes	Drenching
Mycostop	*Streptomycine griseoviridis K61*	Kemira Agro Oy, Finland	Soil-borne pathogens	Drenching, spraying or through irrigation
Bio-Fungus	*Trichoderma sp.*	De Cuester, Belgium	*Sclerotinia, Phytophthora, R. solani, Pythium spp., Fusarium, Verticillium*	Seed treatment, foliar spray, soil application
Monitor SD	*Trichoderma sp.*	M/s Agriland Biotech Pvt Ltd., Baroda, India	Soil-borne plant pathogens	Seed dressing
Monitor WP				Soil application

(continued)

Table 15.1 (continued)

Commercial formulations of bacterial and fungal antagonists for pathogen control and disease management				
Trade name	Bio-control agent	Manufacturer	Target disease/ organism	Method of application
Trichodex	*Trichoderma harzianum* T-39	BioWorks, Inc., USA	*B. cinerea*	Spray
Root Shield or BioTrek T-22G	*T. harzianum* T-22 G	BioWorks, Inc., USA	Soil-borne pathogens	Granules mixed with soil or potting medium, powder mixed with water and added as soil drench
Root Pro	*T. harzianum*	Mycontrol Ltd., Israel	*R. solani, Fusarium spp. Alternaria spp., Sclerotium rolfsii*	Mix with growing media at the time of seedling and transplanting
Trichoderma 2000	*Trichoderma sp.*	Mycontrol Ltd., Israel	Soil-borne pathogens	Seed treatment, tuber or seed dressing, soil drenching
Trieco	*T. viride*	Ecosense Labs Pvt. Ltd., Mumbai, India	Soil-borne pathogens	Seed treatment, tuber or seed dressing, soil drenching
T34 Bio-control	*T. asperellum* T-34	Fargro Ltd., Littlehamptom, West Sussex, UK	*F. oxysporum f.sp. dianthi*	Drenching during sowing and transplanting, root dip of cuttings
Binab T	*T. harzianum* and *T. polysporum*	Bio-innovation AB, Sweden Henry Doubleday Research Association UK	Wilt, take all, root rot	Spray, mixing with potting substrate, as paste painting on tree wound

Bio-control agents *Trichoderma viride* and *Pseudomonas fluorescence* are also useful in bio-priming, seed coating and seedling root dip to control pathogens (Gad et al. 2014; Ananthi et al. 2017). *Trichoderma* and *Pseudomonas* application has several beneficial effects which ultimately improve plant health through increased root and plant growth (Chandra Nayaka et al. 2009; Moeinzadeh et al. 2010). Gad et al. (2014) found inhibition of the rice fungal pathogen *R. oryzae-sativa* along with significant improvement in growth parameters in presence of *P. aeruginosa*. An isolate of *Ulocladium oudemansii* inhibits the growth of *B. cinerea* responsible for botrytis bunch rot in grapes. Its formulation has been developed and commercialized under trade name BOTRY-Zen® in New Zealand (Reglinski et al. 2010; Wurms

Table 15.2 PGPR showing bio-control activity against the plant pathogens

PGPR	Crop	Disease	Target Pathogens	References
Streptomyces sp. KH-614	Rice	Blast	*Pyricularia oryzae*	Rhee (2003)
S. vinaceusdrappus	Rice	Blast	*Curvularia oryzae, Pyricularia oryzae, Bipolaris oryzae, Fusarium oxysporum*	Ningthoujam et al. (2009)
S. aurantiogriseus VSMGT1014	Various	Sheath blight	*Rhizoctonia solani*	Harikrishnan et al. (2014)
Pseudomonas fluorescens	Various	Many	Many (broad spectrum)	David et al. (2018)
Bacillus amyloliquefaciens	Rice	Sheath blight	*M. oryzae and R. solani, and F. graminearum and Bot. cinerea*	Kakar et al. (2018)
Bacillus amyloliquefaciens	Rice	Rice blast	*Magnaporthe oryzae*	Amruta et al. (2018)
Pseudomonas aeruginosa BRp3	Rice	Leaf blight	*Xanthomonas oryzae pv. oryzae (Xoo)*	Yasmin et al. (2017)
Bacillus sp.	Maize	Seed aflatoxin production	*Aspergillus flavus*	Chalivendra et al. (2018)
Bacillus subtilis	Many	Rice blast and others	*Magnaporthe oryzae*	Taguchi et al. (2003)
Bacillus cereus, Brevibacterium Laterosporus, Pseudomonas fluorescens, Serratia marcescens	Sorghum	Root rot	*Pythium ultimum*	Idris et al. (2008)
S. spororaveus RDS28	Various	Collar or root rot, stalk rot, leaf spots, and grey mould rot or botrytis blight	*Rhizoctonia solani, Fusarium solani, Fusarium verticillioides, Alternaria alternata, Botrytis cinerea*	Khair (2011)

et al. 2011). Likewise several bacterial and fungal BCAs useful in the management of various plant pathogens are commercially available. Some BCAs of commercial importance with their trade names are given in Table 15.1.

15.4.3.2 Mechanisms of Action

Bio-control agents deploy several direct and indirect mechanisms to suppress pathogen growth and their activity in the rhizosphere. Direct bio-control mechanisms are parasitism, production of antibiotics and antimicrobial compounds, lytic enzymes (like β-1,3-glucanase and chitinases) and unwanted waste products (like ammonia carbon dioxide and HCN). Direct antagonism requires physical contact between the

Table 15.3 List of antimicrobial compounds produced by major BCAs effective against specific plant pathogens

Antimicrobial compounds	Bio-control agent	Target pathogen/disease
Pseudomonads		
Acetylphloroglucinols (2,4-diacetylphloroglucinol)	*Pseudomonas fluorescens* *P. aurantiaca*	*Pythium ultimum*/damping off *Gaeumannomyces graminis* var. *Tritici/Take all* *Thielaviopsis basicola* *Fusarium oxysporum*
Oomycin A	*P. fluorescens*	*Pythium ultimum*
Phenazine-1-carboxylic acid	*P. fluorescens* *P. aureofaciens*	*Gaeumannomyces graminis* var. *Tritici/Take all*
Phenazine-1-carboxamide	*P. chlororaphis*	*Fusarium oxysporum* f. sp. *radicis-lycopersici*
Pyocyanin	*P. aeruginosa*	*Septoria tritici*
Anthranilate	*P. aeruginosa*	*Fusarium oxysporum*f. sp. *ciceris* *Pythium splendens*
Pyoluteorin	*P. fluorescens*	*Pythium ultimum*/damping off *Rhizoctonia solani* damping off
Pyrrolnitrin	*P. fluorescens* *P. cepacia* *P. fluorescens*	*Rhizoctonia solani* *Aphanomyces cochlioides* *Pyrenophora tritici-repentis*
Pyoverdin	*P. fluorescens* *P.putida*	*Pythium ultimum* *Fusarium oxysporum*
Pyochelin	*P. aeruginosa*	*Pythium splendens*
Cyclic lipopeptides (e.g. Viscosinamide)	*P. fluorescens* (*Burkholderia cepacia*)	*Rhizoctonia solani* *Pythium ultimum*
Hydrogen cyanide Ammonia	*P. fluorescens* *Enterobacter* spp.	*Thielaviopsis basicola*
Bacillus sp.		
Bacillomycin D	*Bacillus subtilis AU195*	*Aspergillus flavus/aflatoxin contamination*
Bacillomycin, Fengycin	*Bacillus amyloliquefaciens* FZB42	*Fusarium oxysporum*/wilt
Fengicin	*B. subtilis* *B. amyloliquefaciens*	*Podosphaera fusca* *Botrytis cinerea* *Fusarium graminearum* *Sclerotinia sclerotiorum*
Plipastatin	*B. cereus* *B. thuringiensis*	
Iturin	*B. amyloliquefaciens,* *B. subtilis* *B. megaterium*	*Rhizoctonia solani* *Pythium aphanidermatum* *Podosphaera fusca* *Xanthomonas oryzae pv. Oryzae*

(continued)

Table 15.3 (continued)

Antimicrobial compounds	Bio-control agent	Target pathogen/disease	
Bacillaene		*Bacillus amyloliquefaciens* *B. subtilis*	*Erwinia amylovora* *E. caratovora*
Difficidin			*E. amylovora* *Pectobacterium carotovorum* *Xanthomonas oryzae*
Macrolactins			
Mycosubtilin		*Bacillus subtilis*	*Fusarium oxysporum* *Botrytis cinerea* *Pythium aphanidermatum/damping off* *Pichia pastoris*
Marihysin A		*Bacillus marinus*	*Alternaria solani* *F. oxysporum* *F. graminearum* *Verticillium albo-atrum* *Sclerotium sp.* *Penicillium sp.* *Rhizoctonia solani* *Colletotrichum sp.*
Surfactin		*B. coagulans* *B. subtilis* *B. polyfermenticus* *B. megaterium* *B. amyloliquefaciens* *B. pumilus* *B. licheniformis*	*R. solani* *Helminthosporium maydis* *F. oxysporium* *Botrytis cinereapers* *Gibberella saubinetii* *Colletotrichum gossypii* *C. capsici* *Physalospora piricola* *Sclerotinia sclerotiorum*
Polypeptin		*Bacillus circulans* *Paenibacillus ehimensis*	*Fusarium oxysporum* *F. graminearum* *F. moniliforme* *Rhizoctonia solani* *Colletotrichum lini*
Polymyxin		*Paenibacillus polymyxa*	*Erwinia amylovora* *Pectobacterium carotovorum*
Pyrrolnitrin pseudane		*Burkholderia cepacia*	*Rhizoctonia solani* and Pyricularia oryzae/damping off and Rice blast
Zwittermicin A		*Bacillus cereus* UW85	*Phytophthora medicaginis* and *P. aphanidermatum*/damping off
Others			
Agrocin- 84		*Agrobacterium radiobacter*	*A. tumefaciens*/crown gall
Gliotoxin		*Trichoderma virens*	*Rhizoctonia solani*/root rot
Xanthobaccin A		*Lysobacter* sp. strain K88	*Aphanomyces cochlioides*/damping off
Herbicolin		*Pantoea agglomerans* C9–1	*Erwinia amylovora*/fire blight

(continued)

Table 15.3 (continued)

Antimicrobial compounds	Bio-control agent	Target pathogen/disease	
Fusaricidin		*Paenibacillus polymyxa*	*Fusarium oxysporum* *Phytophthora sp.* *Aspergillus sp.*
Trichoderma			
Gliotoxin		*Trichoderma virens*	*Rhizoctonia solani*/root rot

pathogen and BCAs like in case of hyper-parasitism shown by obligate parasites of a plant pathogen. Indirect mechanisms of bio-control agents feature surface colonization and competition for space and nutrients (Labuschagne et al. 2010; Martínez-Viveros et al. 2010; Piromyou et al. 2011; Shafi et al. 2017), production and secretion of several antimicrobial compounds and stimulation of plant host defense pathways by induced systemic resistance (ISR) as shown in Fig. 15.2.

Some BCAs use two or more methods at a time so that lines between biological control mechanisms usually appear blurred. The common mechanisms of biological control of a wide range of pathogens are:

15.4.3.3 Production of Antibiotics, Lytic Enzymes and By-Products of Microbial Life

Bio-control agents release antimicrobial compounds such as antibiotics, lytic enzymes, secondary metabolites and unwanted waste products which directly act upon pathogens to suppress their growth and the diseases they cause. Some common antimicrobial compounds produced by BCAs effective against specific plant pathogens are given in Table 15.3.

Genome study of *B. amyloliquefaciens* shows presence of several genes associated with antimicrobial peptides and cyclic lipopeptides such as bacilysin, bacillibactin, macrolactin, bacillaene, fengycin, difficidin, non-ribosomal peptide synthetase, bacyllomicin, lantibiotic subtilin and plipastatin with inhibitory effects on different pathogens (Kakar et al. 2018). Some bio-control strains inhibit pathogens with the production of multiple antibiotics, like *Bacillus cereus* strain UW85 has been found to produce both zwittermycin and kanosamine. Some BCAs like *Pseudomonas putida* strain WCS358r have been genetically engineered to produce both phenazine and 2,4-diacetyl-phloroglucinol to improve its capacity of disease suppression in plants. Some BCAs secrete lytic enzymes and other microbial by-products which interfere with pathogen growth and/or activities. Lytic enzymes hydrolyse polymeric compounds, including chitin, proteins, cellulose and hemicelluloses which are important components of cell wall which directly restrict the infectious activities of plant pathogens. Other microbial by-products such as hydrogen cyanide (HCN) also contribute to pathogen suppression, by blocking the cytochrome oxidase pathway and show high toxicity for all aerobic microorganisms

Table 15.4 Formulations based on the active components of plant extracts

Botanicals	Formulation type	Carrier/s used	Technique	Application	Reference
Sulphoraphane	Sulphoraphane coacervates	Oppositely charged polymers of gelatin/gum arabic and gelatin/pectin	Micro-encapsulation by complex coacervation	–	García-Saldaña et al. (2016)
Coffee leaf extract	Powder	Maltodextrin	Spray-drying	–	Corrêa et al. (2016)
Several aromatics	Bio-compatible micro-particles	Cellulose derivatives (hydroxyl-propyl-methylcellulose-phthalate and ethyl cellulose)	Spray-drying	–	Cortesi et al. (2017)
Rosemary essential oil	Micro-particles with surface depressions	Maltodextrin & modified starch in 1:1 ratio	Micro-encapsulation by spray drying	–	de Barros Fernandes et al. (2016)
Essential oil from *Peumus boldus* (boldo)	Slow release micro-capsules	Gelatin/gum arabic	Micro-encapsulation by complex coacervation	–	Girardi et al. (2016)
Tea tree and palmarosa essential oils	Micro-emulsions	Aqueous suspension of aloe polysaccharides	–	–	Luiz et al. (2017)
Mixture of Azadirachta indica (neem) seed oil and *Cymbopogon nardus* (Citronella) oil	Nano-oil droplets suspended in emulsion	Surfactants	Encapsulation in oil-in-water nano emulsions using high energy methods	–	Ali et al. (2017)
Leaf extract of *Stevia rebaudiana*	Alginate beads	Calcium alginate	Encapsulation by extrusion	–	Arriola et al. (2016)

(continued)

Table 15.4 (continued)

Botanicals	Formulation type	Carrier/s used	Technique	Application	Reference
Eugenol	Controlled release micro-capsules of β-cyclodextrin -eugenol inclusion complex	β-cyclodextrin	Molecular inclusion of eugenol into the lipophilic cavity of β-cyclodextrin	Antifungal against *Peronophythora litchii* Control postharvest fungal attack on fresh litchi fruits	Gong et al. (2016)
Origanum vulgare (Oregano) essential oil	Cellulose acetate based active films	Cellulose acetate and montmorillonite clay	Dispersion of organophilic molecule (montmorillonite clay) in cellulose acetate matrix in presence of plasticizer (oregano essential oil)	Antifungal against the phyto-pathogens *Alternaria alternata*, *Geotrichum candidum*, and *Rhizopus stolonifer*	Pola et al. (2016)
Salicylic acid	Edible film	Cassava starch, tween and glycerol	–	Post-harvest preservation of *Carica papaya*	Castro et al. (2017)
Essential oil of *Eugenia caryophyllata* (clove) and *Lippia berlandieri* (Mexican oregano)	β-cyclodextrin-essential oil micro-capsules	β-cyclodextrin	Micro-encapsulation	Atifungal against *Fusarium oxysporum*	Estrada-Cano et al. (2017)
Phenylpropanoids	Solid complexes loaded with phenylpropanoids	Cyclodextrins	Micro-encapsulation	Increase in solubility and photostability of phenylpropanoids with antifungal activity against *Fusarium oxysporum* and *Botrytis cinerea*	Kfoury et al. (2016)
Thymol essential oil from *Lippia sidoides*	Polycaprolactone (PCL)-coated nano-capsules	Polycaprolactone Kolliphor P 188® and ethyl laurate	Emulsion-diffusion method	Increased stability	Pinto et al. (2016)

Methyl-salicylate	Nano-capsules	Chitosan	Nano-encapsulation	Antifungal against *Aspergillus flavus* and inhibit aflatoxin B_1 (AFB_1) action	Kujur et al. (2019)
Essential oils from *Zanthoxylum riedelianum* fruit	Nanospheres	Poly-ε-caprolactone	Nano-encapsulation	Increase stability with insecticidal and deterrent activity against whitefly (*Bemisia tabaci*)	Pereira et al. (2018)

Table 15.5 Antimicrobial nano-particles and carbon nano-materials in management of plant pathogens

Nano-particles (NPs)/ nano-materials	Comments	Target pathogens/ diseases	Reference
Biosynthesized silver-NPs	• Extracellular biosynthesis of silver nano-particles (Ag-NPs) using culture supernatant of an agriculturally important bacterium, *Serratia* sp. BHU-S4 • Spherical and crystalline bsAgNPs with size range of ~10 to 20 nm exhibit strong antifungal activity by inhibition of conidial germination • Ag-NP increase lignification in plant vascular tissues forming a physical barrier that provides disease resistance to plants against pathogen attack • Bio-fabricated Ag-NPs are effective in the management of spot blotch disease in wheat	*Bipolaris sorokiniana*/the spot blotch disease in wheat	Mishra et al. (2014)
Cu-chitosan NP	• Antifungal Cu-chitosan NPs are most effective at 0.1% concentration in in vitro condition • At the same concentration, Cu-chitosan NPs show maximum inhibition in rate of spore germination of *A. alternata* and mycelial growth inhibition of *M. phaseolina* • Chitosan and Cu-chitosan NPs with increased stability in aqueous solution have tremendous potential for field screening towards crop protection	*Alternaria alternata* *Macrophomina phaseolina* *Rhizoctonia solani*	Saharan et al. (2013)
Colloidal silver NPs	• Spherical and colloidal Ag-NPs size <20 nm synthesized by reduction of silver nitrate solutions	*Colletotrichum gloesporioides*/ anthracnose in a	Aguilar-Méndez et al. (2011)

(continued)

Table 15.5 (continued)

Nano-particles (NPs)/ nano-materials	Comments	Target pathogens/ diseases	Reference
	with glucose in the presence of gelatin as the capping agent • Gelatin interacts with NPs through the amide and hydroxyl group that prevents agglomeration of Ag-NPs • Dose-dependent antifungal activity to reduce the growth of *C. gloesporioides*	wide range of fruits	
Biosynthesized Ag-NPs	• Extracellular biosynthesis of silver nano-particles (AgNPs) using cell free culture supernatant of *Bacillus* sp. strain GP-23 • The biosynthesized silver nano-particles with spherical shape in the range of 7–21 nm exhibit antifungal activity towards *Fusarium oxysporum*	*Fusarium oxysporum*	Gopinath and Velusamy (2013)
Myco-silver NP	• Ag-NPs of different morphological shapes (spherical, cylindrical, agglomerated) with an average size of 2–50 nm. Synthesized from fungal endophyte of *Solanum nigrum* • Myco-Ag-NP exhibit broad-spectrum antifungal activity against wide range of fungal phyto-pathogens by inhibiting the radial growth	*Fusarium graminearum*, *Fusarium udum*, *Rhizoctonia solani* *Aspergillus niger*	Akther and Hemalatha (2019)
Silver NP	• Ag-NP with an average size of 4–8 nm interfere with microbial absorption retarded fungal growth in a dose-dependent manner; • Antifungal Ag-NP inhibit conidial germination and cause damage to the surface of the fungal hyphae that cause release of internal cellular materials resulting in shrinkage of the hyphae	*Raffaelea* sp./oak wilt	Kim et al. (2009)

(continued)

Table 15.5 (continued)

Nano-particles (NPs)/ nano-materials	Comments	Target pathogens/ diseases	Reference
Silica- silver NPs	• Chemically synthesized silica-silver NPs with particle size 1–5 nm • Show antifungal activity against phytopathogenic fungi at lower concentrations with varied degrees while beneficial and or plant pathogenic bacteria are not affected at such low concentrations	*Pythium ultimum* *Magnaporthe grisea* *Colletotrichum gloeosporioides* *Botrytis cinerea* *Rhizoctonia solani*	Park et al. (2006)
Several carbon nano-materials (NMs) (single-walled and multi-wall carbon nano-tubes, graphene oxide, reduced graphene oxide, fullerene and activated carbon)	• Carbon NMs show different degrees of antifungal activity (single wall carbon nano-tubes with strongest activity, followed by multi-wall carbon nano-tubes, graphene oxide and reduced graphene oxide) • Inhibit plant pathogenic fungi by targeting the fungal spores in three steps (1) surface deposition, (2) inhibit water uptake and (3) induce plasmolysis	*Fusarium graminearum* *F. poae*	Wang et al. (2014a, b)
Graphene oxide	• Highly efficient in inactivating the bacteria • Produce considerable changes in the cell membranes caused by the extremely sharp edges of graphene oxide and generation of reactive oxygen species which are fatal for bacteria	*Xanthomonas oryzae pv. oryzae*	Chen et al. (2013)
Several nano-particles (multi-walled carbon nano-tubes, fullerene, and reduced graphene oxide, copper oxide (CuO), ferric oxide (Fe_2O_3), and titanium oxides (TiO_2) NPs)	• Show various degrees of inhibition against *Botrytis cinerea* • Antifungal NPs useful to prevent *B. cinerea* infections in plants during the growth and post-harvest protection of rose and other flowers	*Botrytis cinerea*	Hao et al. (2017)

(continued)

Table 15.5 (continued)

Nano-particles (NPs)/ nano-materials	Comments	Target pathogens/ diseases	Reference
Reduced grapheme oxide	• Sharp edged nano-sheets of reduced graphene oxide • Antifungal activity by inhibiting the mycelial growth of the fungi	*Aspergillus niger* *A. oryzae* *Fusarium oxysporum*	Sawangphruk et al. (2012)
Nano-silica and ZnO NP	• Effective against *Cercospora beticola* • Useful as alternatives fungicides in controlling the leaf spot of sugar-beet	*Cercospora beticola/leaf spot on sugar beet*	Derbalah et al. (2013)
Zinc sulphate NP	• Nano dimensional zinc sulphate particles (size 100 nm) act as a photo catalyst to control and destroy plant pathogenic bacteria • Exhibit antimicrobial activity with formation of inhibition zones and extended lag phase in growth curve of bacterial cultures in the in-vitro conditions	*Xanthomonas campestris,* *X. malvacearum* *Pseudomonas solanacearum* *P. syringae*	Indhumathy and Mala (2013)
ZnO NPs	• Synthetic ZnO NP suspensions with particle size of 70 ± 15 nm • ZnO NPs influence cellular functions in B. *cinerea* which cause formation of unusual bulges on the surface of hyphae. The structural deformities in fungal hyphae inhibit the growth of *B. cinerea* • NPs distort and damage the conidia and prevent the development of conidiophores and formation of conidia in *P. expansum* which eventually led to the death of fungal hyphae	*B. cinerea* *Penicillium expansum*	He et al. (2011)
Sulphur NPs	• Small sized particles of ~35 nm are very effective in preventing the fungal growth • Fungicidal effect is	*Fusarium solani*/ early blight and *Fusarium* wilt diseases *Venturia*	Rao and Paria (2013)

(continued)

Table 15.5 (continued)

Nano-particles (NPs)/ nano-materials	Comments	Target pathogens/ diseases	Reference
	mainly because of the deposition of Sulphur NPs on the cell wall and subsequent damage of the cell wall	*inaequalis*/apple scab disease	
Nano-sulphur	• Spherical Sulphur NPs with an average particle size in the range of 50–80 nm are more efficacious than its elemental form • Nanosulphur is a potent fungicide against food-borne fungal pathogen Aspergillus Niger	*Aspergillus niger*	Choudhury et al. (2010)
Biosynthesized Ag-NPs	• Reduction of silver nitrate using *Acalypha indica* leaf extract as reducing agents • Biosynthesized silver nano-particles of size 35 nm inhibit the growth of several plant pathogenic fungi	*Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* *Curvularia lunata*	Krishnaraj et al. (2012)

even at its picomolar concentrations (Sahu et al. 2018). *P. fluorescens* CHA0 produces HCN to suppress black rot of tobacco caused by *Thielaviopsis basicola*.

