Udai B. Singh
Ravindra Kumar
Harikesh Bahadur Singh *Editors*

Detection, Diagnosis and Management of Soil-borne Phytopathogens

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Preface

About 80,000 diseases have been reported so far on different plants throughout the world, of them majority are caused by soil-borne phytopathogens. These are categorized as soil-borne diseases leading to significant crop losses around the globe. Plant diseases in general and soil-borne diseases in particular affect a wide range of crops and pose a serious challenge to food security at the global level. Soil-borne plant pathogens are characterized as omnipresent, notorious, and difficult to manage as many produce hard resting structure as sclerotia. Early, speedy and reliable detection of plant pathogens is a prerequisite to formulate suitable and accurate management strategies for the management of these catastrophic pathogens/diseases. This book volume is very particular to soil-borne diseases, a complete package having the deep knowledge covering all spheres of soil-borne plant pathogens, viz. soil-borne diseases and their impact on agricultural trade and society, detection of soil-borne plant pathogens, diagnosis of soil-borne diseases, host–pathogen interaction during development of major soil-borne diseases, exploring the microbial resources for management of phytopathogens, and most importantly the integrated management of these soil-borne phytopathogens leading to huge impact on Indian agriculture. Descriptions of cutting-edge techniques and novel approaches for the detection and early diagnosis of soil-borne pathogens are given in detail. In the last few decades, omics approaches (transcriptomics, proteomics, metabolomics, and physionomics) have been widely used to diagnose the early infection and probe the mode of action of phytopathogens and phytotoxin (s) produced by them. Traditionally, the most prevalent techniques used to identify plant pathogens relied upon culture-based morphological approaches; these methods were laborious and time-consuming. Using more than one omics approach enhances the probability of success. In this book, we provide an overview of such omics technologies and focus on methods for their integration across multiple omics layers. As compared to studies of a single omics type, multi-omics offers the opportunity to understand the flow of information that underlies the better disease diagnosis and management strategies. The main focus of the book is on the prevalence of soil-borne disease management on various important crops with use of different strategies, including seed biopriming and microbial inoculation. Further, special attention is given to the emergence of new diseases or the re-emergence of old ones on several crops. This edited book entitled *Detection, Diagnosis and*

Management of Soil-borne Phytopathogens provides a comprehensive overview on recent developments in the area of detection, diagnosis, and management of soil-borne phytopathogens at the global level. It is going to serve as a platform for showcasing the expertise of motivated scientists and researchers working in the area of detection, diagnosis, and management of soil-borne phytopathogens and allied sectors.

In this context, the present book is a topical and timely contribution on plant–microbe interactions and offers a great scope for harnessing the beneficial interactions for agricultural sustainability. This book encompasses and addresses various issues of soil-borne plant pathogens and soil-microbe interrelationship and management of these notorious pathogens that are to be modulated either by resident microbes or by their external application. The role of OMIC in detection and diagnosis of plant pathogens is discussed in detail. Main topics include the detection and diagnosis of fungal, bacterial, and viral pathogens associated with important crop plants, role of microbes in the rhizosphere, below-ground communication among the plant, pathogens, and beneficial microbes including nematophagous fungi, rhizosphere ecosystem functioning with special reference to development of plant disease, positive interaction of the plants with the beneficial soil microorganisms for inducing plant growth, conferring biotic stress tolerance and modulating several pathways of the plants for the proper establishment and protection against major soil-borne pathogens, and host–pathogen interactions leading to the disease development in plants. Further chapters focus on the role of microbial signaling and cross-talk, biofilm formation, and antimicrobial peptides with special reference to the management of plant pathogens in the rhizosphere. The book also discusses the application of microbes in biological control of plant pathogens. Descriptions of cutting-edge techniques and novel approaches make this book unique in the area of plant protection. The book provides the latest understanding of rhizosphere microorganisms for enhanced soil and plant functions, thereby improving agricultural sustainability and food and nutritional security. The aim of the book is to compile high-quality reviews and research articles offering new insight into the application of new and safer molecules, new knowledge about the biology, ecology, and management of soil-borne pathogens, and more attention towards crop and soil health. By bringing all these areas together within the ambit of this special book volume, we hope to build cohesion between conventional and most modern approaches of science to design the future path for managing the soil-borne notorious and difficult to manage pathogens. The book covers (1) impact of soil-borne phytopathogens or soil-borne diseases on agriculture and society, (2) diagnosis and molecular detection of soil-borne pathogens, (3) host–pathogen interaction during the development of soil-borne diseases, (4) understanding the below-ground communication in the rhizosphere for better plant growth, (5) omics approaches to unravel the hidden infection, (6) microbe-mediated induced systemic resistance/tolerance to soil-borne plant pathogens, and (7) microbial inventorization for sustainable crop protection and production. We expect that the book would be useful for students, agricultural scientists, biotechnologists, plant pathologists, mycologists, and microbiologists, the farming community, scientists of R&D organizations, as

well as the teaching community and policymakers to understand the impact of plant pathogens and their role in agricultural production and national economy as a whole and provide directions for the future course of action.