15.4.3.4 Competition for Space and Nutrients

BCAs rapidly proliferate and form intimate associations with plant surfaces. They prevent pathogen establishment and provide protection to infection sites from pathogen attack. Besides, BCAs produce metabolites that suppress pathogen growth. Soil-borne fungal pathogens are more susceptible to competition due to their infection spread through mycelial contact (species of *Fusarium* and *Pythium*).

Iron (Fe) is a co-factor in many cellular enzymes, and a structural and functional component of haeme or non-haeme proteins, cytochrom*es* and iron sulphur clusters (Fe/S) located in cell membranes needed during electron transport that is essential to generate energy for the growth of microbes. BCAs produce low molecular weight (500–1000 Da) iron-chelating ligands called siderophores that bind with ferric iron with high affinity to form tight and stable complexes that reduce availability and prevent Fe^{3+} uptake by pathogenic bacteria and fungi from the soil

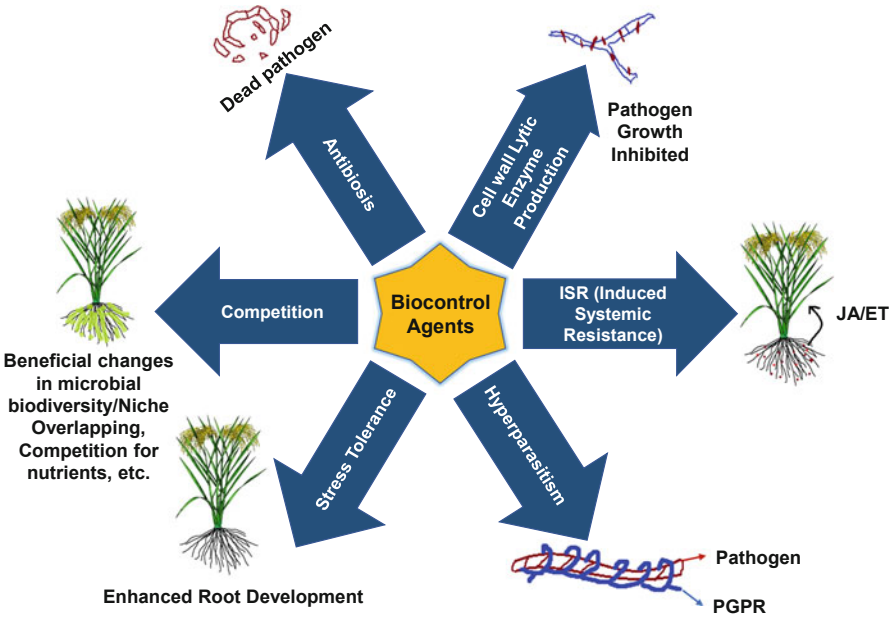

Fig. 15.2 Mechanism of bio-control agents for enhanced productivity in rice

micro-environment. Based on structural moieties, siderophores are either catecholates produced only by bacteria or hydroxymates produced by both yeasts and bacteria (Saraf et al. 2014). Siderophore producing microbial antagonists compete with pathogens for iron resulting in impediment to their growth, spore germination and pathogenesis (Das et al. 2007). Kloepper et al. (1980) were the first to demonstrate siderophore production as the bio-control mechanism against *Erwinia carotovora* by plant growth promoting bacteria *Pseudomonas fluorescens*. Microbial antagonists like fluorescent *Pseudomonas* spp. produce siderophores such as pyocyanin, pyoluteorin, pyrrolnitrin, pyoverdin, pyochelin to inhibit wide range of fungal pathogens (Table 15.3). Siderophore producing *Pseudomonas* sp. enhances activity of plant defense enzymes to provide disease resistance against bacterial leaf blight in rice plants (Yasmin et al. 2016). Similar to bacterial antagonists some yeasts, e.g. *Metschnikowia pulcherrima* and *M. fructicola* produce siderophore pulcherrimin, with the potential to inhibit mycelial growth and conidial germination of pathogen *B. cinerea, A. alternata and Penicillium expansum.* Likewise, *Rhodotorula glutinis* produce rhodotorulic acid, a dihy*droxamate-contai*ning siderophore to prevent growth of *P. expansum.*

15.4.3.5 Hyper-Parasitism

H*yper-para*siti*sm is a* parasitic interaction where parasites are themselves infected by other parasites. They belong to four major classes: obligate bacterial pathogens, predators, hypoviruses, and facultative parasites. For example, *Pasteuria penetrans*,

an obligate bacterial pathogen is known to attack root-knot nematodes. Hypovirulence is a phenomenon, where hyperparasites infect pathogens to limit both the severity and transmission of diseases. A classical example is the mycovirus CHV1 that infects and reduces pathogen growth rate and disease-producing capacity of *Cryphonectria parasitica*, a fungus causing chestnut blight. Fungi such as *Acremonium alternatum, Acrodontium crateriforme, Ampelomyces quisqualis, Cladosporium oxysporum* and *Gliocladium virens* are some of the myco-parasites of plant pathogens. Myco-parasites attack fungal sclerotia *(*e.g. *Coniothyrium minitans*) while others target living hyphae (e.g. *Pythium oligandrum*). Myco-hyperparasites, e.g. *Paecilomyces lilacinus* and *Dactylella oviparasitica* have been reported for their attack on plant-pathogenic nematodes at different stages of their life cycles. Hyperparasites like *Trichoderma* spp. exhibit predatory behaviour in nutrient limited conditions. They destroy cell walls of many different pathogenic fungi and penetrate to obtain nutrition.

15.4.3.6 Induced Systemic Resistance

BCAs produce potential elicitors of plant defense mechanisms and enhance resistance against subsequent infection by pathogens. They cause physiological and biochemical changes in plants, such as activation of defense-related antioxidant enzymes such as chalcone synthase, phenylalanine ammonia lyase, peroxidase, superoxide dismutase; cell wall degradative enzymes like chitinase, β-1,3-glucanase and synthesis of antimicrobial compounds to secure plants against an extensive range of fungal, bacterial and viral pathogens (Rais et al. 2017). Bio-control agents induce accumulation of pathogenesis-related proteins phenolics, callose, lignin and phytoalexins, which cause lysis of pathogen invading cells and reinforce cell wall structure or induce localized cell death to resist infections. Host resistance can be induced locally and/or systemically depending on the type, source and amount of stimuli. Several plant-growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* sp. and *Trichoderma* sp. show strong induction of plant host defense majorly in two forms, viz. Induced systemic resistance (ISR) and Systemic acquired resistance (SAR) (Fig. 15.3); significantly involved in controlling pathogenic diseases like anthracnose (*Colletotrichum lagenarium*), angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*) and bacterial wilt (*Erwinia tracheiphila*). This elevated plant defense is activated by beneficial root microbiome genera, such as *Bacillus*, *Pseudomonas*, *Trichoderma*, etc., which elicit effective cellular responses to resist pathogen attacks (Pascale et al. 2020). *Bacillus amyloliquefaciens* enhances the immune response in rice against sheath blight caused by *Rhizoctonia solani* and *Bacillus* spp. elicit ISR against *Pyricularia oryzae* infection by modulating various physiological, metabolic and molecular functions in rice (Srivastava et al. 2016; Rais et al. 2017). PGPR mediated ISR resembles pathogen induced SAR which enables the plant to acquire resistance not only against the inducing pathogen but also to broad range of other pathogens (Hammerschmidt 2009; Yi et al. 2013). This resistance characteristic response finds biotechnological application for management of pathogenic diseases in crops under field conditions.

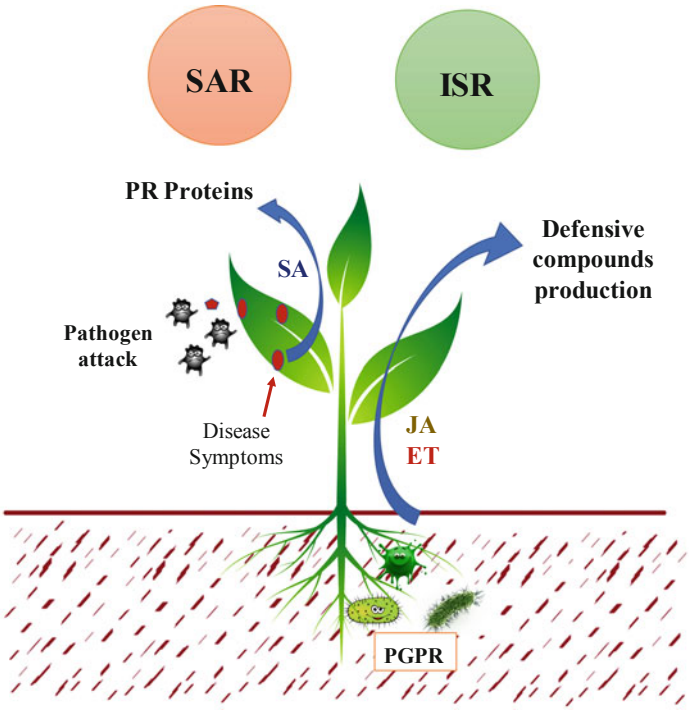

Fig. 15.3 ISR and SAR induced during plant–PGPR–pathogen interaction

Sometimes two or more bio-control agents with antagonistic activity against same or different pathogens or with different inhibitory potential show increased consistency which is also useful for better performance in plant disease control. Combinations of bio-control agents have a broad spectrum of activity, with increased efficacy and reliability that better help in pathogen control (Chaudhry et al. 2012). Microbial consortium comprising of antagonistic bacteria *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bacillus amyloliquefaciens* and one fungi *Trichoderma citrinoviride* with bio-control attributes such as ammonia, siderophore, cell wall-degrading enzymes (like β-1,3 glucanase, chitinase and cellulase) production are more effective against phyto-pathogens like *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* (Thakkar and Saraf 2015. Combination of *B. firmus* and *P. aeruginosa* is more effective against control of both sheath and bacterial leaf blight diseases in rice (Suryadi et al. 2013). Combined applications of *P. fluorescens* and *T. viride* reduce the sheath blight incidence caused by *Rhizoctonia solani* Kühn and increase grain yield in rice (Mathivanan et al. 2005). Combinations of bio-control agents like *Pseudomonas aeruginosa*, *Burkholderia* sp. and *Bacillus* sp. are used together to inhibit leaf curl virus and leaf curl disease incidence in cotton plants (Ramzan et al. 2016).

Fourteen microbial pesticide and 478 products based on their formulations are registered in India since 2009. Out of these, 184 products comprise *Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens* and 18 products of *Bacillus thuringiensis* var. *kurstaki*, 62 products belong to *Beauveria bassiana*, 51 products of *Verticillium lecanii*, 13 products of *Metarhizium anisopliae*, 18 products belong to nuclear polyhedrosis virus (NPV) of *Helicoverpa armigera*, and three products of NPV of *Spodoptera litura*, which are registered for the management of plant pathogens and insect pests. The microbial pesticides based on BCAs are target specific, release no harmful residues and require very less investment in the large scale production. BCAs and their microbial formulation are easily available which can be used repeatedly without any harmful effects on human and animal health and environment. Their broad host-range mechanisms to compete for nutrients and space, direct antagonism for plant pathogen and host plant immunization make BCAs superior over chemical pesticides and other available phytosanitary products. Moreover, combinatorial effect of bio-control agents both on plant growth-promotion and pathogen inhibition offers an advantage to serve the role of a bio-pesticide as well as a bio-fertilizer.

15.4.3.7 Constrains in Application of Bio-Control Agents

The commercialization of BCAs and development of microbial pesticides are strictly regulated which prevent a large number of microbial antagonists elsewhere reported as effective against pathogens, from application in plant systems under natural conditions. Microbial pesticides and their formulations are registered before they are released as product in the market (Chattopadhyay et al. 2017; Mishra et al. 2018). The microbial registration process is lengthy involving strenuous exercise to fulfil the terms and guidelines of Central Insecticides Board in India. Besides laboratory generated data assessment of several features of BCAs, such as their establishment and survival, dispersal, genetic stability and horizontal gene transfer, effects on the resident microbiota and fauna, availability and applicability of safe and an effective containment system and post-release field monitoring are needed. In most of the cases it is mandatory to determine the environmental impacts of BCAs and include these with the bio-safety data and the laboratory predictions as a part of an application for registration and commercial development of not only genetically modified BCAs but also the natural microbial antagonists. To obtain this data on microbial antagonists lengthy experimental procedures are needed which often require well-designed and sophisticated ecological monitoring experimental set-ups over a large scale that need initial investments which being time taking, seem impractical in many situations (Bonaterra et al. 2012; Chattopadhyay et al. 2017). The data generated needs to be validated in living systems through successive field trials in many different conditions. Sometimes it is not possible to get the required data in the lack of sophisticated techniques and experimentation facilities as a result only few BCAs get registered for use as microbial pesticides and many microbial strains with promising antagonistic activity are left. The bio-safety testing of any BCA needs specific analysis at strain level and analysis of the autochthonous microbial population to estimate the qualitative and quantitative alterations in the microbial

community structure caused by the release of BCAs. The methods related with the analysis of BCA at strain level are not available in many BCAs and microbial diversity assessments with complex molecular methods are difficult to perform in many environments. This also limits the application of microbiome engineering which acts as the biomarker of modified plant microbial community with its implication in biological control, as the registration of potent strains is long-term process. Before filing application for registration of any BCAs it is necessary to determine the pathogenicity, virulence, allergenicity of microbe. Also, the generation of toxicological data against mammals and eco-toxicity data for non-targets (fishes, birds, earthworms, honeybees and silkworm) is mandatory. In the race of commercialization, the data with technical formulation of every strain has to be generated. It is also requisite to generate safety data against natural enemies along with bio-efficacy and phyto-toxicity data of formulation on the target crops. Besides, these effects of microbial antagonists need to be evaluated for the clinical opportunistic infections on non-target organisms and/or effects of certain secondary metabolites of concern to guarantee safety to consumers and handlers of the microbial pesticides. For these long-term experiments are performed in mammals and toxicological data thus generated is complicated.

The effective biological control with microbial pesticides is only possible when BCAs are very active and possess features such as high initial cell numbers, increased rhizosphere competence for earlier establishment with production of more antimicrobial/inhibitory compounds. These provide with an additional competitive advantage and are a prerequisite for effective action against the pest or pathogen. The microbial antagonists need to adapt to the fluctuating environmental conditions and be able to survive and grow in the natural conditions to get desirable control on pathogens and prevent disease spread in plants. However, microbial pesticides being living entities are influenced by various biotic and abiotic factors and often show variable efficacy in the natural systems. Their highly specific nature against the target diseases and pathogens sometimes requires multiple microbial pesticides to be used at a time to manage different pests and pathogens. Some BCAs have specific and complex requirements for their growth and activity against pathogens which need to be provided to enhance the growth of BCAs to ensure better performance against pathogens in nature. The bio-safety testing for some well-known BCA species such as *Burkholderia cepacia*, *Pseudomonas putida*, *Pantoea agglomerans* and *Aureobasidium pullulans* shows them as opportunistic human pathogenic strains which prevent their application on crops. The limitations and bio-safety issues associated with BCAs restrict the use of microbial pesticides in plant disease management. The current share of the BCAs is very poor occupying about 4% of the total pesticide market share in India. Immediate actions are needed to overcome these limitations to increase the popularity and safe delivery of BCAs to the agricultural systems.

15.4.4 Biochemical Pesticides

The environmental and toxicological risks associated with intensive use of synthetic chemical pesticides have generated interest in search of alternative chemical sources useful in safe management of plant pests and pathogens. Biochemical pesticides include plant-derived chemicals (botanicals), antibiotics and other microbe and animal-based products. These biochemicals being natural, less toxic and easily bio-degradable are exploited as an eco-chemical and bio-rational approach in crop protection from pests and pathogens and to produce food safe for human consumption. Different plant and microbe based biochemicals of importance in pest and phyto-pathogen management are discussed separately.

15.4.4.1 Plant-Based Products (Botanicals)

Plants naturally produce a wide array of complex organic compounds that are not directly linked with plant growth and development but rather provide strength and protection to plants against biological stress conditions, such as wound and pest/pathogen attack and invasion. These plant extracts (botanicals) have antimicrobial properties and are toxic to several phyto-pathogens which are exploited as biopesticides and bio-fungicides (Mizubuti et al. 2007; Castillo-Sánchez et al. 2015; Chengala and Singh 2017). Some plant extracts with inhibitory action on major plant pest and phyto-pathogens are given in Table 15.4. The bioactive components in plant extracts are developed into products which are useful in the management of plant diseases. One such example is Plant Tonic 9 (EOX-SOV) which inhibits the mycelial growth and conidial germination of pathogenic fungi. Plant Tonic 9 is more effective than fungicide propiconazole against *M. Oryzae and causes* increased accumulation of phenolic compounds and defense enzymes (peroxidase and polyphenol oxidase) in rice plants infected with pathogen (Abed-Ashtiani et al. 2018). Likewise, Achook, Neem Azal T/Z, Neem gold and Tricure with azadirachtin as the active ingredient are more effective in control of blast disease in rice than conventional pesticides carbendazim and ediphenphos (Pandey 2018). A plant oil-based formulation NP2 effective against fungal pathogens responsible for powdery mildew and botrytis bunch rot is commercialized in New Zealand (Wurms et al. 2011). However, bioactive ingredients in some botanicals are volatile and get rapidly degraded that reduce their efficiency under field conditions. Therefore, controlled release of liquid and solid formulation of plant-based extracts increases the stability and shelf life of unstable components (Borges et al. 2018). Some plant extract and or their active component based formulations are developed as given in Table 15.4. Hybrid formulations (plant extract + chemical) are often more efficacious in crop protection, thereby reduce the amount of chemicals which are otherwise needed thus safer for the environment. One such formulation recently developed is Regev™ EC (STK, Petah Tikva, Israel) (Reuveni 2019). It contains difenoconazole and tea tree extract in 1:2 ratio and provides protection against fungal pathogens responsible for diseases powdery mildews (caused by fungi in the order Erysiphales), apple scab (*Venturia inaequalis*), Black Sigatoka in banana

(*Mycosphaerella fijiensis*), and also against species of *Alternaria, Cercospora, Botrytis, Rhizoctonia, Pyricularia, Helminthosporium and Sclerotium.*

15.4.4.2 Microbe and Animal-Based Products

Chitosan, derived from deacetylation of chitin is known to suppress *Fusarium oxysporum* f. sp. *radicis-lycopersici* (soil borne) that causes root rot in tomato. It also shows antifungal activity against *B. cinerea*. A chitosan-based product ARMOUR-Zen® has been commercialized in New Zealand to control botrytis bunch rot in wine grapes and postharvest grey mould of table grapes (Romanazzi et al. 2009; Reglinski et al. 2010; Calvo-Garrido et al. 2013). Although its exact mechanism of action still needs to be explored, it has been observed that chitosan treatment increases pathogenic resistance. Chitosan is bio-compatible and stable in water which is safely used as a carrier to improve the shelf life and antimicrobial activity of plant extracts. A study shows that chitosan capsules containing essential oil from *Citrus bergamia* and *Citrus aurantium* strongly inhibit *Aspergillus flavus* growth and prevent decay of dates during storage in lab conditions (Aloui et al. 2014). Likewise chitosan–cinnamon beads with both antifungal and nematicidal properties were obtained by mixing chitosan with cinnamon powder and cinnamon extract. The chitosan–cinnamon beads *in-vitro* inhibit mycelial growth of fungus *Rhizoctonia solani and prevent egg hatching with juvenile mortality of nematode Meloidogyne incognita* (Seo et al. 2014). Unlike conventional chemical pesticides (Fig. 15.1) biochemical pesticides and their formulations are more specific to target pathogens, have low persistence time and release no residual toxic end products after degradation. The plant and microbe based biochemicals and their residues are less harmful and relatively safe, which, can be applied at times close to crop harvest and also in post-harvest management. The widespread use of biopesticides makes it possible to produce food with no or minimal pesticide residues if any. This is helpful to satisfy consumer desires for more natural, healthy and safe food.

15.4.5 Nano-Pesticides

Nano-pesticides are used to describe any pesticide and/or bio-pesticide formulation that includes entities of size in the nanometre scale (1 nm = 10^{-9} m) ranging from 100 nm to 400 nm. The nano-based pesticides and/or antimicrobial formulations have novel properties associated with the small size. The nano-scale formulations are self-regulatory that allow only the required amount to be delivered into the plant tissue or plant part which is attacked by disease or pest. Nano-pesticides have large surface-area-to-volume which allows fungicides or pesticides to act on a wider area of the infected plant. It reduces the amount of fungicides and pesticides otherwise needed to get the desired outcome thereby minimizing the risk of toxic pesticidal residues in soil, water resources and crops (Prasad et al. 2014, 2017).

Several plant protection chemicals are available in the market as nano-formulations such as nano-emulsions, nano-encapsulations, nano-suspensions which are applied as foliar spray and soil spray. Nano-suspensions consist of poorly

water-soluble pesticide nano-particles (NPs) dispersed in water. Formulations containing pesticide NPs of 100–250 nm are more soluble in water. Nano-emulsions are either water or oil-based formulations and contain uniform suspensions of pesticide or herbicide NPs of size 200–400 nm. Nano-emulsions exhibit greater stability with increased surface coating on leaves and uptake through plant cell walls. Nano-pesticides represent the next-generation to traditional pesticides, and offer benefits such as high efficacy, durability and fewer doses of active ingredients. Nano-formulations of the pesticides are prepared in a simple cost-effective manner which appear safe and environment friendly. Nano-emulsions of a large number of pesticides are commercially available as 'Banner MAXX™', 'Primo MAXX®', Subdue MAXX™ 'Cruise MAXX® Beans' and 'ApronMaxx® RTA®'. Banner MAXX ™ is a systemic fungicide which offers broad-spectrum potential for disease control in turf and ornamental plants. Banner MAXX enters the plant through stem surface or root and prevents fungal growth by inhibiting sterol biosynthesis. Primo MAXX®, a cyclopropyl derivative of cyclo-hexenone, is a plant growth regulator that imparts resistance to plant against abiotic as well as biotic stresses. ApronMaxx® RTA® *is* a seed treatment fungicide that reduces the threat of seed-borne and soil-borne diseases, and protects crop (http://www.syngenta-us.com/seed-treatment/apron-maxx-rta). Syngenta's Karate® ZEON is a quick release micro-capsulated insecticide containing an active compound of lambda-cyhalothrin which is released on contact with leaves to provide control against insect pests on barley, wheat, cotton and other field crops (https://www.syngenta.com.au/product/crop-protection/insecticide/karate-zeon).