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Karnal, Haryana, India — Ravindra Kumar
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Ravindra Kumar is currently working as a Scientist Sr. Scale (Plant Pathology) at the Division of Crop Protection, ICAR-Indian Institute of Wheat and Barley Research, Karnal-132,001 (Haryana). He received his B.Sc. (Ag.) from C.C.S. University, Meerut; and his M.Sc. (Ag.) and Ph.D. (Ag.) from the Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi, India. His areas of research interest are plant disease management, biological control, seed pathology, and plant–microbe interaction. He has published more than 35 research papers and reviews in reputed national and international journals. He has 2 textbooks, 2 edited books (one with Springer), 14 book chapters, 8 technical bulletins, and more than 50 technical/popular articles to his credit. Dr. Kumar is also a life member of the Indian Phytopathological Society, New Delhi; Indian Society of Mycology and Plant Pathology, Udaipur; Indian Society of Seed Technology, New Delhi; and Society for Advancement of Wheat and Barley Research (SAWBAR), Karnal.

Harikesh Bahadur Singh presently a Distinguished Professor, Department of Biotechnology, GLA University, Mathura. Prior to this assignment he served as Professor and Head of the Department of Mycology and Plant Pathology at the Institute of Agricultural Sciences, Banaras Hindu University. He also served the State Agriculture University, Central University, and CSIR institutes in teaching, research, and extension roles. His major research focus is on bioinoculants, biological control of plant pathogens, and nanobiotechnology. Professor Singh has been honored with several national awards for his key role in popularizing organic farming and translating agriculturally important microorganisms from lab to land. In recognition of his scientific contributions and leadership in the field of plant pathology, he has been honored with several prestigious awards, notably the CSIR Technology Prize for Biological Sciences, M. S. Swaminathan Award, Vigyan Bharti Award, Prof. V. P. Bhide Memorial Award, Scientist of Excellence Award, BRSI Industrial Medal Award, Sangam Jyoti Award, Akshyavat Samman Award, Distinguished Scientist Award, Prof. Panchanan Maheshwari Medal, Rashtriya Gaurav Award, Plant Pathology Leader Award by IPS, CSIR Award for S&T Innovation for Rural Development (CAIRD), Environment Conservation Award, Vigyan Ratna by CSTUP, and C N R Rao Award by BHU. Dr. Singh has been a fellow of the National Academy of Agricultural Sciences. Currently, he is serving as an associate/academic/board editor for journals of international repute. Dr. Singh has more than 350 publications to his credit, 34 edited books from CABI, Springer, CRC, and Elsevier, and 20 patents (USA, Canada, and PCT). He has successfully transferred several microbe-based technologies for commercial production of biopesticides to several industrial houses in India.

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Soil-Borne Viruses: Outlook on Community and Recent Advances in Detection

1

Shikha Sharma, Dalvir Kaur Dhadly, Neeta Pathaw, Konjengbam Sarda Devi, Raghuveer Singh, and Susheel Kumar Sharma

Abstract

Plant viruses are transmitted via various means, and a number of them belonging to different genera are transmitted through soil. The soil-borne viruses are found throughout the world and infect a variety of economically important crops including wheat, potato, fruit crops, barley, etc. Control strategies to minimize the losses caused due to viruses in general are very few, and the very persistence nature of these viruses makes them more difficult to be understood and managed. Research on the diversity of soil-borne viruses is still lacking. Early and reliable detection of plant pathogens is prerequisite to design an effective and sustainable disease management strategy. Traditionally, symptomatology, indexing, or visual methods were used for detection of viruses. But these techniques are time-consuming and laborious. Recent advances in molecular detection strategies

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offer to improve accuracy and reliability and overcome the abovementioned limitations. The nucleic acid and protein-based detection techniques as well as development of onsite detection assays, viz., next-generation sequencing, DNA fingerprinting, isothermal amplification, serology, and biochemical assays, have revolutionized the outlook on detection of plant viruses due to their high degree of specificity and reliability.

Keywords

Soil-borne Viruses · ssRNA · Virus indexing · Next generation sequencing · DNA fingerprinting · Isothermal amplification · Serology

1.1 Introduction

The transmission of the majority of the plant viruses is via biological vectors mainly arthropods onto the aerial parts of the plant (Hull 2013). However, some viruses that are also transmitted via soil are referred to as soil-borne viruses (SBVs), which cause diseases in many important crops, viz., potato, wheat, fruit crops, barley, groundnut, sugar beet, etc. SBVs are ubiquitous in nature and if once established in the field, their eradication can be very difficult, hence causing yield losses in many crops (Roberts 2014). Similar to other proteins, viruses, due to their nucleo-proteinaceous properties, can be adsorbed by colloidal particles in the soil, e.g., clays, and this phenomenon keeps them infective for longer periods of time. Currently, there are few effective arsenals against SBVs at our disposal like cultivation of resistant varieties and chemical control, while resistant cultivars are limited in number. Moreover, chemical control is expensive and leads to environmental and health hazards (Roberts 2014). The transmission of SBVs can be either via abiotic means or via biotic means including soil-inhabiting organism, viz., fungi, plasmodiophorids, and nematodes. A total of 15 genera of viruses including two unassigned genera are soil-borne belonging to families *Secoviridae*, *Potyviridae*, *Ophioviridae*, *Tombusviridae*, and *Virgaviridae* or unassigned family.