15.4.6 Antimicrobial Nano-Particles (NPs)

Like pesticides nano-particles, several metals, metal oxides, metalloids, non-metals and carbon compounds possess many bactericidal and fungicidal properties and are being nanotized. These NPs are more useful in the control of plant pathogens. Metal NPs exert inhibitory effect on microbes which are generally lethal for plant pathogens. The metals such as silver (Ag) and copper (Cu) directly exert their toxic effect on pathogens while (Ali et al. 2020) others indirectly act by altering the host nutritional status and activating defense mechanisms in the host plant. Toxic ions such as Zn^{2+}, Ag^{+} released from metal NPs bind to various sulphur-containing proteins and enzymes which accumulate in cell and prevent their proper functioning. They interrupt electron transport, cause membrane potential collapse, generate reactive oxygen species (ROS) and cause ROS-mediated cellular damage that destroy nucleic acid which leads to cell death (Aziz et al. 2014, 2015, 2016, 2019). Unlike pesticides, metal NPs simultaneously use more than one mechanism to fight more effectively against different plant pathogens. Some nano-materials with antimicrobial potential commonly used in plant pathogen control are discussed below.

15.4.7 Silver Nano-Particles

Silver (Ag) is widely known to possess antimicrobial activity against numerous pathogens and is generally non-toxic to humans. Silver nano-particles (Ag-NPs) and other silver containing nano-structures are one of the most commonly used inorganic nano-materials in plant pathology (Ali et al. 2020). Ag-NPs have large surface-area-to-volume ratio which enables their increased contact with microbes and their permeability into microbial cells making them more effective against pathogens (Aziz et al. 2014, 2015, 2016). As a result, antimicrobial formulations containing Ag-NPs are needed in small amounts to get desirable control on the growth of pathogens. The antifungal activity increases with the increasing Ag-NPs concentration, for example, excellent inhibition is observed under in-vitro conditions at 15 mg concentration of Ag-NPs against pathogenic fungi, namely *Alternaria alternata, Sclerotinia sclerotiorum*, *Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea* and *Curvularia lunata* (Table 15.5). Silver NPs adhered to graphene oxide (AgNP-graphene oxide composites) are more stable that effectively decrease cell viability of pathogenic bacteria *Xanthomonas perforans* causing bacterial spot on tomato in both culture and green house conditions (Ocsoy et al. 2013).

The Ag-NPs also improve the efficiency of synthetic antifungal agents, like fluconazole against *Phoma glomerata, P. herbarum, F. semitectum, Trichoderma* sp. and *Candida albicans*. In-vitro tests reveal that Ag-NPs differently inhibit the hyphal growth and sclerotium germination of fungal phyto-pathogens, namely *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor* in a dose-dependent manner with maximum inhibition on hyphal growth of *R. solani*, followed by *S. sclerotiorum* and *S. minor* (Min et al. 2009). Even the low concentrations of Ag-NPs can inhibit sclerotium germination of *S. sclerotiorum* effectively. The nano-Ag liquid inhibits *Sclerotium cepivorum* that causes the white rot of the green onion (Jung et al. 2010). *Fusarium culmorum* spores treated with Ag-NPs show deeply decreased mycelial growth with reduction in the number of germinating fragments and sprout length from the spores (Kasprowicz et al. 2010). It is believed that metallic Ag ions released from NPs interact with the sulphydryl groups that cripple the enzymes needed during aerobic respiration in fungi and bacteria, which prevent cellular metabolic processes resulting in the death of susceptible microorganisms. Metallic Ag alters membrane permeability with the cell consequences of detachment of plasma membrane from cell wall. Beside these many other ways are possible by which Ag-NPs exert the inhibitory effect against specific microbial targets. The various antimicrobial mechanisms and the detrimental effect of Ag on microbial cellular processes make Ag-NPs as important tools to destroy and prevent growth of a wide range both bacterial and fungal pathogens.

15.4.8 Copper Nano-Particles

Copper (Cu) is an active ingredient in many agro-chemicals (fungicides and or bactericides) commonly employed to prevent fungal or bacterial infections and

control plant diseases. These compounds typically contain micron-sized metallic copper as hydrophobic copper hydroxide and copper oxide (CuO) particles, which, usually aggregate in aqueous medium that decreases their antimicrobial activity. The nano-particles of Cu and CuO with small size and high surface area have higher antibacterial and antifungal activity. The Cu-based NPs deliver Cu to target pathogens and plants at higher rate without any deleterious effect on plants (El-Abeid et al. 2020). Study shows that foliar spray of CuO NPs prevents *Fusarium* and *Verticillium* wilt and enhances growth of tomato and egg plants grown in disease infested soil and soilless medium more than bulk equivalents, or their sulphate salts (Elmer and White 2016). Synthetic copper based NPs such as CuO, Cu_2O and Cu/Cu_2O nano-composites of different morphology are more effective in control of *Phytophthora infestans* and prevent its infection on the tomato leaves than commercial Cu-based products (Giannousi et al. 2013). Small sized chemically synthesized Cu-NPs coated with cetyltrimethylammonium bromide show significant antifungal activity against fungal pathogens, namely *Fusarium oxysporum*, *Curvularia lunata*, *Alternaria alternata* and *Phoma* destructive (Kanhed et al. 2014). These Cu-NPs show more activity than broad-spectrum fungicide bavistin against pathogenic fungi. The nanotized copper compounds dissociate to release Cu particles which act upon the pathogenic fungi. Nanotized forms of Cu compounds like $Cu_3\ (PO_4)_2{\cdot}3H_2O$ nano-sheets rapidly breakup in natural conditions and release Cu particles that increases their antifungal character (Borgatta et al. 2018).

Silica nano-composites consist of Cu and/or CuO NPs embedded in hydrophilic silica gel matrix that reduces particle–particle interaction and aggregation. Sol–gel silica is hydrophilic and negatively charged, thus serves like a weak chelator of copper ions and increases its availability. Copper nano-composites such as core-shell copper, multivalent copper and fixed quaternary ammonium copper are effective bactericides more active against *Xanthomonas perforans* causing bacterial spot on tomato (Strayer-Scherer et al. 2018). Study shows that these copper nano-composites are more effective than metallic micron-sized copper and reduce bacterial spot disease severity using 80% less metallic copper as compared to copper-mancozeb in the field conditions. Cu-based nano-scale materials with inhibitory effects on wide range of fungal and bacterial pathogens prevent their growth that helps in plant disease control.

Metals and their oxides have inhibitory effect on broad range of both fungal and bacterial pathogens. Also, their nanotized forms are needed in very small amounts which are generally non-toxic for human and plant health. These NPs thus offer a sustainable and effective strategy in the crop disease management.

15.4.9 Carbon Nano-Materials

Carbon is the most abundant element naturally found in larger amounts in many easily available sources. The allotropic character of carbon allows formation of nano-materials with several different morphologies and structure. Different carbon nano-materials, namely carbon nano-tubes (single-walled and multi-walled),

graphene oxide (oxidized and reduced) and fullerenes have shown to restrict growth of pathogens such as *Xanthomonas, Aspergillus spp., Botrytis cinerea* and *Fusarium spp* as given in Table 15.5. Carbon-nano-materials (CNM) tightly contact with fungal spores forming CNM-spore aggregates which effectively suppress germination of spores. Their sharp edges and coating on microbial surface damage the cell wall and produce oxidative stress (ROS) that inhibit pathogen growth. Numerous antibacterial carbon nano-tubes dispersed in solution act as sharp and fast moving 'Nano-darts' that constantly attack on the pathogenic bacteria, disrupting their cell integrity and causing the cell death (Liu et al. 2009). Foliar spray of metal oxide (Fe_2O_3 or TiO_2) NPs and carbon- nano-materials (multi-wall carbon nano-tubes or fullerene) promote plant growth and resistance against Turnip mosaic virus infection in tobacco (Hao et al. 2018). The carbon nano-materials and their use in plant disease suppression are relatively new and very little is known about the environmental fate of these materials. However, their antimicrobial properties and inhibitory effect on wider range of pathogens make CNMs important tools in plant disease control and protection from phyto-pathogens.

15.4.10 Green Nanotechnology in Plant Disease Management

Various microbial species aggregate inorganic materials either within or outside their cells to form nano-particles (NPs). Green nanotechnology is based on the use of microorganisms and/or plant extracts for the synthesis of nano-particles. Biological biosynthesis of many NPs such as gold, silver, gold–silver alloy, selenium, platinum, palladium, silica, zirconia, magnetite and uraninite by bacteria, actinomycetes, fungi, yeasts and viruses have been reported earlier (Prasad 2014; Prasad et al. 2016, 2018). Many of these biosynthesized NPs act as potent antimicrobial agents which find application in crop pest and pathogen management. Several fungal species, e.g. *Fusarium sps., Aspergillus sps.*, *Verticillium sp.*, *Penicillium sp.* and *Trichoderma* sp. are promising resources in NP fabrication, often termed as myco-nano-particles. Mycelia-free water extracts of *Amylomyces rouxii* strain, KSU-09 isolated from date palm roots suspended in silver nitrate, facilitate production of stable, predominantly monodispersed and spherical Ag-NPs (Musarrat et al. 2010). These myco-AgNPs exhibit antimicrobial activity against many bacterial pathogens like *Shigella dysenteriae* type I, *Staphylococcus aureus*, *Citrobacter* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and fungal pathogen *Fusarium oxysporum.* Nano-pesticides and antimicrobial NPs provide green alternatives with safe applications on wide range of crops in fields and also during storage to prevent post-harvest pest and pathogen attack (Bhattacharyya et al. 2016). However, the toxicity of nano-particles and nano-pesticide carriers, and their biological effects on plant health need to be addressed before their application in agriculture.

15.5 Plant-Incorporated Protectants (Genetically Modified Plants)

Considering the demand to devise new strategies for the development of new resistant plant varieties against infectious diseases, several advancements have been made through molecular approaches. These approaches mainly involve: (1) gene introduction for detoxification of microbial compounds (fungal pathogenicity factors), (2) gene expression, producing antifungal proteins and other antimicrobial products, (3) gene editing and gene silencing approaches.

15.5.1 Transgenic Approaches

Hydrolytic enzymes responsible for the degradation of fungal cell wall have been widely used in transgenic plants for the stimulation of gene overexpression (Shin et al. 2008). Introduction of chitinase genes for production of transgenic plants, imparting disease resistance has been reported by Melchers and Stuiver (2000). Many transgenics have been produced using these enzymes (alone/synergy) which include rice, tomato, carrot and tobacco enhancing fungal resistance in plants. There are also reports of introducing enzyme such as the RC24 chitinase gene of rice into wheat that showed resistance against *Puccinia graminis* f. sp. *tritici* (Huang et al. 2013). Likewise, the chitinase class-I gene (RCH10) of rice, when introduced in *lilium*, showed elevated resistance against infection by *Botrytis cinerea* (de Cáceres González et al. 2015). Chitinase gene (*chi*A) from *Serratia marcescens* was the first gene incorporated in plants to develop disease resistance against *Alternaria longipes* and *Rhizoctonia solani* (Kamble et al. 2016). Another chitinase gene *chit42* from *Trichoderma harzianum,* with 400 fold increased chitinase activity, after incorporation in potato showed absolute resistance against *Alternaria alternata*, *Alternaria solani*, *Botrytis cinerea* and *R. solani* and *Sclerotinia sclerotiorum* (Zhang et al. 2016).

15.5.2 Genome Editing

Gene editing technologies (GET), being a versatile approach, play a crucial role in the plant immunity. Progress proceeds by altering the genotype and phenotype of organisms by introducing specific changes in the DNA sequence of a target gene (crop/specific plant species) via insertion, deletion, modification and replacement (Zhang et al. 2017; Van Eck 2020). In such technologies, site specific endonuclease system enables targeted genome modifications through DNA double-stranded breaks (Khandagale and Nadaf 2016). Similar four different systems have been used which include clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9), zinc-finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs) and

meganucleases (Osakabe et al. 2010; Baltes et al. 2015; Zaidi et al. 2016; Mushtaq et al. 2018).

CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) is an indispensable tool for basic plant research and crop improvement (Sarma et al. 2021). It has been a promising approach against plant viruses as many bacteria possess antiviral defense machinery. This system requires an RNA-guided nuclease (often a Cas protein), which cleaves at specific target sites governed by base complementarity between CRISPR RNA and target DNA/RNA (Wu et al. 2014). Some examples include RNA-guided endonuclease Cas9 from *Streptococcus pyogenes* (SpCas9), RNA-guided RNases Cas13a from *Leptotrichia shahii* (LshCas13a) or *Leptotrichia wadei* (LwaCas13a) (Abudayyeh et al. 2017; Cox et al. 2017) and Cas9 from *Francisella novicida* (FnCas9) (Price et al. 2015). Ilardi and Tavazza (2015) have proposed gene editing as a potent tool against Plum pox virus.

The CRISPR/Cas9 technology has opened a new opportunity in the fields of functional genomics and crop improvement (disease resistant) by either stacking of resistant (R) gene(s) or disruption/deletion of susceptible (S) genes (Li et al. 2020). This method is now extensively used to modify the plant immunity at different levels in several crops. It is achieved by alteration in (1) susceptible genes (S-genes), (2) resistance genes (R-genes), (3) genes regulating the interaction between effector and target and (4) genes regulating plant hormone balance (Andolfo et al. 2016).

By using genome editing on mildew-resistance locus O (MLO) of wheat through TALEN- and CRISPR/Cas9, powdery mildew disease-resistant genotypes have been developed for wheat (Wang et al. 2014a, b). Similarly, CRISPR/Cas9 was used to modify pathogenicity gene Avr4/6 in *Phytophthora sojae*, an oomycete pathogen of soybean (Fang and Tyler 2016). It has also been used in developing plant resistance to bacterial leaf blight caused by *X. oryzae* pv. *oryzae* (Li et al. 2012). Wang and colleagues have used CRISPR/Cas9 technology to develop mutagenized rice lines with enhanced blast resistance (Wang et al. 2016). Similarly, Jiang et al. (2013) have evaluated disruption of susceptibility genes *OsSWEET11* and *OsSWEET14* using CRISPR/Cas9 against rice bacterial blight. Recently, Ma et al. (2017) used CRISPR/Cas9 to knockout rice *OsSEC3A* gene and showed that absence of *OsSEC3A* gene provide improved defense response and resistance against fungal pathogen *M. oryzae*.

15.5.3 Gene Silencing

RNA interference (RNAi), a method of gene silencing, involves the blocking of a gene function (Younis et al. 2014). The term RNA interference (RNAi) was first coined in *Caenorhabditis elegans,* however, originally known to occur in plants and fungi (Hannon 2002; Nawal et al. 2016). It is a post-transcriptional sequence-selective technique which regulates a gene expression by destroying the corresponding mRNA, suppressing its translation, with chromatin remodelling. RNAi is not used to knock out some gene expression but it is only a way to suppress

its effect by the binding of small RNA fragments to messenger RNA thus causing cleavage thereby reducing the expression.

RNAi, due to its specific mode of action, opens new avenues to control pests and diseases, introducing novel plant traits by gene silencing to increase crop yields. It may have a wide host range and involves the gene expression silencing of both specific endogenous genes and those of pathogens as well, thus assisting in crop protection. It is therefore used to turn off genes with a specific mode of action compared to other strategies such as the use of chemicals. Using this approach many resistant plant varieties have been developed against root-knot nematodes, corn rootworm and cotton bollworm. Many new crops have been developed using RNAi such as nicotine-free tobacco, decaffeinated coffee, nutrient fortified maize, etc. (Tang et al. 2007).

Host-Delivered RNAi (HD-RNAi), also known as Host-induced gene silencing (HIGS) produces double-stranded RNA molecules which target pathogen genes present in host by cleaving it into short interfering RNA molecules (siRNAs). These siRNAs get utilized by pathogens resulting in induced RNAi causing pathogen gene silencing (Fairbairn et al. 2007). HD-RNAi has shown significant results against some fungal diseases (Tiwari et al. 2017) such as barley powdery mildew caused by *Blumeria graminis* (Nowara et al. 2010), wheat stripe rust fungus by Puccinia striiformis (Yin et al. 2011). Yin et al. (2011) reported *Barley stripe mosaic virus* (BSMV)-induced gene silencing against *Puccinia striiformis* f. sp. *Tritici* (*Pst*) genes to screen RNAi targets for rust diseases. Control of the devastating disease of wheat scab has also been reported to control by this technique (Koch et al. 2013). Synthetic siRNAs are known to downregulate the key fungal genes involved in toxin production in *Aspergillus* and *Fusarium* spp., using a hairpin RNAi approach in plants to control mycotoxigenic fungi (Abdel-Hadi et al. 2011). Virus-induced gene silencing (VIGS) is another versatile tool for triggering RNAi silencing (Purkayastha and Dasgupta 2009). Ding et al. (2006) have developed a brome mosaic virus (BMV)-induced RNA interference (RNAi) system for rice VIGS work, which is easy to operate and produce designed siRNA against internal rice target genes.

15.6 Biopesticides: A Feasible Alternative

Nowadays, biopesticides are gaining importance for disease management, as they are economic, feasible and eco-friendly substitutes to chemical pesticides (Bhattacharyya et al. 2016; Samada and Tambunan 2020; Kesho 2020). They may allow sustainable control of severe pathogens by their non-toxic mode of action. Biopesticides are classified in three categories: plant-incorporated protectants, microbial pesticides and biochemical pesticides (Senthil-Nathan 2015). Plant-incorporated protectant (PIP) are biopesticides expressed in genetically modified crops which induce plants to produce a pesticide on its own (Parker and Sander 2017). The Cry proteins from the bacterium *B. thuringiensis* (*Bt*) were the first-generation insecticidal PIP expressed in genetically modified (GM) crops (Clark et al. 2005). Numerous lines of Cry gene-expressing rice have been shown to confer

resistance to lepidopterous pests (Cohen et al. 2008). Other than Cry protein, a new class of PIP-based GM crops has been developed expressing double-stranded RNA (dsRNA), based on RNAi (Parker and Sander 2017). Plant-incorporated protectant GM crops are mostly used in Americas, Asia, Australia, whereas their use is still restricted in Europe (ISAAA 2016). In search for new biomolecules and efficient biopesticides, recombinant DNA technology has been used to develop biopesticides with the use of novel fusion proteins in order to improve their efficacies. This technology involves the fusion of a toxin (non-toxic to higher animals) with a carrier protein making it toxic for insect pests if consumed orally (Fitches et al. 2004). A fusion gene/protein constructed of DI and DII domains of *Bt* Cry1Ac and lectin from garlic allowed the development of rice varieties resistant to different groups of insect pests such as yellow stem borer, leaf folder and brown plant hopper (Boddupally et al. 2018).

Another fusion protein, super-Blad, consists of the blad gene, an active ingredient of biological fungicides and peptides with a potential as antibacterial agents. It was constructed in *Lupinus albus* as a broad-spectrum antifungal tool against both plant and human pathogens (Monteiro et al. 2015; Pinheiro et al. 2017). Recently, its mode of action was determined to be a multi-site fungicide which disturbs cell homeostasis and leads to microbial cell death (Pinheiro et al. 2017). Therefore, the fusion protein can be developed as recombinant protein in microbial systems, which can facilitate its industrial and commercial formulations.

Microbial pesticides comprise biological agents (bacteria, fungi and viruses) with a capability to control different pathogens. They are applied in a manner similar to chemical pesticides, but they work in an eco-friendly way as discussed earlier. On the other hand, biochemical pesticides are generally synthetic materials that either inactivate or kill the pest. These are naturally occurring substances including pheromones, plant extracts and fatty acids. They interfere with growth or mating and sometimes attract insect pests to trap (Senthil-Nathan 2015). Use of biopesticides in agriculture has its own advantages. They are inherently less harmful and eco-friendly, perform effectively in small concentrations, are more specific towards their targets, naturally decompose in the environment without any deleterious impact.

15.7 Conclusion and Future Perspective

Plant diseases pose a severe risk to global agricultural production and economy. In the past farmers shifted worldwide towards the use of chemical fertilizers to overcome many plant protection issues. Excessive and continuous usage of agrochemicals on-farm resulted in human health hazards, environmental pollution and reduced agricultural production. There have been many cases of intoxication of farmers, rural workers and their families reported to occur during pesticide application on-farm, and of poisoning of these synthetic chemicals on human health as well. Due to these challenges the need roused to devise eco-friendly strategies for disease management. Numerous researches have been done to develop GM crops which

have potential to prevent themselves against pathogens but their usage is restricted to some countries only, due to opposition by public opinions and government issues. These technologies should also be introduced in developing countries in order to minimize the knowledge gap between researcher and farmers. Other than GM crops, common and feasible practices such as those involving bio-control agents are also of immense importance. Approaches to increase their popularity among farmers through awareness programs, under different government schemes, are deeply needed. The drawback of quality production should also be taken care for usage of bio-control agents to achieve a sustainable crop production. The development of molecular-based novel technologies should not be confined only to laboratories, as there is a need for awareness among common people to bring these tools at the field level.

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Host Plant Resistance: An Eco-Friendly Approach for Crop Disease Management

16

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Abstract

The climate change and ever-increasing population has stressed the plant scientists to devise economically viable, ecologically safe and socially acceptable agricultural practices that can keep the menace of plant disease losses under economic threshold levels. In the recent past, the philosophy of crop protection has shifted from the use of environmentally unsafe chemical pesticides to the eco-friendly approaches. The conventional methods of plant disease management like cultural practices, biological control, chemical control, and natural resistance are still in practice, but are not adequate to control many destructive diseases. The sustenance of disease resistant varieties under variable eco-systems remained a challenge to the breeders due to the fast-evolving nature of many plant pathogens resulting in the breakdown of the R-genes. This emphasized the plant breeders to have a wide range of genetic options to diversify the resistance traits having potential to reduce the pressure of pest evolution. Hence the approaches for the genetic improvement of crops are expanded from simple selection to the genome editing. The recombinant DNA technology and genetic transformation techniques offered unique opportunities to transfer resistance genes beyond crop plant gene pools. The effective strategies for engineering disease resistance include exploitation of genes related to pathogenesis-related protein (PR proteins), upregulation of plant structural defense mechanism, disarming host susceptibility genes and to express proteins or antimicrobial compounds that are harmful to the pathogens.

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The RNA-mediated gene silencing is another approach to switch off specific genes by inserting double-stranded RNA and has been successfully exploited in disease management especially the viral pathogens. With the advent of genome editing technologies, now it is possible to edit crop genome by editing the sequences of a specific gene instead of complete gene deletion and it can be exploited to modify the genes associated with plant immunity. To achieve the targets of developing eco-friendly and durable pathogen management, there is a need to design strategies that can complement conventional and modern techniques.

Keywords

Genome editing · Host · Hybridization · Molecular breeding · Resistance · RNAi transgenic

16.1 Introduction

Production of food grains has been increased significantly after our independence along with population and technology. Further, increase in population and industrialization has led to climate change which resulted in the emergence of several new pests and diseases. Despite of several advances in disease management strategies, global food security is threatened due to multitude of pests and pathogens causing 30% production losses annually. According to FAO estimates, annually 20–40% of global crop production are lost to pests and plant diseases cost the global economy around $220 billion every year (Anonymous 2019). To increase the production of food grains to feed ever growing population, heavy use of chemical fertilizers and pesticides along with high yielding varieties, was practiced. But their injudicious use resulted in the emergence of pesticide resistant population of various insect-pests and pathogens, besides polluting our natural resources and causing many health hazards. Moreover, the chemical control of plant diseases is beyond the reach of resource poor farmers of developing countries. So, the efforts of plant scientists were directed towards other alternative methods for the management of various plant diseases which were responsible for huge pre- and post-harvest losses. The eco-friendly and sustainable conventional practices for controlling diseases include the development of resistant varieties, application of cultural practices and biological control agents. These conventional methods are still in practice but are not adequate to control many destructive diseases. Biological control measures have received considerable attention, but their effective application is limited due to quality, problem of supply, and complex soil-pathogen-environment interactions under field conditions (Thakur 2007). Exploiting host plant resistance is important for sustainable crop production which is the need of the hour as it reduces the environmental damage by reducing the application of agrochemicals. Disease management through host plant resistance is based on simple and easily transferable seed-based technology that does not cost additional amount to the farmers, although it is a time

consuming and exhaustive exercise for researchers. Disease resistant varieties have been developed by implementing various conventional and modern plant breeding approaches. The sound knowledge of biology and epidemiology of the disease, host–pathogen interaction, effective and rapid screening techniques to identify resistant sources, exploitation of identified resistance sources to develop agronomically desirable lines, evaluation of their agroecological adaptation and yield potential are major steps involved in developing a disease resistant cultivar (Thakur 2007). The major limitations of conventional breeding approaches are slow transfer of desirable traits to develop superior genotypes and the linkage drag which results into the transfer of some undesirable traits along with desirable ones into an otherwise superior variety (Sanghera et al. 2011). Also, the time required for the transfer of desired gene depends on the donor of desired gene as well as evolutionary relationship between the donor and recipient plant. It is easy to transfer desired traits from donors belonging to primary gene pool of the crop. Secondary and tertiary gene pools are the reservoirs of several important biotic stress resistance traits, but it is difficult to transfer resistance genes from a donor belonging to secondary and tertiary gene pools and may take appreciably long time to transfer the genes due to pre- and post-fertilization barriers (Jauhar 2006). During natural host–pathogen interaction some pathogens evolved to parasitize large number of hosts while others remained specific to certain hosts and developed races that are specific to the varieties within a host crop. The resistance in plants is categorized as monogenic, oligogenic, polygenic, systemically acquired resistance, and post-transcriptional gene silencing. In most of the crop plants major resistance genes based on classical gene-for-gene concept have been exploited in conventional breeding programmes that have rapidly been accompanied by resistance break down. Polygenic resistance is no doubt a durable resistance but its exploitation is limited due to complex inheritance. The constant struggle between host and pathogen resulted in the evolution of new races of pathogens and the disease resistant varieties having major genes become obsolete after 7–8 years or even early because these genes can be defeated by a single loss of function mutation in corresponding avirulence (Avr) gene. For example, in the recent past emergence of super virulent wheat stem rust race *Ug99* threatened the wheat breeder's worldwide (Singh et al. 2011). Plant scientists employed different strategies to enhance the durability of resistance using conventional methods, but due to evolution of super races of pathogens and frequent break down of resistance with the passage of time, there is continuous challenge for breeders to develop new varieties in short time span to protect the crops from new pathogens. It is expected that world population will exceed nine billion by 2050 and to feed the whole population 70% more grain production is required (Anonymous 2017). The great challenge of global food security directed the plant scientists to develop and use modern biotechnological approaches to speed up the process of varietal development with resistance to biotic and abiotic stresses. Molecular marker assisted selection, back cross breeding and gene pyramiding have been exploited by the breeders for development of durable disease resistant varieties in short time span with greater efficiency against many diseases. Due to recent advancements in sequencing techniques, the genome sequences of many crops are available and for generation

of new variations specific DNA sequences in the genomes can be targeted. Molecular biology of disease resistance has deciphered the role of various proteins (PR proteins), phytoalexins, hydrolytic enzymes, and antimicrobial compounds in host plant defense mechanism. These defense responses are genetically programmed, and activated only upon invasion of a plant by the pathogen (Somssich and Hahlbrock 1998). The understanding of genetically controlled defense responses and the genetic basis of products (enzymes or proteins) being produced during defense actions has opened new vistas of research on plant diseases at molecular level. Availability of genome sequences of many crop plants and knowledge of disease development at molecular level enables genome editing techniques to modify the target gene sequences to develop disease resistant varieties (Andolfo et al. 2016). This book chapter will elaborate the various breeding methods to exploit resistance genes for the management of various crop diseases with special reference to cereal crops. In addition, the reader will be provided with an insight into various aspects of plant defense response and host resistance.

16.2 Defense Mechanisms in Plants Against Diseases

In nature, plants are continuously challenged by different pathogens where few of them are successful in getting entry into a host. The plants have developed a variety of constitutive and inducible defense systems for protection from different biotic factors. Constitutive defense system of plants comprises of multiple preformed barriers such as waxy cuticles, cell walls, and bark as host surface barriers. Induced defense include the production of toxic chemicals, pathogen degrading enzymes, anti-nutritional effects and deliberate cell suicide (Freeman and Beattie 2008). Plants oftenly do not produce toxic compounds or defense-related proteins until pathogens are detected due to metabolic cost associated with production and maintenance of these compounds (Saskia and Jorunn 2011).

The first line of defense in plants present on their surface comprises of several characters that act as barriers to pathogen penetration and are required to be breached to enter the host. If the pathogen succeeds in penetration; it encounters pre-existing internal structural barriers. Plants either possess or liberate chemicals, which interfere with pathogen activities performed during the process of pathogenesis, thereby preventing or interfering with infection. Plants surface cells also contain variable amounts of hydrolytic enzymes such as glucanases and chitinases, which may cause breakdown of pathogen cell wall components.

The first step in infection process is the cell-to-cell communication between host and pathogens. The plant species or varieties may not be infected by pathogen if their surface cells lack specific recognition factors like oligosaccharides, polysaccharides, and glycoproteins. If the pathogen does not recognize the plant as one of its hosts it may not adhere to the host surface or it may not produce infectious substances such as enzymes or structures like appressoria, haustoria, etc. In many host–parasite interactions, the pathogen produces host specific toxins, which are responsible for symptoms and disease development. Many facultative saprophytes and most of the

obligate parasites are host specific and sometimes so specialized that they can grow and reproduce only on certain varieties having essential nutrients and growth factors required for the establishment of parasitic relationship with the host. The activation or induction of defense mechanism may be both specific and non-specific type. Several structural changes are known to be induced by a range of biotic or abiotic elicitors. These dynamic defense mechanisms prevent further colonization or spread of pathogen. The active defense in plants involves cellular defense that rely upon preformed surveillance systems encoded by resistance genes. The receptor-proteins are strategically located in cell membrane to detect the pathogen factors. The ability of plant to mount an active defense response is again under genomic control.

Primarily, defense systems of plants can be categorized into two classes, basal defense (pattern triggered immunity, PTI) and specific defense systems (effector-triggered immunity, ETI). In general, PTI is quantitative in nature and ETI is qualitative in nature (Kou and Wang 2010; Zhang and Wang 2013). The basal defense system is the first line of preformed and inducible defense system, also known as innate immunity (Jones and Dangl 2006; Freeman and Beattie 2008; Wally and Punja 2010) which protects the plants from the pathogens (Freeman and Beattie 2008). It provides immunity at the beginning of infection and triggered when plant recognized the microbe-associated molecular patterns (MAMPs) using host pattern recognized receptors (PRRs). This defense system is much effective against necrotrophic pathogens. If basal defense system is failed, then plants respond with hypersensitive response (HR), specific defense system (Freeman and Beattie 2008). This specific defense mechanism operates effectively against biotrophs and hemibiotrophs and in this response plant limits the access of water and nutrients to the pathogen by sacrificing few cells at the infection site, i.e. programmed cell death. It is triggered in the presence of disease-causing effector molecules. The plant tissues become highly resistant to broad range of pathogens when HR is triggered. This is known as systemic acquired resistance (Freeman and Beattie 2008; Nelson et al. 2017) a whole plant resistance response occurred following a localized exposure to a pathogen. It involves communication with the rest of the plant using the hormones jasmonate, ethylene, abscisic acid, and the accumulation of endogenous salicylic acid (Shah 2009). If the gaseous hormones are released from the injured tissue, it is possible for neighbouring plants to take part in the resistance response as well. Plant resistance is entirely dependent on a network of signalling pathways involving innate immunity and a class of resistance genes (Dangl and Jones 2001; Ausubel 2005; Chisholm et al. 2006).

The basal defense system or innate immunity is a generalized barrier which does not discriminate between pathogens, unlike the specific defense system which is mediated by a highly specific set of genes called resistance (R) genes. Several studies have proven that expression of defense response (DR) genes like Chitinases and Phenyl alanine ammonia-lyase (PAL) can directly correlate with host resistance (Chassot et al. 2007; Tonnessen et al. 2015).

16.3 Host Plant Resistance and Resistance Hypothesis

In nature, only few plant pathogenic microbes are able to cause diseases in plants in spite of large number on the earth. Plants have developed different mechanisms to combat various pathogenic microbes resulting tolerance to different pathogens. We know each and every trait in organisms is controlled by genes, like wise resistance against different pathogenic microbes is also controlled by different genes present in the organism. There are two types of resistance reported in the plants for various biotic stresses, vertical and horizontal resistance controlled by major and minor genes, respectively, in the crop plants. Vertical resistance is race specific resistance, whereas horizontal resistance is race non-specific resistance. Due to complex inheritance of horizontal resistance, genes controlling vertical resistance are exploited in many crops by developing disease resistant varieties. The resistance controlled by major genes break down frequently by loss of function mutation in single gene. So, in order to develop disease resistant varieties with durable resistance, horizontal or quantitative resistance is of importance. Durability of quantitative resistance may be due to number of genes involved in controlling the trait (Vanderplank 1982). Singh et al. (2008) reported that even 4–5 minor genes can control the wheat rust caused by new race *Ug99* to negligible level under high disease pressure. Apart from number of genes involved, other mechanism includes selection coefficient against individual gene controlling horizontal resistance will be less as compared to those for major resistance genes. Further, interactions between host, pathogen, and environment could also play an important role for genes with small effects (Kulkarni and Chopra 1982). Researchers sometimes quantify horizontal resistance via different components of resistance like latent period (time taken from pathogen invasion to development of disease symptoms), infection efficiency and sporulation, etc. which are highly correlated (Parlevliet 1979; Parlevliet 1989) and there are evidences of pleiotropic control of genes involved in HR (Parlevliet 1986; Wang et al. 1994). Development of near isogenic lines of barley containing different combinations of three QTL (Richardson et al. 2006) provided strong evidence for pleiotropic control of latent period, infection efficiency, lesion size, and pustule density (a surrogate for sporulation) for stripe rust of barley.

The classical work of Flor (1971) on genetics of interaction between flax and flax rust (*Melampsora lini*) resulted in substantial understanding of gene- for- gene hypothesis. According to this hypothesis, plant contains single dominant gene resistance gene (R gene) that specifically recognize the complementary avirulence gene in pathogen. This interaction results in defense gene expression, i.e. hypersensitive response (HR) and inhibition of pathogen growth, means resistant reaction (Fig. 16.1). On contrary, if host plants do not contain the R-gene, the pathogen will be able to infect the host even though it contains Avr gene (Fig. 16.2). This hypothesis was followed by multiple cases of R-Avr interactions, i.e., a single NLR gene recognizes its counterpart Avr effector and imparts resistance to the pathogen (Jones and Dangl 2006; Bernoux et al. 2011). This gene-for-gene system occurs frequently in biotrophic pathosystems like rusts, smuts, powdery mildews, and downy mildews of cereals. This type of direct interaction has been

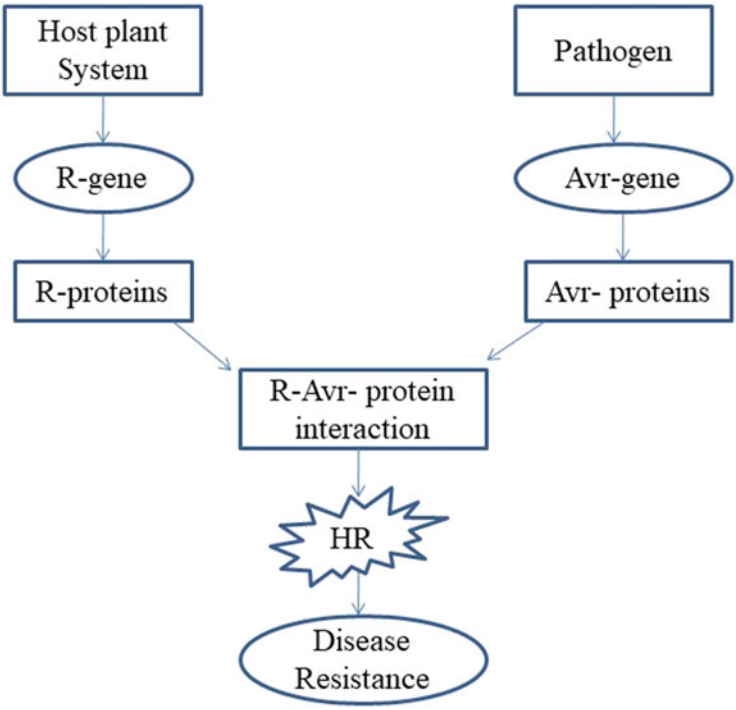

Fig. 16.1 Host–pathogen interaction as per gene-for-gene hypothesis

reported in the case of the rice blast resistance protein Pi54 and its counterpart Avirulence protein, Avr Pi54 (Ray et al. 2016). The resistance of this system is race specific and also gets easily break down by new races of pathogens. It has become evident that many Avr proteins contribute to pathogen virulence on plants lacking the cognate R-gene. However, for a number of R-Avr combinations, physical interactions have not been observed, and perception is thought to be indirect.

During last three decades tremendous advancement has been observed in the field of agriculture both in terms of technology and plant genomic resources that has led to the understanding of host–pathogen interaction phenomenon and the defense mechanisms operating in various biotic stress responses. The knowledge about the genetics of resistance and its implications in breeding for resistance varieties has given boost to the agriculture sector to develop varieties that possess resistance to both biotic and abiotic stresses. In the current scenario, the development of resistant varieties involves the use of both conventional and modern innovative approaches for exploiting host plant resistance.

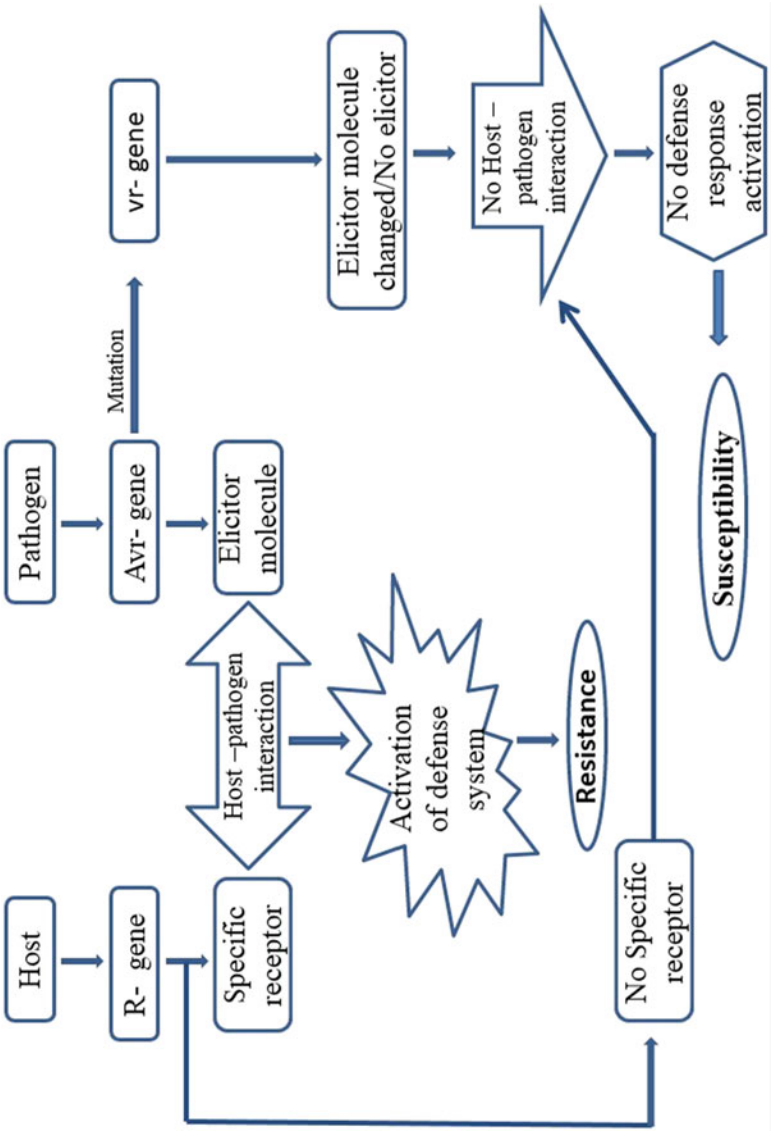

Fig. 16.2 Host–pathogen specific interaction at cellular level, absence of one of both products results susceptibility

16.4 Conventional Breeding Approaches for Exploiting Host Plant Resistance

Resistance breeding has been exploited to protect crop plants from various biotic and abiotic stresses that hamper the crop production. The constant search for resistance genes in diverse sources and their incorporation into elite susceptible varieties and deployment over time and space has protected important crops from deadly diseases, for example, wheat from rust and potato from late blight. The techniques employed in disease resistance breeding includes introduction of disease resistant varieties, hybridization, back cross breeding, multiline breeding, gene pyramiding by introgression of disease resistance genes and their deployment through gene rotation. A brief account of each of the conventional strategies is given hereunder:

16.4.1 Introduction of Disease Resistant Varieties

Plant introduction is the simplest and easiest breeding method to develop new varieties with desirable traits. This method is used when desired traits (resistance gene) are not available in the indigenous germplasm. In this method, the exotic material procured from outside state or country resources is first screened for required traits both under controlled and multi-locations for its suitability and stability in the target environments, then released for cultivation. The plant introduction may be primary or secondary. When introduced/exotic genotypes are released for cultivation in a country where it was not grown before without any alteration in its genetic composition, then it is called primary introduction. This type of introduction is common in the developing countries where well developed breeding programme for the target crop do not exist. For example, spring wheat varieties Sonora 64 and Lerma Rojo 64A introduced from Mexico, having broad spectrum of rust resistance apart from other desirable traits were released for commercial cultivation in India in 1965 (Singh 2015). While the secondary introduction is more common in developed countries which have well-defined breeding programmes and the introduced genetic stocks/germplasm are used for selection, hybridization or in other breeding programmes. For example, Kalyan Sona and Sonalika wheat varieties developed from the segregating materials introduced from CIMMYT, Mexico and were resistant to rust (Singh 2015).

Though, plant introduction is the quick method to resolve the problem of certain areas where all local varieties become susceptible to the pathogen strains, it also serves as source of resistance breeding programme. For example, African pearl millet (*P. americanum*) introductions have been used for developing Downy mildew resistant male sterile lines (Tift 23A cytoplasm) for use in hybrid pearl millet production. This is an important development in the hybrid pearl millet programmes since the original male sterile lines Tift 23A and 23D2A were extremely susceptible to downy mildew. Limitations in sharing germplasm globally due to the enforcement of various laws of exporting/importing countries limits the use of this method in disease resistance breeding programmes. However, with the advent of WTO treaty,

such problems have been solved to greater extent in the member countries. There has been frequent exchange of genetic material during the past few decades that has helped the exploitation of diverse resistance sources in developing resistant varieties. Sharma et al. (1999) reported that exotic common bean accessions procured from CIAT Columbia were resistant to majority of races of bean anthracnose pathogen (*Colletotrichum lindemuthianum* L.) prevalent in north western Himalayan region. Similarly, Thippeswamy et al. (2016) also found resistance in many rice genotypes procured from International Rice Research Institute (IRRI) against local races of blast fungus prevalent in south India. However, the introduction of Co475 variety of sugarcane in Mumbai has defeated the red rot disease but brought leaf rust and whip smut diseases in the country.

16.4.2 Selection

The present day cultivated plants are derived from wild weedy species through the process of domestication by prehistoric man. After domestication, crop species are exposed to both natural as well as artificial selection. Artificial selection is practiced by farmers in ancient time for selecting plants for different traits as per their needs by exploiting natural variability present in the crop species. For developing disease resistant varieties, this method is better than plant introduction and has more chances of success in getting disease resistant plants. Sometimes segregating materials of different crops were also introduced in the country to further develop useful varieties after selection. The selection is carried out under natural and artificial epiphytotic conditions. To ensure the resistant character of a plant, large population of crop plants which were found resistant under natural epiphytotic conditions may be exposed to the attack of pathogens under artificial conditions and the non-infected plants may be chosen which may be released as new variety after proper evaluation for agronomic traits and agroecological adaptation. The varieties developed through either mass selection or pure line selection in self-pollinated crops, whereas in cross-pollinated crops, mass selection, recurrent selection, recurrent selection for GCA, recurrent selection for SCA, and reciprocal recurrent selections are used for developing varieties. For example, rust resistant Kanred variety of wheat was developed by pure line method of selection (Allen 1921). Kufri Red, a potato selection from Darjeeling Red Round is a disease resistant variety. The resistance in such plants will occur in nature by mutation. Sugandh of Bihar is a selection from Basmati rice of Orissa tolerant to bacterial leaf blight. Suvarnamodan rice of Kerala is a pure line of ARC 11775 and is highly tolerant to blast. For example, Kalyan Sona wheat (*T. aestivum*) resistant to brown rust (leaf rust) developed through selection. The limitation of this method is that genotypes with different traits must present in the crop species for the selection of plants with desirable traits and variability cannot be generated by using this method.

16.4.3 Hybridization for Exploiting Host Plant Resistance

16.4.3.1 Combination Breeding

Combination breeding means developing new crop varieties by combining the economic traits of two or more varieties through hybridization. This method involves intervarietal, interspecific, and intergeneric hybridization. In combination breeding, intervarietal hybridization is the most common method to be followed due to fertility barriers and various chromosomal abnormalities in case of interspecific and intergeneric hybridization which require the help of biotechnological tools to generate successful F_1 generation. This method involves two steps, the first step involves the development of breeding populations segregating for traits of agronomic interest (resistance and high yield) by selecting two or more parents with desirable traits that complement each other. The strengths of one parent have the capacity to supplement the weaknesses of another parent. Here, generally two or more parents are crossed with each other to develop segregating populations through sexual recombination. The second step involves the selection of the individual plants containing the target/desired traits amongst the progenies developed from a given cross combination. In this method, selections are made in F_2 generation for superior genetic traits including disease resistance. By continued selfing, selections are made through F_3 to F_5 or F_6 generations either by using pedigree selection (Fig. 16.3) or

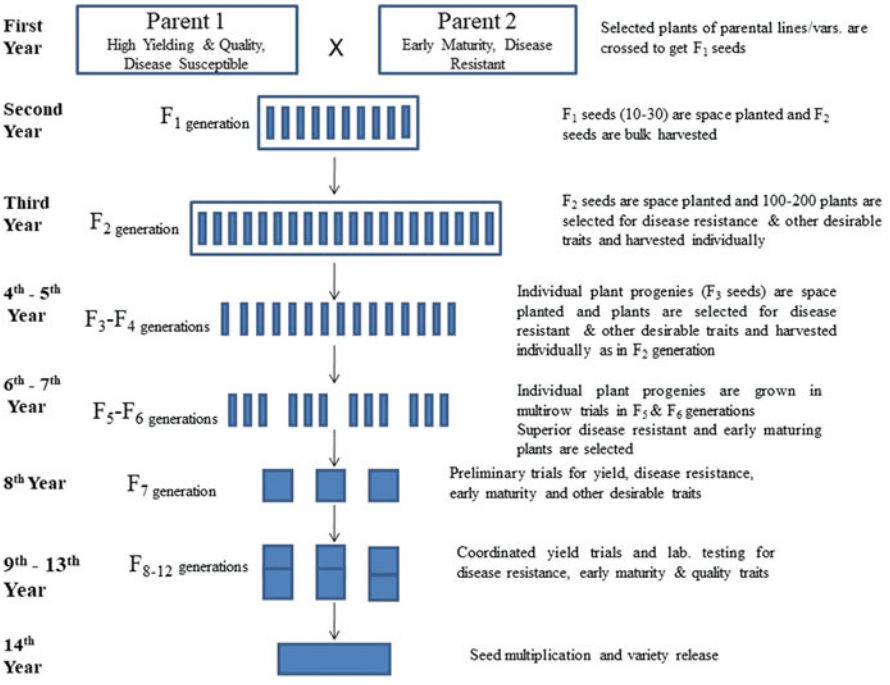

Fig. 16.3 Generalized scheme of pedigree selection for developing variety

bulk method or their modifications and the best variety is selected. There are many examples of varieties developed through this strategy that possess durable and high level of resistance. However, there are chances of linkage drag and resistance may not last long as it takes 10–12 years or more to develop disease resistant cultivar by these methods and by that time pathogen develops the ability to infect resistant plant due to evolution of new races/strains. Interspecific cotton rust resistant hybrids were developed by exploiting rust resistance genes from *Gossypium anomalum* and *Gossypium arboreum* by transferring these genes into *G. hirsutum* (Anjum et al. 2015).

16.4.3.2 Back Cross Breeding for Introgression of Disease Resistance Genes

It is well known fact that improved modern varieties of different crops are less tolerant to different stresses as compared to their wild relatives or local landraces due to the loss of useful genes during the process of evolution and selection for higher yield (Reif et al. 2005). Many wild species or wild relatives of crop plants and germplasm belonging to secondary and tertiary gene pool are considered as important sources of certain desirable traits including disease resistance. But apart from desirable traits, these wild species or relatives have a number of traits which are important for their survival but not desirable as per our needs, also inherit during hybridization. So, to avoid introgression of undesirable traits, the back cross breeding method is used that has advantage of introducing only single trait (for example, disease resistance gene) from wild weedy sp./donor into the cultivated elite susceptible variety. This method was first proposed by Harlan and Pope (1922). Now-a-days this method is employed in improvement of both self and cross-pollinated crops for transferring a single simply inherited character like disease, frost or drought resistance, and earliness from an undesirable variety to a good commercial variety. The desirable variety is called as recurrent or recipient parent and it is crossed to otherwise an undesirable variety but containing trait of interest, called as donor or non-recurring parent (called donor because the desirable genes are transferred). The F_1 progeny obtained is again backcrossed to recurrent parent repeatedly and after 5–6 generations of back crossing, more than 99% genome of recurrent parent is recovered along with traits of interest (disease resistance gene) from donor parent (Fig. 16.4). Examples are the transfer of leaf rust resistance (*Lr9* gene) to *Triticum aestivum* from *Aegilops umbellulata* and powdery mildew resistance (*Pm12* gene) from *Ae. speltoides* by using backcross method of breeding. Back cross method is generally used to improve one or two traits/defects of cultivated variety without altering other traits of the variety. However, many undesirable traits are also incorporated due to linkage drag which is very difficult to remove even after several generations of back crossing (Young and Tanksley 1989). But in spite of this drawback, several varieties have been developed using this method in many crops (Hussain 2015).

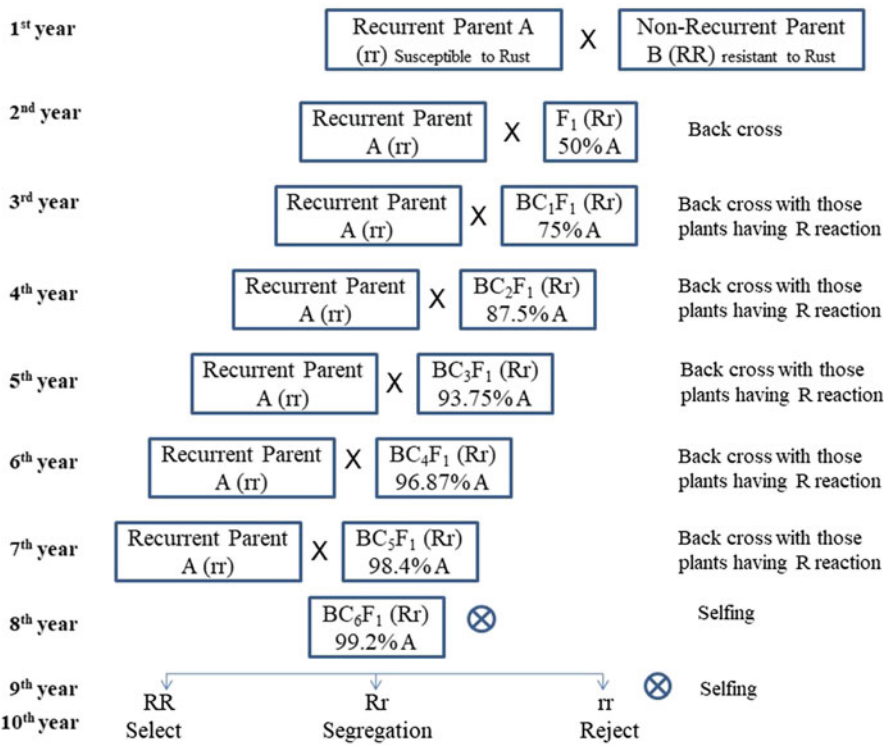

Fig. 16.4 Generalized backcross breeding scheme for transferring disease resistant dominant gene

16.4.4 Multiline Breeding

This breeding strategy is utilized to enhance the durability of resistance to diseases by deploying different resistance genes against same disease in the form of varietal mixture (Keneni et al. 2012; Mundt 2014; Sattari et al. 2014). The varieties developed are basically mixture of different lines of same crops developed through either back cross breeding or combination breeding. These lines are mostly near isogenic lines (NILs) which have the same genetic composition except for disease resistance genes. These are morphologically similar with each other for all agronomic and phenological traits but may differ genetically (Keneni et al. 2012). These lines are bulked together to form elite variety called multiline variety containing separate resistance genes. There are several examples in cereals where multiline varieties have improved resistance against different diseases as compared to individual component isolines (Gill et al. 1980; Wilson et al. 2001). If new races of the pathogen are identified at a later stage, additional isolines resistant to the newly arisen races may be constituted and incorporated. It has been reported since long that rusts and mildews of cereals are controlled through dilution of inoculums in multilines (Chin and Wolfe 1984; Wolfe 1985) and local reduction of intrinsic

rates of disease increase (Browning and Frey 1969; Mundt and Browning 1985). Multiline variety appears to be a useful approach to control diseases like rusts where new races are continuously produced. The first multiline variety in wheat, "Miramar 60" was developed and released in Columbia to combat the attack of yellow rust. "Miramar 63" and "Miramar 65" were resistant to stem rust and stripe rust. In India, three multiline varieties KSML3, MLKS11, and KML7406 have been released in wheat (*T. aestivum*). Kalyan Sona, one of the most popular varieties in the late sixties, was used as the recurrent parent to produce these varieties. Variety 'KSML 3' consists of eight lines having rust resistance genes from Robin, Ghanate, Kl, Rend, Gabato, Blue Brid, Tobari, etc. Multiline 'MLKS 11' is also a mixture of eight lines; the resistance is derived from E 6254, E 6056, E 5868, Frecor, HS 19, E 4894, etc. The third variety, KML 7406 has nine lines deriving rust resistance from different sources. Similarly, Sonalika lines MLSKA9, MLSKA11, and MLSKA 12 resistant to brown and yellow rust were developed in India. It is quite convincing and logical that a resistance gene will last long in a mixture than in pure stand of crop owing to reduced exposure to the pathogen and slow evolution of new races of pathogen. This method is quite effective against rust and powdery mildew diseases in small grain crops. In rice also several multiline varieties with improved resistance to rice blast has been developed by rice breeders (Ishizaki et al. 2005; Sattari et al. 2014). Major limitation in the development of multiline variety is to find out the correct combination of different lines and low quality produce due to mixture of lines.

16.4.5 Resistance Gene Pyramiding

Although varieties having single major gene for resistance can protect the variety from pathogen but large-scale use of that variety results in resistance breakdown due to its exposure to diverse pathogen populations. Since the development of an improved variety takes around 10–12 years and the breakdown of resistance by a given pathogen strains emphasized the necessity of developing multigene varieties that possess more than one R-gene. Watson and Singh (1952) proposed the concept of multigene varieties to minimize the frequent break down of resistance. The pyramiding of R-genes from diverse resistance donors into a single elite susceptible genotype offer long-term resistance against pathogens and has turned into an important technique to develop broad spectrum disease resistance cultivars. In this method, resistance genes from different sources (local landraces, wild spp., wild relatives, and genetic resources belonging to secondary and tertiary gene pools of crop) have been transferred either by hybridization or by backcross breeding (Fig. 16.5). Resistance gene pyramids are expected to considerably extend the durability of resistance because of the low probability for the pathogen to assemble multiple, rare virulence genes by mutation and/or recombination (Pearson et al. 1983). But bringing together two effective major genes in an agronomically successful cultivar is difficult through conventional approaches (Hogenboon 1993). In wheat, varieties with stem and leaf rust resistance has been developed using this

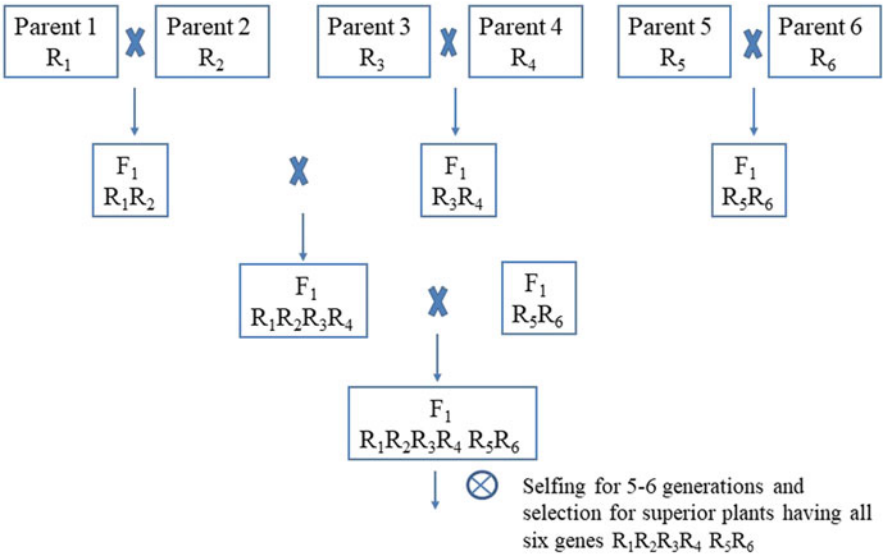

Fig. 16.5 Gene pyramiding through conventional hybridization to enhance durability for disease resistance (R_1, R_2, R_3, R_4, R_5, R_6 are six different disease resistant genes combined from six different parents)

method (Green and Campbell 1979; McIntosh and Brown 1997; Samborski 1985; Schafer and Roelfs 1985). The gene pyramids in wheat have kept stem rust of wheat under control since 1950s till 1990s when emergence of new race of stem rust *Ug99* in Uganda threatened the durability of resistance genes. This has indicated that resistance due to gene pyramids is also not permanent. It has been reported that certain combination of resistance genes will be more durable as compared to others and identification of most durable combination can be done by evaluating the fitness effect of individual gene (Leach et al. 2001; Fabre et al. 2009; Janzac et al. 2009; Khatabi et al. 2013).

16.4.6 Gene Rotation

This is another method which is exploited to increase the durability of single disease resistance gene in a variety. Gene rotation is a strategy which involves the deployment of resistance gene against a particular race of pathogen and after the appearance of new races of pathogen, it is replaced with another resistant gene and then again, the reuse of original resistance gene when the frequency of new race is sufficiently reduced over the period of time (Crill 1977; Mehrotra 2000). We can also call it as varietal rotation. However, the use of this approach requires constant survey and surveillance of new virulences of the pathogen in a given area over time. Gene rotation schemes have been implemented against rice blast (Crill et al. 1981) and rice tungro disease (Manwan et al. 1985; Sama et al. 1991). This approach was used to

control stem rust of wheat in Australia between 1938 and 1950. Gene rotation would be effective only in areas of intensive agricultural production where plant pathologists maintain adequate and intensive disease surveys in cooperation with plant breeders to gather information on disease intensity and pathogen populations (Harahap and Silitonga 1988).

16.5 Innovative Approaches for Exploiting Host Plant Resistance

Although traditional breeding approaches are still used in disease resistance breeding, but several limitations of these approaches have led plant scientists to develop end explore new innovative approaches to overcome the short comings of conventional breeding techniques. Conventional breeding methods take long time to develop new varieties, or gene transfer, more labour intensive, undesirable traits transferred together with the valuable resistance genes, frequent break down of resistance due to pathogenic variability, and emergence of new pathogen races. Use of multiline varieties has biggest disadvantage of finding out the correct combination of different lines and threat of development of super race of pathogen. The quality of the produce is also limitation in multiline varieties; hence this strategy can be exploited only in the countries where quality of produce is not the priority. Similarly, it is difficult to combine more than two genes in a variety and identification of desirable gene combinations through conventional breeding methods. Further, it is difficult to develop resistance varieties through traditional methods for diseases where resistance is controlled by many genes due to complex nature of horizontal resistance.

The process of co-evolution of host and pests evolved strategies to avoid the defense mechanism of each other (Seidl and Thomma 2017). The meager information about resistance mechanism through conventional methods necessitates the use of various innovative and efficient approaches against various fungi, bacteria, and viruses causing deadly diseases in crop plants. Over the past few decades, advances in molecular genetics, gene mapping, precision in plant selection using molecular markers, identification of genome sequences of different crop plants, genetic engineering, and gene transfer technologies resulted in development of several biotic stress resistant varieties in less time. Pyramiding of desirable genes can also be done using these approaches. Genetic engineering or transgenic approach directly modifies the qualitative and quantitative traits by inserting desirable genes of unrelated organisms into plant cell asexually, thus allow access to an unlimited gene pool without the constraints of sexual compatibility. Gene silencing technology is extensively exploited in the development of disease resistant varieties especially against viruses. With the invention of genome editing tools, modification in target gene sequences is possible without affecting other agronomic traits and these modified genes can be further exploited in the development of various biotic stress resistant varieties. So, various innovative approaches are used to develop disease resistant varieties to overcome the problems associated with traditional breeding

strategies. Innovative approaches used in exploitation of host plant resistance are discussed below:

16.5.1 Mutagenesis

Mutations are ultimate source of variation. When resistance sources are not available in germplasm, one of the strategies to create variation is mutagenesis in which mutations are induced in crop plants and then rare mutants having resistance genes for specific biotic stress are selected. Mutations may occur spontaneously in nature or may be induced using various mutagens. For inducing mutation, various physical and chemical mutagens are used. Physical mutagens include x-rays, gamma-rays, and UV radiations whereas chemical mutagens include EMS (Ethyl methane sulphonate), MMS (Methyl methane sulphonate), colchicine, etc. are used for inducing mutation. Mutations at molecular level resulted due to alterations in DNA sequences by causing transition and transversions. The main drawback of mutation breeding is that mutations are random, lethal, and recessive in nature. For example, in wheat NP836, Sharbati Sonora, Pusa lerma varieties were developed through induced mutation and NP-11 by spontaneous mutation. Many rice blast resistant lines have been developed using mutation breeding method (Miah et al. 2013).

Nowadays classical mutagenesis is used in functional genomics to identify the functions of the genes controlling different traits. In this technique, mutations are induced to create mutants and genes controlling a particular trait are identified by analysing the mutants. Map based cloning is used to detect the sequences of the gene responsible for the changed phenotype. Although, this is time consuming and labour-intensive method but it has been successfully used for cloning of several genes in different crop species (Krattinger et al. 2009). With the advancement in genome sequencing techniques, reverse genetics approach has replaced the forward genetics approach to detect the functions of the genes. In this approach, first the sequence of gene with unknown function is altered and then analysed for the associated change in the phenotype. Nowadays, biological mutagens like transposable elements and T-DNA insertion mutagenesis have been widely used by researchers in plant functional genomics (Alonso and Ecker 2006). Targeting Induced Local Lesions IN Genomes (TILLING) is one of the reverse genetics strategies introduced in 2000 and it takes the advantage of classical mutagenesis, sequence availability, and high-throughput screening for nucleotide polymorphisms in a targeted sequence. It combines the high frequency of mutations induced by traditional mutagenesis with sensitive techniques for discovering single nucleotide mutations. This technique can be applied to any plant species, regardless of its genome size, ploidy level or method of propagation which are difficult targets for insertional mutagenesis. Another advantage of TILLING is that insertional mutagenesis results mostly gene knock outs whereas chemical mutagens used in TILLING generate a series of alleles such as gain of function and hypomorphic alleles in addition to loss of function alleles, thus provides a range of phenotypes

(Alonso and Ecker 2006). Further, transformation is required in RNAi technology and insertional mutagenesis using transposon tagging or T DNA, but not required in TILLING, so TILLING has become a valuable alternative approach to transgenic technology in crop breeding. There are many examples where site directed mutagenesis is exploited in disease resistance breeding. In resistance conferring R-genes, leucine-rich repeats (LRRs) are present and a mutation in the *RPS5* gene of Arabidopsis showed interaction of the LRR region with other plant proteins. The resultant mutant *rps5–1* resulted in the replacement of lysine with glutamate amino acid in the LRR region and this altered plant response against downy mildew and bacteria (Warren et al. 1998; Huang et al. 2010; Zhu et al. 2013; Wang et al. 2014). Chujo et al. (2014) reported that phosphomimic based mutation in one of the rice WRKY transcriptional factors (TFs) conferred resistance against rice blast disease. In rice, *Les* (*Spl*) mutants activate SAR against rice blast caused by *Magnaporthe grisea* fungus (Yin et al. 2000; Zeng et al. 2004). In maize, the *Rp1* based *Les* mutant provided resistance against *Puccinia sorghi* rust pathogen and *Cercospora zeae-maydis* causing grey leaf spot disease (Hu et al. 1996; Johal 2007). So, it can be concluded that mutagenesis not only used to create genetic variation for biotic stress resistance but also helps in understanding the resistance mechanism in crop plants. Mutagenesis combined with molecular genetics has ability to cope up with genetic erosion of crop plants.

16.5.2 Somaclonal Variations in Disease Resistance Breeding

Somaclonal variations (SVs) are genetic or epigenetic changes induced in plant cell and tissue cultures. Induction of somaclonal variations is an alternate approach to conventional breeding and transgenic approaches to introduce desirable genetic variability in the gene pool (Anil et al. 2018). Somaclonal variations that occur spontaneously in culture induce changes in a range of plant characters. However, the probability of improving a key agronomic trait such as disease resistance can be cumbersome when left to chance alone. The efficiency of developing disease resistant somaclonal variants is better when an appropriate in vitro selection pressure is imposed. Selection agents that have been applied include pathogen elicitors, pathogen culture filtrate, and purified pathotoxins. This method of somaclonal variant selection has been successful in enhancing disease resistance in several crops. For example, fusaric acid is used to select *Helminthosporium sativum* resistant plants in *Hordeum vulgare*, culture filtrates for selection of *Helminthosporium oryzae* resistant plants in rice, deoxynivalenol for *Fusarium graminearum* resistant plants in wheat and phytotoxin is used to select *Colletotrichum falcatum* resistant sugarcane plants (Jalaja et al. 2006). The somaclonal variant approach is based on changes resulting from internal mutations and thus does not face acceptability issues nor pose any known biosafety concerns. Callus cultures can be used to recover somatic mutants because the in vitro culturing encourages the division of individual cell and regeneration of whole plant. Somaclonal variants can be somatically or genetically stable. The genetically stable variations can be termed mutations.

However, because of the possibility of reversible epigenetic variations this area broadly uses the term 'variations' instead of 'mutations' (Meins 1983). Genetically stable somaclonal variations are due to point mutations, variations in chromosome number and structure, recombinations, DNA methylation, deletions and transpositions in nuclear, mitochondrial or chloroplast genomes (Lee and Phillips 1988; Phillips et al. 1990). Somaclonal variants generated from wheat varieties displayed improved resistance to spot blotch disease and enhanced resistance over the source varieties HUW-206 and HUW-234 (Arun et al. 2003). A high yielding somaclonal variant of sugarcane (var. Co 671), Co 94,012, released as Phule Savitri in Maharashtra (Jalaja et al. 2006), has better sucrose content and resistance to red rot and smut diseases. Although, selection for disease resistance in cell cultures is a simple process, however, regeneration of plantlets from somaclonal variant calli is usually difficult. Multiple subculturing, prolonged exposures to auxins and selection pressure may result loss of regeneration potential of cells, so to avoid this, large number of somaclonal variants are developed to select a few with desirable traits amongst them.

16.5.3 Somatic Hybridization

Sexual hybridization in higher plants is a valuable tool for the conventional breeding to improve cultivated crops. It involves the artificial cross fertilization between the genetically dissimilar individuals to combine several desirable traits present in different varieties into one single variety. Unfortunately, conventional hybridization is limited to only very closely related species. Sexual hybridization is a total failure for distantly related plant species as well as sexually incompatible plants. Therefore, protoplast culture has developed as a potential biotechnological system for transferring genetic information between widely different plant species. It would also provide the basis for a technology to overcome the limitation of conventional sexual hybridization. Thus, protoplast culture is a most suitable mode for somatic hybridization bypassing sexual incompatibility barriers.

Somatic hybridization or protoplast fusion involves isolation of protoplasts, fusion of protoplast obtained from different species to produce heterokaryons, cell wall regeneration by fused product, fusion of nuclei, division of hybrid cells and their subsequent growth, identification, selection of hybrid cells, induction of organogenesis in callus tissue derived from hybrid cells and finally raising of mature plants from regenerated shoots. So, the in vitro fusion of plant protoplasts derived either from somatic cell of the same plant or from two genetically different plants is called somatic hybridization. *O. sativa* (AA type chromosomes) is readily hybridized with common AA type wild rice, but not with non-AA types (such as *O. officinalis* of CC type and *O. meyeriana* of GG type). In the latter cases, conventional crossing methods have failed to generate fertile hybrids. However, this problem can be overcome by subsequent embryo-rescue of hybrids (Jena and Khush 1990). Because the non-AA type wild rice generally possess desirable traits not found in cultivated rice or closely related wild rice species, it is important to develop techniques to

generate hybrid plants between *O. sativa* and non-AA types of wild rice species. The ability to produce and fuse rice protoplasts has allowed the generation of interspecies hybrid clones between the wild rice and *O. sativa* (Hayashi et al. 1988) as well as intergeneric hybrid clones between rice and *Panicum maximum* (Zhang et al. 1999), rice and *Hordeum vulgare* L. (Kisaka et al. 1998), and rice and *Porteresia coarctata* (Jelodar et al. 1999). Bacterial blight (BB) resistance gene(s) for rice was (were) introduced into a cultivated japonica rice variety *Oryza sativa* (cv. 8411), via somatic hybridization using the wild rice *Oryza meyeriana* as the donor of the resistance gene(s) (Yan et al. 2004). Tiwari et al. (2010, 2011) have developed different interspecific, somatic hybrids of potato such as hybrids of *Solanum pinnatisectum* + *S. tuberosum* having resistance for late blight disease and on the other hand hybrids of *S. etuberosum* + *S. tuberosum* having resistance for *potato virus Y*. These somatic hybrids were confirmed through molecular (RAPD & SSR markers) and phenotypic assessments.

16.5.4 Polyploidy Breeding

Polyploids are organisms with multiple sets of chromosomes in excess of the diploid number (Acquaah 2007; Chen 2010; Comai 2005; Ramsey and Schemske 1998). Polyploidy is common in nature and provides a major mechanism for adaptation and speciation. Polyploidy is a major force in the evolution of both wild and cultivated plants. Approximately 50–70% of angiosperms, which include many crop plants, have undergone polyploidy during their evolutionary process (Chen et al. 2007). Polyploids may be classified based on their chromosomal composition into either euploids or aneuploids. Euploids constitute the majority of polyploids. Euploids are polyploids with multiples copies of the complete set of chromosomes specific to a species. Depending on the composition of the genome, euploids can be further classified into either autopolyploids or allopolyploids. Tetraploidy is the most common class of euploids (Comai 2005). Autopolyploids are also referred to as autoploids, contain multiple copies of the basic set (x) of chromosomes of the same genome (Acquaah 2007; Chen 2010). Autoploids occur in nature through union of unreduced gametes and can be artificially induced (Chen 2010). Allopolyploids also called alloploids are a combination of genomes from different species (Acquaah 2007). They result from hybridization of two or more genomes followed by chromosome doubling or by the fusion of unreduced gametes between species (Acquaah 2007; Chen 2010; Jones et al. 2008; Ramsey and Schemske 1998). Economically important natural alloploid crops include strawberry, wheat, oat, upland cotton, oilseed rape, blueberry, and mustard (Acquaah 2007; Chen 2010). An example of a man-made interspecies allopolyploid hybrid is triticale. It is derived from crossing two cereals, hexaploid bread wheat (*T. aestivum*) and rye (*Secale cereale*). Triticale was developed to combine good qualities of wheat including high yield and grain quality with the hardiness (disease and stress tolerance) of rye (Acquaah 2007; Chen 2010). Aneuploids are polyploids that contain either an addition or subtraction of one or more specific chromosome(s) to the total number of chromosomes that

usually make up the ploidy of a species (Acquaah 2007; Ramsey and Schemske 1998). Aneuploids result from the formation of univalents and multivalents during meiosis of euploids (Acquaah 2007). For example, several studies have found that 30–40% of progeny derived from autotetraploid maize are aneuploids (Comai 2005). Aneuploidy has been applied in breeding to develop disease resistant plants through the addition of an extra chromosome into the progeny genome. For example, first perennial wheat cultivar, Montana-2 (MT-2) was developed by crossing durum wheat (*Triticum turgidum* L. var. *durum*) and *Thinopyrum intermedium* at Montana State University in Bozeman, MT, USA in 1987 (Lammer et al. 2004; Schulz-Schaeffer and Haller 1987). Lines selected from segregating generations of crosses between octoploid wheat–*Thinopyrum intermedium* hybrids and durum wheat–*Thinopyrum intermedium* were resistant to cereal cyst nematodes, *Puccinia striiformis* Westend f. sp. *tritici* and *Blumeria graminis* f.sp. *tritici*. In addition to perennial growth habit, wheat–*Thinopyrum intermedium* partial amphiploids, possess multiple resistances to other pests and pathogens including *wheat streak mosaic virus* and its vector, the wheat curl mite (*Aceria tosichella* Keifer), *barley yellow dwarf virus*, eyespot (caused by *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams and *Oculimacula acuformis* (Boerema, R. Pieters & Hamers), and the cereal cyst nematode (*Heterodera spp*.) (Sun 1981; Li and Wang 2009; Li et al. 2012). Many of the desirable traits like perennial growth habits and resistance to various biotic and abiotic stresses from *Thinopyrum spp*. have been used to develop wheat cultivars by introgression breeding (Lei et al. 2018).

16.5.5 Molecular Breeding Approach

The term molecular breeding is used to describe the several modern plant breeding strategies involving molecular biology such as Marker Assisted Selection (MAS), Marker Assisted Backcrossing Breeding (MABB), Marker Assisted Recurrent Selection (MARS), and Genomic Selection (GS). With advances in gene tagging, mapping, isolation, and cloning techniques, several disease resistance genes have been identified, tagged, mapped, isolated, and transferred into different genetic backgrounds to develop disease resistant varieties. Different approaches are elaborated under the following heads:

16.5.5.1 Marker Assisted Selection

Traditional breeding methods for developing disease resistance varieties mostly depend upon the phenotypic symptoms of disease which are strongly influenced by the environmental conditions. Marker assisted selection used for identification of resistant plants in the absence of pathogen and it is not influenced by the environmental conditions. Marker assisted selection provides the opportunity to increase the efficiency of selection by using markers tightly linked to the trait of interest at seedling stage with less cost and more precision and even in the absence of pathogen. DNA or molecular markers are not influenced by the environment, making the selection process accurate and efficient. Marker Assisted Selection (MAS) is a

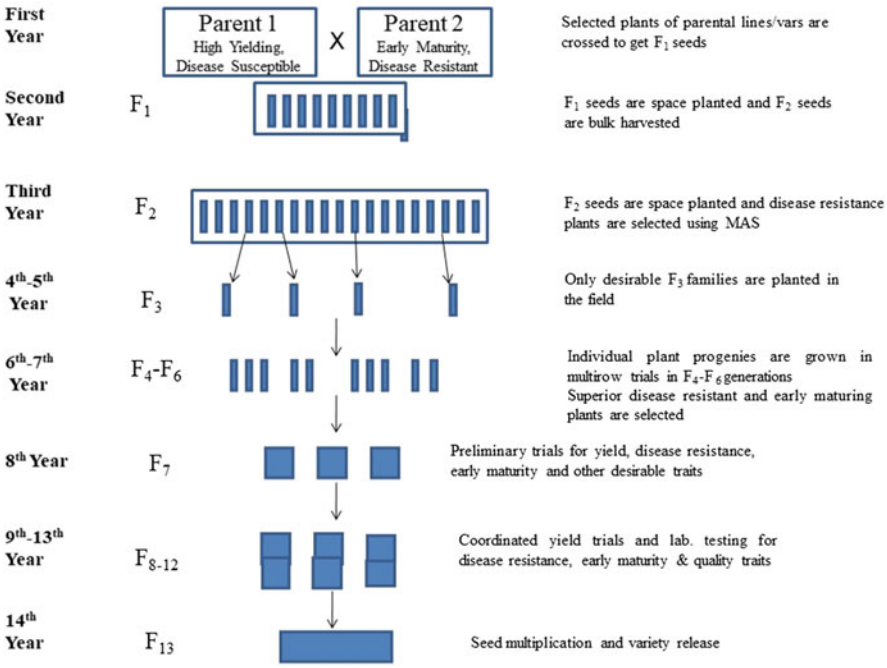

Fig. 16.6 Molecular marker assisted selection in developing variety

selection scheme which involves an indirect selection of a genotype carrying desirable genes through linked marker(s). It allows scoring for the presence or absence of a desired plant phenotype indirectly based on DNA banding pattern of linked markers on a gel or autoradiogram depending on the marker system. The rationale is that the banding pattern revealing parental origin of the bands in segregants at a given marker locus which indicates the presence or absence of a specific chromosomal segment carrying the desired allele.

With the help of molecular markers breeder can efficiently select plants during early segregating generations like in F_2 and F_3 generations, hence help in discarding majority of unwanted plants during initial stages of breeding programme (Fig. 16.6). Another advantage of marker assisted selection is that germplasm can be screened for various disease resistance genes simultaneously and linkage of these markers to target alleles. Various hybridization based, PCR based, and DNA sequence based molecular markers like RFLP, RAPD, AFLP, SSR, ISSR, VNTR, and SNPs linked to disease resistance genes have been utilized by plant breeders to select disease resistance plants. Marker assisted selection is very useful in host–pathogen interaction where resistance and avirulence genes interacted as per gene-for-gene concept (Petit-Houdenot and Fudal 2017). In some cases, disease resistance is controlled by more than one gene, making its inheritance complex. Such traits are called quantitative traits and the regions in the genome where genes of a specific trait are located are termed as quantitative trait loci (QTLs). Molecular markers are also effective in

identifying quantitative trait loci (Langridge et al. 2001) and by QTL mapping various QTLs linked to disease resistance genes have been discovered by plant breeders which helped in the efficient selection of desirable plants (Hussain et al. 2012). RFLP and RAPD markers helped to locate the s*hs* gene linked to head smut resistance in sorghum (Oh et al. 1994). QTLs linked to *Fusarium* head blight (Yang et al. 2005), foliar disease (Chu et al. 2008), and leaf rust resistance (Huang et al. 2003) have been mapped in wheat. QTL mapping has helped in identifying QTLs linked with yellow, leaf and stem rust resistance in wheat (Chu et al. 2009; Prins et al. 2011). Several molecular marker systems are used to map major genes and QTLs for disease resistance in different crops are reviewed by different workers (Miedaner and Korzun 2012; Pathania et al. 2016; Singh et al. 2018). Examples in which MAS has been successfully applied to practical breeding are the wheat rust resistance genes *Lr34* and *Yr36*, the eyespot resistance gene *Pch1*, the recessive resistance genes *rym4/rym5* to barley yellow mosaic viruses, *mlo* to barley powdery mildew, and two QTL for resistance to *Fusarium* head blight in wheat (*Fhb1* and *Qfhs.ifa-5A*) (Miedaner and Korzun 2012). There are certain limitations of MAS that selection is done for only the gene(s) of trait(s) for which markers are linked, but total dependency on MAS can excludes other genes and thus use of parents that do not show DNA polymorphism when used in MAS whereas, in phenotypic selection different genetic options for a desired genotype can be selected (Blair et al. 2007) and reproducibility of different marker systems in different genetic backgrounds also limited the use of MAS. The limited application of MAS for QTLs is due to more influence of environment on QTLs, non-transferability of marker-QTL associations across different breeding populations, non-availability of QTLs with major effect on target traits and difficulties is QTL detection and mapping. The major disadvantage of applying MAS at early generations is the cost of genotyping a larger number of plants. Although markers can be used at any stage during a typical plant breeding programme, MAS is a great advantage in early generations because plants with undesirable gene combinations can be eliminated. This allows breeders to focus attention on a lesser number of high-priority lines in subsequent generations. There are several instances when phenotypic screening can be strategically combined with MAS. In the first instance, 'combined MAS' may have advantages over phenotypic screening or MAS alone in order to maximize genetic gain (Bohar et al. 2020). This approach could be adopted when additional QTLs controlling a trait remain unidentified or when a large number of QTLs need to be manipulated. In India, the first product of MAS in crop breeding included the bajra hybrid HHB-67 with resistance to downy mildew disease caused by *Sclerospora graminicola*, released by ICRISAT, Hyderabad. Likewise, MAS has been applied to develop different resistant varieties in many crops (Ragimekula et al. 2013; Singh et al. 2013).

16.5.5.2 Marker Assisted Back Crossing

MAS is successfully used in introgression of disease resistance genes by backcross breeding also called as Marker assisted backcross breeding (MABB) to reduce the time taken in transferring the gene of interest. Conventional back cross breeding method used to transfer disease resistance genes from resistance sources took

8–10 years to develop the resistant varieties. Using molecular markers in back cross breeding programme reduced the time required for transfer the resistance gene. In marker assisted back crossing programme generally molecular markers are used to select plants carrying target gene which is tightly linked to the flanking markers called foreground selection and to recover the genome of recurrent parent through background selection. This will help in reducing number of generations from six to two or three to recover the genome of recurrent parent through conventional backcross breeding programme. MABB was used to transfer bacterial blight resistance gene in rice (Chen et al. 2001), leaf rust resistance gene in barley (van Berloo et al. 2001), and yellow rust resistance gene *Yr15* in wheat (Randhawa et al. 2009).

16.5.5.3 Molecular Markers Assisted Pyramiding of Resistance Gene

Gene pyramiding is basically combining more than one gene for a trait from different parents into a single line or variety by back cross breeding to generate durable resistance. Through conventional back cross breeding, it will take long time to develop a disease resistant variety and also due to linkage drag many undesirable genes along with targeted one are also transferred into new genetic background which may further delay the varietal development programme. The selection for more than one different gene for a specific virulent race is also difficult phenotypically through conventional breeding methods. By using MAS, pyramiding of different resistance genes in single genetic background can be done in less time and selection for target genes with the help of markers will overcome the difficulty of selection of different genes as observed in conventional combination or backcross breeding programmes. With the help of molecular markers, the genes of interest can be selected in F_2 generation. It is difficult to identify two genes for the same traits through conventional methods and sometimes one gene mask the effect of other gene, so in that situation marker assisted selection will help in selecting plants containing more than one gene for trait of interest. Three highly effective alien genes for leaf rust resistance, *Lr24*, *Lr28,* and *Lr9* were selected for pyramiding in the background of a susceptible but well adapted bread wheat variety HD2329 conferring high degree of seedling and adult plant resistance (Charpe et al. 2012). The use of molecular markers confirmed the presence of the linked genes resistance genes, *Lr24*, *Lr28,* and *Lr9* in the three rust resistant near isogenic lines (NILs) of HD2329 and the application of molecular markers facilitated identification of individual plants in three-way cross (HD2329 + *Lr24* × HD2329 + *Lr28*) × HD 2329 + *Lr9*, F_1 and F_2 generations possessing the targeted genes and finally, one bulked progeny in F_8 generation was identified carrying the desired resistance genes, *Lr24*, *Lr28,* and *Lr9* in homozygous condition in the background of HD2329. Many wheat lines carrying *T. timopheevii*-derived linked gene *Sr36/Pm6* in the background of many commercial Indian bread wheat cultivars were developed by employing simple back cross technique assisted by MAS using the donor line 'Cook*6/C 80–1' an Australian cultivar which carried *Sr36/Pm6* and in addition *Lr19/Sr25*. Some genes have masking effects, for example, *Xa21*, which confers resistance to many races of bacterial blight. It is difficult to distinguish between plants having *Xa21* alone and those having *Xa21* and other recessive genes through

conventional methods. Marker assisted selection allows the identification of plants contain multiple genes. The resistance genes *xa5*, *xa13*, and *Xa21* have been pyramided into an *indica* rice cultivar (PR106) using MAS that expressed strong resistance to BB races of India (Singh et al. 2001). Successful marker assisted pyramiding of disease resistance genes has already been reported in wheat with respect to three leaf rust genes *Lr13*, *Lr34,* and *Lr37* (Kloppers and Pretorious 1997) and three powdery mildew genes *Pm2*, *Pm4a,* and *Pm21* (Liu et al. 2000).

MABB coupled with phenotypic selection for agronomic, grain, and cooking-quality traits has been used to incorporate bacterial blight resistance genes *xa13* and *Xa21* into 'Pusa Basmati 1'. One improved line was released as 'Improved Pusa Basmati 1' for commercial cultivation in 2007 (Gopalakrishnan et al. 2008). A three-gene pyramided line, RPBio-226 (IET 19,046), containing bacterial blight resistance genes *Xa21*, *xa13*, and *xa5*, developed through marker assisted breeding named 'Improved Samba Mahsuri', was released for commercial cultivation (Sundaram et al. 2008). Shanti et al. (2010) introgressed bacterial blight resistance genes *Xa4*, *xa13*, *xa5*, and *Xa21* into the parental lines KMR3, PRR78, IR58025B, Pusa 6B of hybrid rice and the popular cv. Mahsuri by MABB. Likewise, a number of resistance genes have been introgressed into many elite rice varieties across the world (Ragimekula et al. 2013).

Molecular marker assisted pyramiding of major genes governing resistance to *turcicum* leaf blight and *Polysora* rust in elite five Indian maize lines, viz., CM137, CM138, CM139, CM140, and CM212 has been achieved at Indian Agricultural Research Institute (Prasanna et al. 2010). *Turcicum* leaf blight resistant genes, i.e., *Htn1*and *Ht2* along with a QTL (*RppQ*) for *Polysora* rust from four resistant donors, viz., NAI 147, SKV 21, NAI 112, and SKV18 were pyramided together by generating seven different backcross populations.

16.5.5.4 Marker Assisted Recurrent Selection (MARS)

Phenotypic recurrent selection involves cycles of selection, evaluation, and recombination that aims at increasing the frequency of favourable allele within the population commonly in cross-pollinated crops. In Marker Assisted Recurrent Selection (MARS), markers associated with trait of interest are first identified and selection is based on several genomic regions involved in the expression of complex traits to assemble the most superior genotype within a population (Ribaut et al. 2010). MARS may prove to be one of the most important strategies in the molecular breeding programme as this can be used for multiple QTLs controlling the expression of a complex trait (Knoch et al. 2020) which is a limitation in case of Marker Assisted Selection (MAS) and Marker Assisted Backcross Breeding (MABB) (Gokidi et al. 2016). Marker Assisted Recurrent Selection (MARS) consists of four main steps, training population development, QTL analysis and selection, recombination, and fixed lines development. The training population is genotyped using genetic markers and evaluated for phenotype in field trials. QTL analysis correlates phenotypic variance to genotypic variance and also identifies potential genomic regions involved in quantitative traits. After QTL identification in progeny

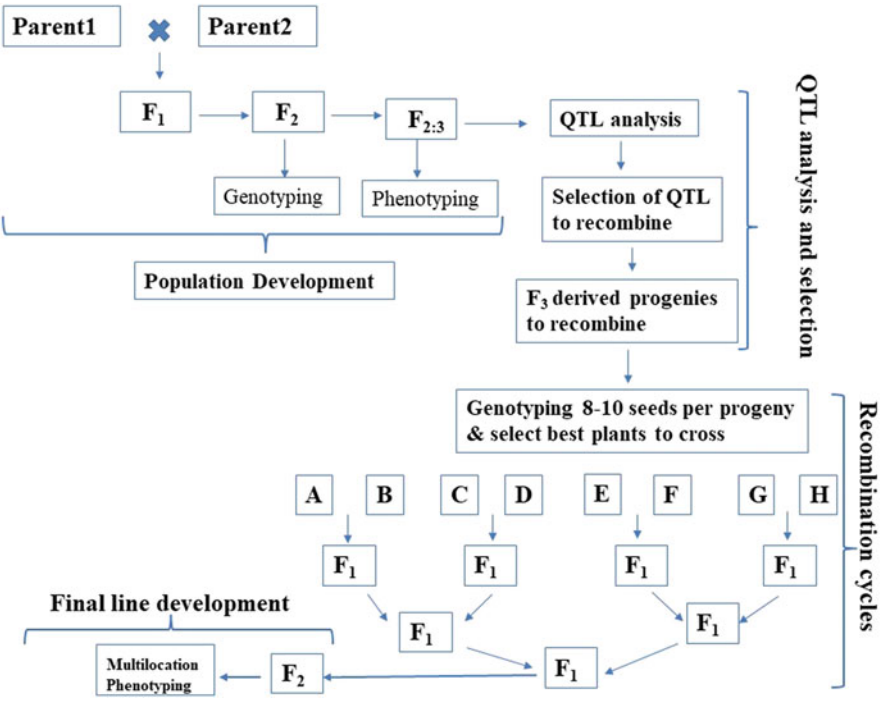

Fig. 16.7 Basic steps in molecular markers assisted recurrent selection

from training population, selected progenies are allowed to intermate and finally recombinants obtained are selfed to get homozygosity in the breeding population.

The procedure of MARS involves estimation of marker effects from genotyping of F_2 or F_3 population and phenotyping of F_2 or F_3 derived F_4 or F_5 progenies, followed by two to three recombination cycles based on presence of marker alleles for small effect QTLs (Eathington et al. 2007). In the first step of MARS, fingerprinting of the progeny from a given breeding population derived from biparental cross with specific molecular markers is performed. This means that the QTLs are identified in the 200–300 or more than 300 progenies from F_3 population with the help of specific molecular markers to enable the calculation of a genotypic value for each progeny. In the second step, about 200–300 progenies from the F_3 derived population, i.e., $F_{3:4}$ or $F_{3:5}$ are evaluated at different locations in multilocation trails to get phenotyping data. Based on the genotyping followed by phenotyping data, few plants are selected and allowed for controlled pollinations or intermating for two or three cycles. Subsequently, the recombined lines (F_3 or $F_{3:4}$) produced by two or three cycles of recombination or intermating among the selected plants are selfed for 2 or 3 years and are then subjected to a final phenotypic screening at multilocation trials to select the best lines to release as varieties (Fig. 16.7) (Eathington et al. 2007; Gazal et al. 2015).

16.5.6 Genomic Selection (GS)

Marker assisted selection is suitable for selecting traits controlled by major genes, but it has limited applications for selecting traits such as yield and related traits which are controlled by many genes with small effects. In such situation, genomic selection which incorporates genome-wide markers for selection of all traits' loci is reliable selection approach. As the genetic architecture of resistance shifts from single major R-genes to a diffused architecture of many minor genes, the best approach for molecular breeding will shift from marker assisted selection to genomic selection. Genomic selection (GS) is a form of marker-based selection, which was defined by Meuwissen (2007) as the simultaneous selection for many (tens or hundreds or thousands) markers, which cover the entire genome in a dense manner so that all genes are expected to be in linkage disequilibrium with at least some of the markers. In GS genotypic data (genetic markers) across the whole genome are used to predict complex traits with accuracy sufficient to allow selection on that prediction alone. Selection of desirable individuals is based on genomic estimated breeding value (GEBV) (Nakaya and Isobe 2012), which is a predicted breeding value calculated using different methods based on genome-wide dense DNA markers (Meuwissen et al. 2001). Several statistical approaches have been proposed for the prediction of genomic estimated breeding values (EBVs), such as best linear unbiased prediction (BLUP) and a Bayesian framework. GS does not involve significant testing and identifying a subset of markers associated with the trait (Meuwissen et al. 2001). In other words, QTL mapping with populations derived from specific crosses can be avoided in GS. However, it does first need to develop GS models, i.e. the formulae for GEBV prediction (Nakaya and Isobe 2012). In this process, phenotypes and genome-wide genotypes are investigated in the population called training population (a subset of a population) to predict significant relationships between phenotypes and genotypes using statistical approaches. Subsequently, GEBVs are used for the selection of desirable individuals in the breeding phase, instead of the genotypes of markers used in traditional MAS (Fig. 16.8). For accuracy of GEBV and GS, genome-wide genotype data is necessary and require high marker density in which all quantitative trait loci (QTLs) are in linkage disequilibrium with at least one marker. In genomic selection, selection is based on two separate populations, a training population and a breeding population. Training population is related to breeding population and is used for estimation of parameters of the genomic selection model. This population is genotyped for a large number of markers covering whole genome, and precisely phenotyped for the target trait(s) in replicated trials. The genotypic and phenotypic data is analysed with the help of suitable GS model to estimate the effects of individual marker on the target trait. These estimates of marker effects are used to compute the genomic estimated breeding values (GEBVs) of all the plants in breeding population. The marker genotypes of plants are used to calculate GEBVs, which is the sum of the effects of all marker alleles present in a given plant. The selection in breeding population is based on GEBVs, and there is no phenotypic evaluation of this population (Meuwissen et al. 2001). Genomic selection plays important role in crop improvement programmes including

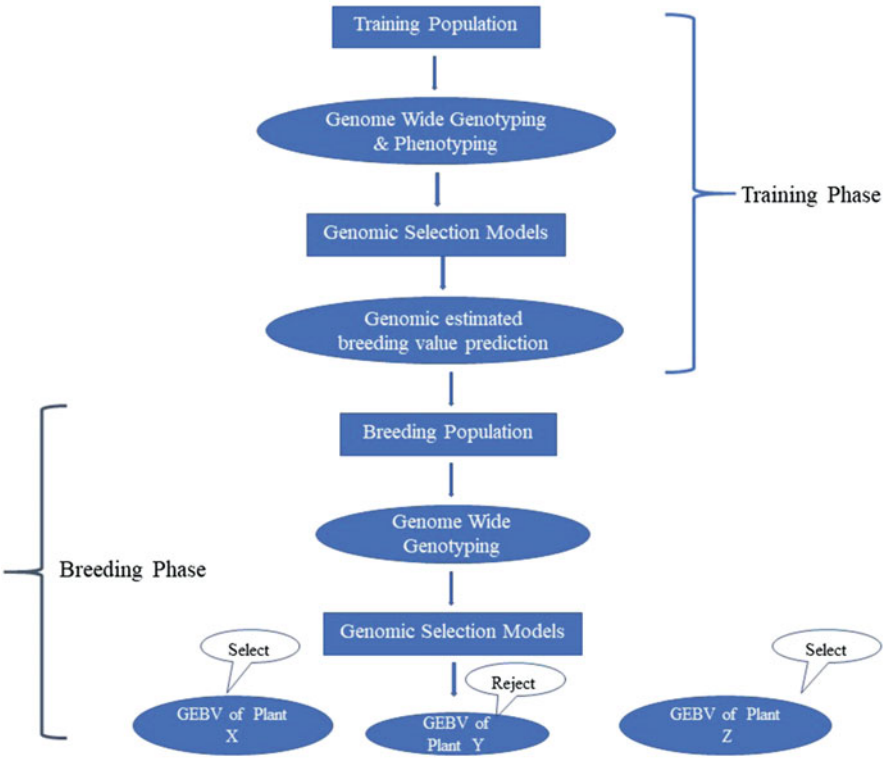

Fig. 16.8 Generalized scheme of genomic selection for desirable traits

disease resistance breeding. For example, genomic best linear unbiased prediction (GBLUP) and Bayesian regression methods are used to predict rust resistance in 206 landraces of wheat (Daetwyler et al. 2014). In maize genome-wide selection is used to identify lines with resistance to *Stenocarpella maydis* causing ear rot in maize.

16.5.7 Association Mapping in Disease Resistance Breeding

Association mapping is also known as linkage disequilibrium mapping. It is a method of mapping QTLs that take advantage of linkage disequilibrium to link phenotypes to genotypes. The diverse lines from natural populations or germplasm collections are used and markers linked to gene controlling the target traits are discovered. Association studies are based on the assumption that a marker locus is closely linked to the target trait so that some markers alleles would be travelling along with the target trait through many generations during recombination (Rodriguez-Murillo and Greenberg 2008). Association mapping is of two types, genome-wide association mapping search whole genome for genetic variation. A

large number of markers are tested for association with various complex traits and it does not need any prior information about the candidate gene. Second type is candidate gene association mapping which dissect out the genetic control of complex traits based on available results from genetic, biochemical or physiology studies (Mackay 2001). Wang et al. (2012) used GWAS for identifying genetic factors to control head smut disease in maize. Genome-wide association mapping has been used by Juliana et al. (2018) to identify the candidate genes linked to leaf rust, stripe rust, and tan spot resistance in wheat.

16.5.8 Genetic Engineering in Resistant Breeding

With advances in the understanding of genetically controlled defense responses and genetic basis of products being produced during defense response opened new horizons of molecular research on plant diseases. Molecular mapping of disease resistance genes, their cloning and generation of transgenic lines using genetic engineering methods are very promising approaches. The most important contribution of genetic engineering in varietal development is through gene isolation and transformation technology using transgenic resistance against disease pathogens. When resistance genes are not found in a particular species or even in its wild relatives and land races, resistance cannot be introgressed through conventional hybridization. In this situation, genes of resistance are introduced from unrelated species through recombinant DNA technology to overcome the genetic barriers. Foreign genes are transferred to crop plants using different transformation tools like gene gun or particle bombardment, electroporation, floral dip (direct transformation methods), and *Agrobacterium* mediated transformation (in direct transformation methods). Cisgenic plants, on the other hand, have inserted gene(s) from the same species; however, as the inserted gene is a recombinant DNA they are also considered as GMOs (Genetically modified organisms). Transgenic technology aimed at engineering for the expression of many antifungal genes including pathogenesis-related (PR) proteins, phytoalexins, hydrolytic enzymes, antimicrobial peptides, and resistance (R) genes. The expression of these antifungal genes was successfully incorporated into plants via, transgenic technology contributing to significant resistance against fungal pathogens. For disease resistance, candidate genes involved in plant–microbe interaction should reduce the virulence traits of the pathogens, e.g., pathogen cell wall degrading enzymes and toxins. Such genes are introduced to plants that enhance the production of plant defense molecules and confer resistance to plants against different diseases (Strange 2005). In rice transgenic having *NH1* gene, an *NPR1* gene orthologue, provided resistance against *Xanthomonas oryzae* pv *oryzae* causing bacterial leaf blight disease in rice (Chern et al. 2005). Various genes involved in pathogenesis and plant immunity have been dissected and used for developing durable disease resistant crops through transgenic approaches like over-expression/gene complementation tests, Small RNA (microRNA), RNA interference (RNAi), CRISPR/Cas systems (Gurr and Rushton 2005a, b; Singh et al. 2016). A broader and in depth understanding of plant resistance along with

transcriptomics, proteomics, metabolomic, and protein interaction studies have led to the identification of several candidate genes from plants, bacteria, viruses, and fungi that potentially can enhance resistance. These candidate genes can be constitutively over expressed, induced to express under biotic threat, tissue-specifically expressed, knocked out or silenced by RNAi to obtain the desired resistance trait. Plant molecular biology and biotechnology techniques have taken a rapid progress in the identification and cloning of genes involved in plant defense responses. In the following sections, various transgenic approaches being used for the development of disease resistance in different crops are explained:

16.5.8.1 Pathogenesis-Related Proteins (PR Proteins)

Pathogenesis-related proteins (PR) are a set of pathogen-induced proteins called as stress proteins. Genes encoding for PR proteins provide a promising source of resistance against fungal pathogens. PR proteins are activated during hypersensitive response (HR) and systemically acquired resistance (SAR). PR proteins are grouped into 17 protein families PR-1 to PR-17 (Sels et al. 2008; Balconi et al. 2012) based on their structure, amino acid compositions, and biochemical responses. PR-1 and PR-5 (thaumatin-like proteins and osmotins) are termed as permatins as they target the membrane. Osmotin or thoumatin protein has in vitro antifungal activity against *Sclerotinia, Rhizoctonia, Botrytis, and Fusarium* (Woloshuk et al. 1991). PR-2 (b − 1,3-glucanases), PR-3, PR-4, PR-8, and PR-11 (chitinases) target the pathogen cell wall (Wally and Punja 2010; Honee 1999), PR-6 proteins (proteinase inhibitors) may target nematodes, whereas the PR-7 protein (an endoproteinase) may be involved in microbial cell wall dissolution (Jordá et al. 2000). The PR-9 family may enhance resistance to multiple pathogens by catalysing lignifications which helps in cell wall reinforcement (Passardi et al. 2004). PR-10 family has weak ribonuclease activity therefore may target pathogen RNA or play a role in defense against viruses (Park et al. 2004), PR-12 (defensins), PR-13 (thionins), and PR-14 (lipid transfer proteins) predicts antibacterial and antifungal activities (Epple et al. 1997), PR-15 (oxalate oxidases), and PR-16 (oxalate oxidase-like proteins) generate hydrogen peroxide and are toxic to pathogens and pests (Hu et al. 2003). PR-17 (uncharacterized) was detected in infected tobacco, wheat, and barley (Christensen et al. 2002)**.** Pathogenesis-related (PR) genes could increase the level of pre-existing barriers. Naturally occurring PR proteins are constitutively expressed at low levels and are induced to high levels challenged by pathogens or application of either salicylic acid or jasmonic acid (Ferreira et al. 2007)**.** Over-expressing thaumatin-like protein (TLP) in a rice line showed an enhanced level of Sheath blight resistance compared to the control plants (Rajesh et al. 2016).

16.5.8.2 Hydrolytic Enzymes in Host

Cell wall of fungal pathogens is made up of chitin and glucan molecules which can be degraded using chitinase and glucanase enzymes produced as a result of fungal infection. These enzymes cause degradation of fungal cell walls by breaking chitin and glucan molecules. In transgenic plants this phenomenon is utilized by inducing overexpression of genes encoding hydrolytic enzymes (Shin et al. 2008). Chitinases

and glucanases belonging to group of PR proteins and over-expression of these PR proteins in plants are assumed to cause lysis of fungal hyphae and inhibit the fungal growth. These enzymes are expressed in both resistant and susceptible tissues, their role in non-transgenic plant resistance are difficult to prove. These enzymes are also activated by stress (Punja and Zhang 1993). Transgenic plants introduced with genes for chitinase production have been evaluated in vivo exhibited reduced fungal infection leading to disease resistance (Grison et al. 1996). The synergic effect of chitinase and glucanase prevented the development of many fungal pathogens (Van den Elzen et al. 1993; Zhu et al. 1994) and hence transgenic expression of more than one hydrolytic enzyme in combination can provide broader resistance as compared to expression of a single enzyme (Lamb et al. 1992; Evans and Greenland 1998; Melchers and Stuiver 2000). Varying levels of resistance towards powdery mildew were observed in transgenic wheat lines carrying a barley chitinase or a barley β-1, 3-glucanase (Bieri et al. 2003). Chenault et al. (2005) expressed a rice chitinase and an alfalfa glucanase in transgenic peanut and observed enhanced resistance against *Sclerotinia* blight in the transgenic plants. Expression of barley oxalate oxidase in transgenic peanut also enhanced resistance to *Sclerotinia minor* (Livingstone et al. 2005). Transgenic peanut expressing a tobacco chitinase gene was shown to possess enhanced resistance to the late leaf spot caused by *Phaeoisariopsis personata* (Rohini and Rao 2001; Anuradha et al. 2008). Transgenic wheat expressing the *Arabidopsis NPR1* gene, a gene that regulates defense responses, was shown to exhibit a high level of resistance to fusarium head blight in greenhouse evaluations (Makandar et al. 2006). Three genes, *ech42*, *nag70,* and *gluc78*, encoding hydrolytic enzymes, from a biocontrol fungus *Trichoderma atroviride* were introduced in rice. *Gluc78*-overexpressing transgenic plants showed enhanced resistance to *Magnaporthe grisea*, while transgenic plants over-expressing the *ech42* gene encoding for an endochitinase, increased resistance to *R. solani*, resulting in 62% reduction in sheath blight disease index (Liu et al. 2004; Shah et al. 2009). Plant scientists have discovered a chitinase gene from an antifungal biocontrol fungus species (*Trichoderma viride*), which infers transgenic resistance to rice against sheath blight pathogen. Shao et al. (2008) introduced a harpin encoding gene *hrf1*, derived from *X. oryzae* pv. *oryzae*, into rice and generated transgenic rice lines with overexpression of the *hrf1* gene. Disease assays revealed that the *hrf1* overexpressing transgenic rice plants were highly resistant to all major *M. grisea* races. Rice RC24 chitinase gene was introduced into wheat to confer resistance against *Puccinia graminis* f. sp. *tritici* (Huang et al. 2013). Transgenic expression of Rice chitinase class-I gene (*RCH10*) in lilium increased its resistance against infection of *Botrytis cinerea* (de Cáceres et al. 2015). Richa et al. (2017) also developed transgenic rice plants harbouring novel chitinase gene (*LOC_Os11g47510*) through genetic transformation resulting in higher resistance against sheath blight (ShB) disease.

16.5.8.3 Inhibition of Cell Wall Degrading Enzymes in Pathogens

Plant pathogens are categorized as biotrophs, hemibiotrophs, or necrotrophs depending upon their mode of infection and nutrient uptake (Laluk and Mengiste

2010). Necrotrophic and hemibiotrophic pathogens produce large quantity of cell wall degrading enzymes to kill host cells for nutrients or to invade the host cell. Sometimes bacteria also cause the degradation of host cell wall during infection process to acquire nutrients. There is direct relationship between disease severity and degree of damage to cell wall and its components. The main components of plant cell wall comprised of cellulose, hemicelluloses, pectin, lignin, and other structural proteins. Peroxidase and laccases enzymes of fungal pathogens stimulate the degradation of lignin. Hydrolases such as cellulase and hemicellulase secreted by both fungal and bacterial pathogens hydrolysed glycoside bonds of cellulose and hemicelluloses (Kubicek et al. 2014). During host–pathogen interaction, there is significant multiplication of genes coding cell wall degrading enzymes in fungi and such genes are present as gene clusters in fungal genomes. Most of the fungi cause differential infection on monocot and dicot plants. Genes encoding pectin lyase enzyme are more in number in the genome of fungi which are pathogenic for dicots. The mode of infection of pathogens also regulated the differential expression of cell wall degrading enzymes. The expression of cell wall degrading enzymes was upregulated in hemibiotrophic pathogens, whereas the expression of pectinases and xylanases was upregulated in necrotrophs during infection and colonization of host plants (Fernández-Acero et al. 2010; Zhao et al. 2013). Since biotrophic pathogens depend upon living cells of host for nutrition comparatively a smaller number of genes encoding cell wall degrading enzymes are reported in the genomes of these pathogens (Zhao et al. 2013) whereas the mechanism of pathogenicity of necrotrophic and hemibiotrophic pathogens can be exploited through genetic engineering by developing engineered plants to inhibit the plant cell wall degrading ability of the pathogens. The polygalacturonases are another group of fungal and bacterial enzymes which degrade polygalacturonan constituent of pectin. Transgenic plants overexpressing the genes encoding for polygalacturonase inhibitory proteins have shown remarkable reduction in disease symptoms caused by *B. cinerea* and *Bipolaris sorokiniana* and disease symptoms were restored upon antisense suppression of these genes (Wally and Punja 2010). The cassette comprising the constitutive promoter of maize ubiquitin gene *Ubi1* and the bean *Pvpgip2* gene was used to transform durum and bread wheat and the modified wheat showed a significant reduction in foliar spot blotch symptoms caused by the fungal pathogen *B. sorokiniana* (Ferrari et al. 2012).

16.5.8.4 Phytoalexins

Phytoalexins are low molecular weight secondary metabolites possessing antimicrobial resistance against many fungal and bacterial pathogens. Phytoalexins are produced as a result of pathogen's attack or mechanical injury. The phytoalexin accumulation is a part of host defense mechanism and is less toxic than chemical fungicides. The elicitors molecules signal plants to synthesize phytoalexins during host–pathogen interaction. These elicitors are high molecular weight substances which are the constituents of fungal cell wall and are released by host plant enzymes. Most of these elicitors are non-specific and induce phytoalexin accumulation irrespective of plant cultivars. Many phytoalexins have been characterized in

different plant families, for example, terpenoids in *Solanaceae* family, oflavonoids in *Leguminosae* family. Diterpenoid phytoalexins are produced by the plants of Poaceae family. Corn plants produce diterpenoid phytoalexin when infected with pathogens such as *Aspergillus flavus*, *Aspergillus sojae*, *Cochliobolus heterostrophus*, *Colletotrichum sublineolum*, *Fusarium graminearum*, *Ostrinia nubilalis*, *Rhizopus microspores,* and *Ustilago maydis.* The speed of accumulation of phytoalexins is associated with resistance in plants to diseases, though genetic information of their synthesis is found in the susceptible and resistant plants (Singh and Chandrawat 2017). Phytoalexins have been expressed transgenically to provide resistance against many fungal pathogens (Leckband and Lorz 1998). The synthesis of phytoalexins undergoes a complex biochemical pathway (Dixon et al. 1996) including shikimic acid pathway. To achieve genetic manipulation of these pathways in order to suppress or enhance phytoalexin production is quite difficult. Similarly, as in the case of hydrolytic enzymes, it has always been difficult to precisely explain the involvement of phytoalexins in enhancing disease resistance against many fungal pathogens.

16.5.8.5 R-Genes Mediated Resistance

The development of disease resistant varieties is an alternative approach for the use of pesticides or other chemical control methods, which minimizes the bad effect of chemicals on social animals and environment. For the development of resistant varieties, the nature of R-genes and corresponding Avr genes, detailed knowledge of disease resistance, its types and of the different breeding methods is must. The plant immune system is known to activate itself in response to invading pathogen due to the recognition of pathogen-associated molecular patterns or microbe-associated molecular patterns (PAMPs/MAMPs) by corresponding resistance (*R*) genes (Liu et al. 2007). At molecular level, the Flor's gene-for-gene concept served as a base to demonstrate and understand the mechanism of host–pathogen interactions, which stated that 'For each resistant gene in a host there is a corresponding gene of avirulence in pathogen conferring resistance and vice versa'.

In plants, circulating antibody system is absent therefore to fight or to maintain constant vigilance against invading pathogens they are known to express large arrays of R-genes. Most of the R-genes have conserved motifs/domains such as nucleotide-blinding site (NBS), leucine-rich repeat (LRR), interleukin-1 receptor (TIR), a coiled-coil (CC), leucine zipper (LZ), transmembrane domain (TM), and protein kinase domain (PK). The majority of R-genes encodes for proteins having at least three domains, viz., a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide binding site (NBS) domain, and an N-terminal domain that either contains homology to cytosolic domains of the Drosophila Toll or animal interleukin-1 receptors (TIR) or a potential coiled-coil (CC) domain (TIR-NBS-LRR or CC-NBS-LRR).

Based on the organization of amino acid motif and membrane spanning domains, plant disease resistance genes are divided into eight groups, viz., NBS-LRR-TIR (*N, L6, RPP5*), NBS-LRR-CC (*I2, RPS2, RPM1*), LRR-TrD (Cf-*9,* Cf-*4,* Cf-*2*), LRR-TrD-Kinase (*Xa-21*), TrD-CC (*RPW8*), TIR-NBS-LRR-NLS-WRKY (*RRS1R*),

LRR-TrD-PEST-EC (*Ve1, Ve2*), and enzymatic R-genes (*Pto, RPG1, Hm1*) (Gururani et al. 2012). And out of these, the NBS-LRR genes represent the largest class of *R*-genes, which encodes for proteins with variable N-terminal domains. R-genes recognizes Avr gene products and trigger multiple responses like production of reactive oxygen species, accumulation of various inhibitory metabolites, salicylic acid (which leads to systemically acquired resistance—SAR), induction of PR proteins, etc. which at the end results in hypersensitive response or programmed cell death (Ryals et al. 1996; Kombrink and Schmelzer 2001).

With the advancements in genetic engineering, numerous R-genes were discovered and cloned against several pathogens and their discovery has proved as a breakthrough in plant protection. The first *Avr* gene was cloned by Staskawicz et al. (1984) from *Pseudomonas syringae*. Thereafter, so many R-genes were identified conferring excellent amount of resistance against many fungal pathogens (Dixon et al. 1996). The first R-gene *Hm1* was cloned from maize which encodes for NADPH dependent reductase to inhibit a toxin produced by *Cochliobolus carbonum* (Johal and Briggs 1992). The continuous selection pressure in *avr* genes of fungal pathogens and sequence diversification had overcome R-gene mediated host resistance in a many of the newly engineered crops (Fawke et al. 2015; Kumari et al. 2017) and despite of various advantages, it may confers threat to the plant by evolving an alternate *Avr* gene affecting overall fitness of the plant (Collinge et al. 2008). To triumph over failure of engineered varieties, gene stacking methods like gene pyramiding for combing two or more genes are being used. The utilization of R-genes for biotic and abiotic stress management may serve as an excellent alternative of chemical control methods and could be exploited effectively in near future to meet the demands of growing world by protecting plants.

16.5.8.6 S-Gene Mediated Resistance/Concept of Loss of Susceptibility Leading to Resistance

The molecular interaction between microbial effectors and host factors either leads to susceptibility or resistance in plants and by going through this molecular intricacy of plant–pathogen interaction, the development of disease-free crops is possible. Jones and Dangl (2006) explained the various steps involved in plant–pathogen interaction and also describe how both of them evolve in response to each other. Various host (fatty acids of plant cuticle, galacturonan molecules of host pectin, phenolic compounds such as strigol, isoflavones, amino acids, and sugars) and pathogen components (activation of the cutinases and pectin lyases, etc.) are reported to be involved in recognition process. Although, it is still unclear how both of them recognize each other, i.e., the nature of early event is not known (Agrios 2005). Targeting or altering host components which otherwise favour its susceptibility/predisposition towards invading pathogen will provide comprehensive and durable resistance. In host plants, there exist preformed passive barriers, i.e., structural characteristics that act as physical barriers and biochemical reactions that take place in the cells and tissues of the plant to induced defense reactions (Agrios 2005). Similarly, on the other side, pathogens either with the help of mechanical forces exerted on host tissues or chemical weapons infects host. As discussed in

earlier section, both host and pathogen survive over considerable periods of time to maintain dynamic equilibrium of resistance and virulence. From long back, most of the studies were based on R-genes and their role in plant protection but in 2002, a gene coding for a susceptibility factor, i.e., *PMR6* was discovered, for promoting growth of powdery mildews. Nowadays, the research is moving towards the exploitation of plant susceptibility genes (S-genes) for durable and broad spectrum resistance. The concept of loss of susceptibility (LOS) is in limelight from last few years and it is generally considered to be as recessively inherited resistance. The loss of susceptibility (LOS) is also known as recessively inherited resistance and can be utilized by effectively exploiting genes responsible for susceptibility in plants (Hückelhoven et al. 2013). Plant pathogens exploit plant's susceptibility (S) genes to cause disease and disruption of these S-genes may lead to the incompatible reaction between both of the interacting partners, thereby provide broad spectrum and durable disease resistance (Zaidi et al. 2018). These susceptibility genes (S-genes) code for effector targets that function as negative defense regulators or susceptibility factor and these S-genes are dominant genes which when mutilated will lead to recessive resistance. The importance of susceptibility genes in developing resistant crops was first elucidated by Eckardt (2002). In Arabidopsis-powdery mildew interaction, some mutants were found which do not support normal growth of *Erysiphe cichoracearum* and was reported to be because of *PMR6* which encodes for a pectate lyase—like protein with a novel C-terminal domain. A mutation in *PMR6* alters the composition of the plant cell wall and it was also reported that, *pmr6*-mediated resistance does not require the activation of well-defined defense-related pathways (Vogel et al. 2002). S-gene mediated resistance approach has limited application in developing resistant crops as these genes are also involved in other important processes of plants and silencing of these genes will be lethal for plants.

16.5.8.7 Manipulation of Interactions Between Defense-Related Signalling Molecules

In nature, changes in the host resistance appear to be continually balanced by shift in virulence of the pathogen (Longdon et al. 2015). Detailed knowledge about how pathogens attack plants and how plants respond/defend against invading pathogen will help in developing broad spectrum disease resistant crops. In response to pathogenic invaders, plants activate different defense mechanisms. Jones and Dangl (2006) explained in detail about two interconnected branches of plant immunity, i.e., PTI (pathogen-associated molecular pattern (PAMP)-triggered immunity), and ETI (effector-triggered immunity). PTI is initiated by the recognition of molecular signs of many pathogens which further leads to the activation of downstream mitogen-activated protein (MAP) kinase cascades and defense genes, whereas ETI is because of plant disease resistance proteins (product of major R-genes) which actually recognizes directly or indirectly pathogen-derived effectors. Systemic defense responses also called as systemic acquired resistance (SAR) are activated by these PTI and ETI responses of plants. The cross-talk between different signalling molecules involved in defense-related pathways leads to the production of plant

hormones like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Other hormones such as abscisic acid, gibberellin, cytokinin, and brassinosteroid have also been emerged as modulators of plant immunity (Denancé et al. 2013). Recent advances in plant defense signalling research open doors in the development of new varieties which are genetically engineered. Development of new chemicals which mimic the action of various signalling compounds involved in biosynthetic pathways through genetic engineering provides useful tools for the development of new strategies for crop protection. However, the activation of a plant defense response is not specific to a particular pathogen, it helps in developing resistant crops against number of pathogens. Due to the expression of cloned transgenes which encodes for signalling mimickers induce the activation of a complete arsenal of defense-related components. MAPK (Mitogen Associated Protein kinase) components, certain transcription factors (TFs) and non-pathogenesis-related protein 1 (NPR1), all can be utilized in the development of disease resistant crops. Nowadays, transcriptional reprogramming is also gaining importance because of conserved regions present in transcription factors. There are several TFs such as ethylene response factor (ERF), WRKY, Myb, TGA-bZIP, Whirly, NAC, and TGA2 have been reported to be involved in plant defense against biotic factors and these can be used in the manipulation strategy with the aim to get resistant varieties (Eulgem 2005; Ryu et al. 2006; Naoumkina et al. 2008; Alves et al. 2013). The WRKY transcription factors and MYB transcription factors are among the most studied TFs during the last decade and in addition to these TFs containing a basic leucine zipper domain (bZIP) also gaining importance. A bZIP domain is among the largest families of transcription factors in plants and in plants, these factors regulate genes in response to abiotic stress, seed maturation, flower development, and pathogen defense. The role of bZIP TFs in plant growth and its defense against various factors has been studied in many crops and nearly about 127 bZIPs are known in Arabidopsis, 70 in cotton, 266 in soybean, 47 in tobacco, 70 in tomato, 140 in rice, 102 in wheat, and 218 in maize (Ali et al. 2016). Another group of transcription factors, i.e., Whirly proteins, also contributes to defense against invading pathogens. In arabidopsis and potato, orthologs of Whirly proteins are reported to act as TFs which regulates the expression of defense-related genes (Desveaux et al. 2005).

NPR1 is considered as the positive regulator of SA mediated immune responses in plants, i.e., induces long-lasting immune response called systemic acquired resistance (SAR) similar to the adaptive immunity of animals (Silva et al. 2018). NPR1 is a receptor of salicylic acid (SA) which modulates multiple immune responses in plants especially activation of induced and systemic acquired resistance (SAR). Although, AtNPR1 homologs were first discovered in Arabidopsis, but has been successfully isolated and utilized in many agriculturally important crops. Ali and associates (2017) developed BjNPR1 transgenic lines which showed enhanced resistance to *Alternaria brassicae* and *Erysiphe cruciferarum*. In these genetically engineered lines, there is delay in symptoms and reduced disease severity as compared to non-transgenic plants. According to them, the overexpression of NPR1 in *Brassica juncea* actually confers broad spectrum resistance to fungal

pathogens (Ali et al. 2017). Although, its overexpression enhances disease resistance but, it also resulted in yield losses. Therefore, it is important to study in detail the physiological and molecular mechanisms involved in resistance elicitation.

16.5.8.8 RNAi Technology

The origins of RNA silencing are that of an ancient mechanism that directly defends host cells against foreign nucleic acids, including viruses and active transposable elements. This defense is stimulated by double-stranded RNA (dsRNA), a signature molecule derived from amplification of invasive nucleic acids, which is processed by the host into small RNAs (sRNAs) that are 20–24 nucleotides (nt) in size. These sRNAs are then used to guide the silencing of the viral or transposable element RNA or DNA through transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS), respectively (Peragine et al. 2004). Host-induced gene silencing (HIGS) is a highly conserved process that targets messenger RNA (mRNA) transcript and degrades it in all eukaryotes to silence the gene (Kamthan et al. 2015). Because the regulation occurs at a transcriptional level, the process in plants is also called post-transcriptional gene silencing. This RNA silencing process is called quelling in fungi (Duan et al. 2012). RNA-mediated gene silencing involved in switching off the expression of specific genes of fungi responsible for pathogenicity and is an advanced approach for enhancing resistance against fungi (Sanghera et al. 2009). Plant pathogenic fungi develop direct connections with their host plants via a specialized structure known as haustorium which act as an interface for signal exchange as well as nutrient uptake (Panstruga 2003). It allows fungi to uptake dsRNA's or siRNA's during nutrient uptake from targeted host plant to activate RNA-mediated gene silencing. Gene silencing by introduction of dsRNA has been successfully employed against many fungal pathogens including *Magnaporthe oryzae, Neurospora crassa, Venturia inaequalis,* and *Aspergillus nidulans* (Kadotani et al. 2003; Fitzgerald et al. 2004; Goldoni et al. 2004; Hammond and Keller 2005). Nakayashiki et al. (2005) silenced *mpg1* gene and polyketide synthase genes. *Mpg1* gene is a hydrophobin gene which triggers the development of appressorium, hence plays an important role in pathogenicity (Talbot et al. 1996) and these genes were successfully silenced by p-Silent-1 based vectors in up to 90% transformants. This concept of RNA-mediated gene silencing was observed in case of barley powdery mildew caused by *Blumeria graminis* (Nowara et al. 2010).

RNAi plays critical roles in developmental regulation, stress response, and host defense against transposons and viruses. The natural defensive aspect of this approach is scientifically exploited to develop disease resistant crops. In this approach, a siRNA is produced inside the plant and it moves into the pathogen to silence pathogenesis-related genes (Govindarajulu et al. 2015). RNAi technology works as (1) the entry of double-stranded RNA which may be an introduced transgene or a viral intruder, triggers the RNAi pathway of cells which results in the production of enzyme Dicer, (2) Dicer cleaves the dsRNA into short, 20–25 basepairs long fragments called small interfering RNA (siRNA), (3) an RNA induced silencing complex (RISC) which distinguishes between the two strands of small interfering RNA as sense or antisense. The sense strand which has exactly the

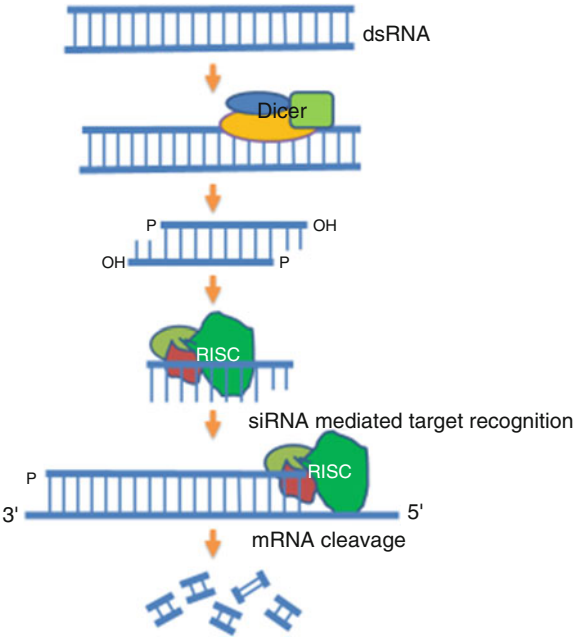

Fig. 16.9 General mechanism of RNAi pathway

same sequences as the target gene, (4) the antisense strands are incorporated to the RISC which are used as guide to target messenger RNAs (mRNA) in a sequence specific manner, (5) messenger RNAs (mRNA), which codes for amino acids are cleaved by RISC (Sherman et al. 2015 Fig. 16.9). The activated RISC can repeatedly cause mRNA degradation, thus inhibiting protein synthesis (Fire et al. 1998; Meister and Tuschl 2004; Borges and Martienssen 2015; Wagh et al. 2016). The target genes of pathogens are expressed and dsRNA is generated using the plant machinery. This dsRNA is used as a precursor for generating smaller RNA fragments complementary to the genes expressed distantly in the pathogen (Nowara et al. 2010). Due to the presence of variations in precursor RNA for the generation of siRNA, a diverse targeting approach has been designed for gene silencing which include sense/antisense RNA, small/long hairpin RNA, and artificial miRNA precursor (Duan et al. 2012).

RNAi can provide broad spectrum resistance against highly variable pathogens like viruses and also with this technology it would be possible to target multiple genes for silencing by using a thoroughly-designed single transformation construct.

Development of host-induced RNAi system has also been reported in wheat stripe rust fungus (*Puccinia striiformis* f.sp. *tritici*) where gene fragments from the rust fungi *Puccinia striiformis* f.sp. *tritici* or *P. graminis* f.sp. *tritici* were delivered to plant cells through Barley stripe mosaic virus system and some reduced the expression of the corresponding genes in the rust fungus. This is associated with fungal gene expression patterns (Yin et al. 2011). 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). Transgenic rice lines carrying a hybrid RNAi construct targeting two pathogen genes MAP kinases RPMK1–1 and RPMK1–2 increased sheath blight resistance compared to the control lines (Tiwari et al. 2017). VIGS (Virus Induced Gene Silencing) is an important tool for triggering RNAi silencing with the use of viral vectors like BMV (Brome mosaic virus). VIGS acts as an efficient and rapid tool for assigning gene function in plants. Using BMV-HIGS, Zhu et al. (2017) reported that *MoABC1*, *MoMAC1,* and *MoPMK1* genes of *M. oryzae* were responsible for disease development.

16.5.9 Genome Editing Technologies

Genome editing is the beginning of new era to make precise changes in the genomic DNA by site-specific mutagenesis. Basically, in all genome editing techniques, sequence specific nucleases are used for the recognition of specific DNA sequences and these nucleases produced breaks in both the strand of DNA at targeted sites. Cellular DNA repair mechanism is of two types, nonhomologous end joining and homologous recombination (Voytas and Gao 2014). Non-homologous end joining is most common way of DNA repair but it resulted into insertion or deletion mutations due to errors. Double-stranded DNA breaks were supposed to be repaired by homologous recombination in the presence of donor DNA template resulting into specific base changes or replacement of genes. The field of functional genomics has been revolutionized with the advent of genome editing technologies in which engineered nucleases including zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPER)/CRISPER-associated protein 9 (Cas9) are used for gene editing (Han et al. 2020). Use of such nucleases offer opportunity for site directed mutagenesis without possible effects of background mutations in crop plants as in case of random mutagenesis (Pabo et al. 2001; Boch et al. 2009; Moscou and Bogdanove 2009; Qin et al. 2020). First generation genome editing tools like ZFNs and TALENs are expensive and time consuming as they require protein engineering. CRISPR/Cas9 genome editing involves the use of the same CAS9 with different guide RNAs for targeting multiple sites in the genome (Jaganathan et al. 2018). In this method the transgene construct can be eliminated through successive crossing to generate plants with desired mutated nucleotides without transgene. CRISPR targets the endogenous genes which is not possible using RNAi technology where gene regulation is governed by endogenous micro RNAs (miRNAs). Any displacement in miRNAs from exogenous miRNAs (miRNAs) can lead to hypomorphic mutations and off-target phenotypes (Khan et al. 2009). CRISPER technology is further advanced to include options for various genetic alterations like making precise modifications, generating knockouts, multiplex genome engineering, activation, and repression of target genes (Arora and Narula 2017). Wang et al. (2014) introduced mutations using site-specific endonucleases in homeoalleles encoding mildew resistance locus (MLO) proteins of hexaploid bread

wheat. Some examples include the resistance against rice bacterial blight disease by targeting *OsSWEET13* gene (Zhou et al. 2015), the resistance against rice blast disease by targeting *OsERF922* gene in rice (Wang et al. 2016). The stacking up of multiple nucleases as one transgene by CRISPR/Cas9 system also leads to the targeted cleavage of multiple infections by viruses (Iqbal et al. 2016). Protospacer adjacent motif dependent cleavage of target system is a major bottle neck of using CRISPER/Cas9 system. However, many CRISPER/Cas systems with different protospacer adjacent motif specificity have been identified (Wrighton 2018) and further, RNA targeting CRISPR/Cas systems have also extended the applicability of CRISPER toolbox (Yin and Qiu 2019).

Molecular information about target gene and known host genome sequences are prerequisite for successful genome editing. With the advent of next generation sequencing platforms, full genomes sequences of an increasing number of plants species have been available. Further, molecular and genetic studies about innate plant immunity revealed increasing numbers of candidate genes for the management of pest and diseases. Host susceptibility genes which regulate plant disease resistance negatively are good targets for genome editing.

Ethylene responsive factors (ERFs) of the APETELA2/ERF (AP2/ERF) superfamily play important role in rice adaptation to various biotic and abiotic stresses (Mizoi et al. 2012). The expression of OsERF922 is induced by rice blast pathogen *Magnaporthe oryzae*. Knockdown of OsERF922 by RNAi leads to increased resistance to *M. oryzae*, indicating that OsERF922 is a negative regulator of rice blast resistance (Liu et al. 2012). Targeted modification of OsERF922 using CRISPR/Cas9 generated rice Oserf922 knockout mutants (Wang et al. 2016). These mutants showed enhanced resistance to rice blast without affecting other major agronomic traits. Therefore, the targeted knockout of negative regulators or/and susceptibility genes via genome editing represents a powerful approach for plant disease resistance breeding. Presently, majority of disease resistant crops against various pathogens except viral pathogens have been developed using genome editing by targeted mutagenesis of susceptibility genes. The functional conservation of S-genes across plant species is exploited to generate desired S-gene mutants in most of plants for breeding without species barriers. Mutations in S-genes resulted adverse effects on plant growth as these S-genes are involved in plant growth and development which may limit its applicability.

Many times, R-genes are used for transferring disease resistance from wild species to cultivated elite varieties have single nucleotide variations. Newly developed base editors are used to generate specific base changes in cultivated improved varieties and recently synthetic immune receptors has open new horizons for breeding crops resistant to phylogenetically divergent pathogens (Giannakopoulou et al. 2015).

Disease resistance breeding is not limited to gene replacement or gene dispersion, but also gene regulation through genome editing. For example, in rice japonica rice plants carrying *Xa3* gene exhibited enhanced resistance spectrum as compared to indica rice plants due to increased expression of *Xa3* in japonica rice (Cao et al. 2007). Similarly, *Hm2* gene in maize provides dosage dependent resistance against

leaf spot and ear mould disease (Chintamanani et al. 2008). In contrast, dose of *GhLMMD* gene in cotton regulates programmed cell death and immunity and downregulation of this gene resulted in resistance to *Verticillium dehliae* infection (Chai et al. 2017). Multiple disease resistance plants have been obtained using CRISPR/Cas9 technology. Recently, developed CRISPR activation (CRISPRa) or CRISPR interference (CRISPRi) approaches can regulate target genes specifically and allow multiple gene regulation. These approaches can be used for breeding crops with broad spectrum resistance in future. Further, mRNA translation of target genes having upstream open reading frames can be modified by genome editing (Zhang et al. 2018).

16.6 Conclusion and Future Prospects

It has been a long journey of crop improvement from conventional practices to the novel strategies of plant genetic engineering for developing disease resistant crop plants. The various options available worldwide to control the spread of diseases are by various crop cultural and management interventions, breeding of resistant cultivars of crops, and by application of agrochemicals at the time of disease occurrence. Even after the adoption of various agricultural practices and agrochemicals, every year plant diseases account approximately 12% yield loss at the field level, to which is added 9–20% during post-harvest stages (Agrios 2005). Durable pest and disease resistance achieved so far by traditional breeding and chemical practices have been undergone through many scientific challenges and modifications till now. These conventional approaches are successful in controlling only some diseases and to overcome the limitations of conventional breeding approaches, new innovative approaches have been exploited to develop disease resistant varieties like mutation breeding, somaclonal variants, TILLING, MAS for disease resistance genes, marker assisted disease resistance gene pyramiding and genetic engineering (Fig. 16.10). These approaches also have certain limitations in applicability and used under specific situations. With the advent of next generation sequencing techniques, isolation and cloning of genes and transformation technology, genetic engineering technology has been most widely and successfully implemented in enhancing resistance against numerous fungal diseases. Designing tools to deliver genes directly into the tissue and in a stress-specific manner without disturbing the normal metabolic cycle of crops is an important aspect of genetic engineering (Gurr and Rushton 2005a, b). Genetic engineering for genes related to defense including PR proteins, hydrolytic enzymes, antimicrobial peptides, and phytoalexins have provided significant amount of resistance against fungal diseases. The combined expression of all defense-related genes have served as a remarkable source of resistance. Another approach includes RNA silencing 'switching off' of the expression of specific genes by introducing double-stranded RNA's is gaining huge importance since last decade. Although many fungal genes encoding for pathogenicity factors have been sequenced successfully but the application of RNA silencing against fungal pathogens is still limited. Transgenic technology

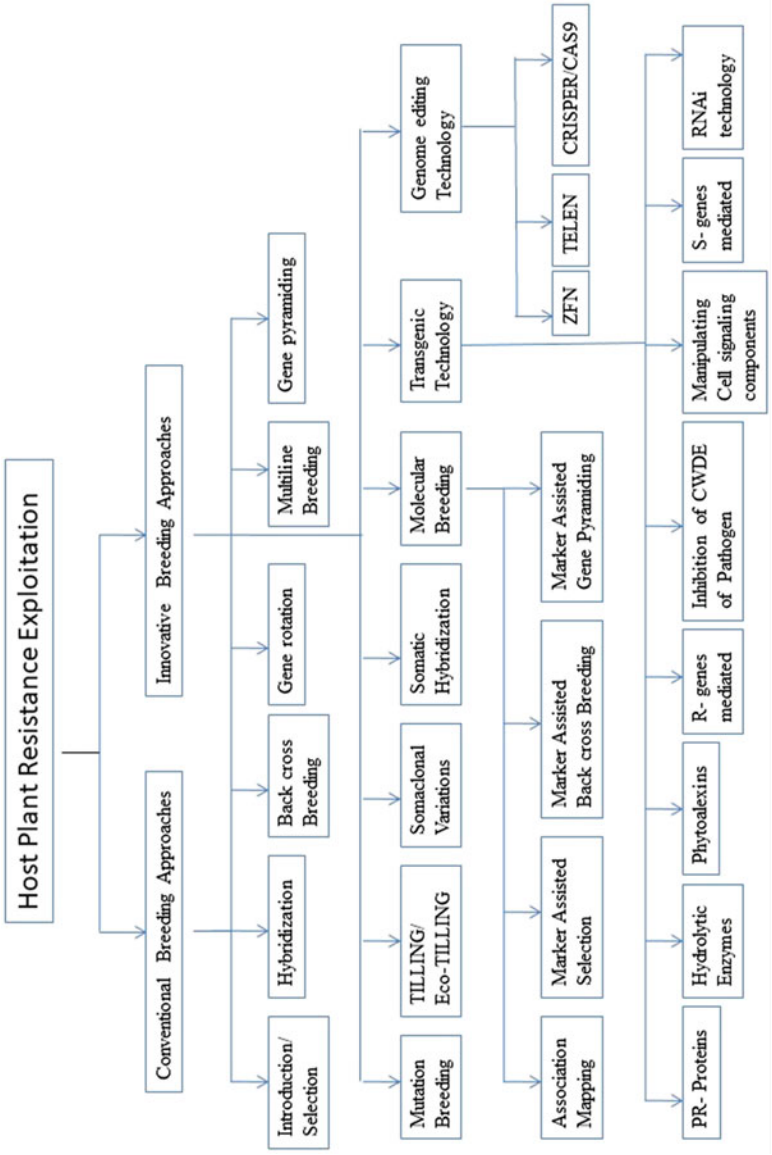

Fig. 16.10 Comprehensive view of different methods/techniques for exploiting host plant resistance to develop disease resistance varieties

integrated with classical breeding has revolutionized agricultural research and opened new vistas of research to enhance resistance and productivity of economically important agricultural crops. Although a large number of genetically engineered crops against different plant pathogens have been developed but majority of genetically engineered plants are confined to the laboratory. They have not undergone field trials and some genetically modified crops that have cleared field test are not available for cultivation because of biosafety concerns. To encourage the development of more GM crops, issue related to acceptance of these should be resolved and emphasis should be given on agronomically important crops. The field of RNAi is moving at an impressive pace and generating exciting results associated with RNAi, transgene silencing and transposon mobilization. This technology can be considered an eco-friendly, biosafe and ever green technology as it eliminates even certain risks associated with development of transgenic plants. The scopes are further widened with the advent of genome editing tools like CRISPR—Cas9 (Sander and Joung 2014) and new digital phenotyping technologies (phenomics), to develop a more sustainable crop production in scenario of changing climate.

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