Hewitt et al. (1958) were the pioneers in discovering that soil-borne fanleaf virus of grapevine, vectored by *Xiphinema index* in 1958. This discovery started the search on nematodes vectoring SBVs. However, earlier in 1886, Mayer (1886) proposed the idea of soil transmission of viruses. The soil-borne viruses are distinct from other viruses because they are subjected to different anatomy, patterns of gene expression, external environmental conditions, and anti-viral defense in the roots than the shoot region of the plant (Andika et al. 2016). Surprisingly, all the known SBVs transmitted by vectors have RNA as their genomic nucleic acid (positive sense (+) single-stranded RNA (ssRNA) genome) except for the two genera of viruses, viz., *Ophiovirus* and *Varicosavirus*, whose member viruses are composed of negative sense (−) ssRNA genomes (Verchot-Lubicz 2003; Kormelink et al. 2011). The difficulty in studying these viruses leaves us with scanty knowledge *w.r.t.* their biology, and it is highly likely that there can be more unknown genera of viruses

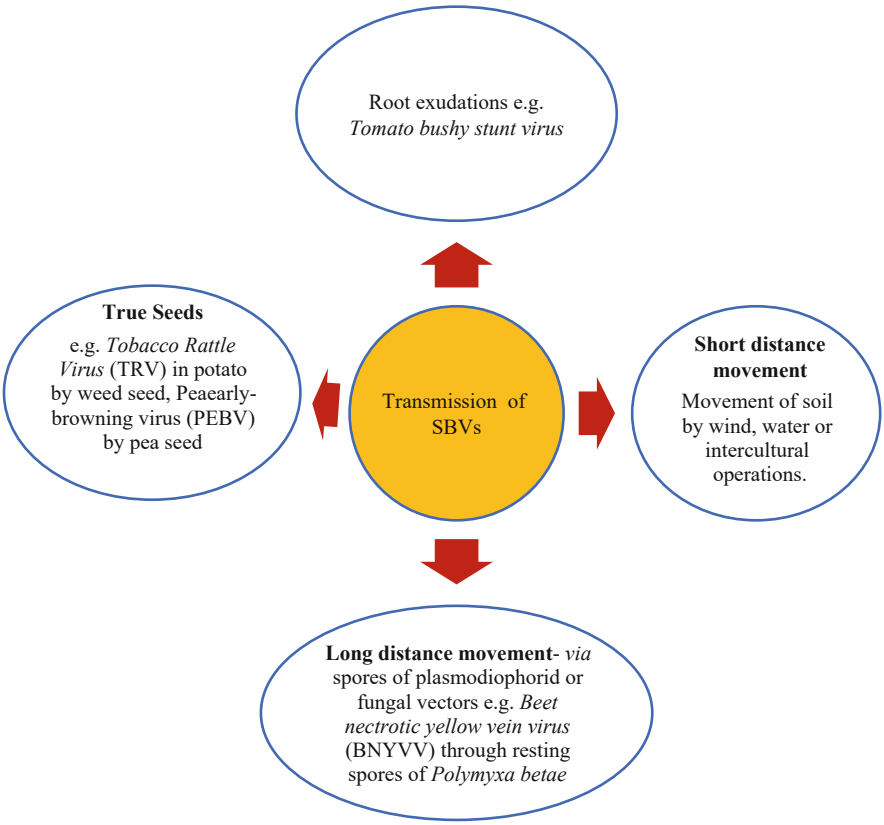

Fig. 1.1 Diagrammatic representation of transmission and movement of soil-borne viruses (SBV)

which may belong to category of soil-borne in nature (Roberts 2014; Andika et al. 2016).

The viruses enter the plant system either via injury on roots created by feeding of nematodes or during colonization of soil-inhabiting fungi. Upon entering the plants, the virus travels upwards using plant's vascular system after producing disease symptoms on the roots, e.g., *Beet necrotic yellow vein virus* (BNYVV; genus *Benyvirus*) causes Rhizomania disease in sugarbeet. Rhizomania disease causes excessive growth of side roots and rootlets while taproots remain stunted (Tamada et al. 1999). Symptoms can also be produced on aerial parts of the plant due to pathogenesis of roots, e.g., yellow mosaic symptoms accompanied with stunting of plants are produced on winter cereal crops due to infection of *Barley yellow mosaic virus* (BaYMV; genus *Bymovirus*) (Kühne 2009). Another example of aerial symptomatology due to virus infection is by *Peanut clump virus* (PCV) which belongs to genus *Pecluvirus*. The infection of PCV leads to appearance of mottling and chlorotic rings on leaves and stunting of infected plants (Thouvenel and Fauquet 1981; Dieryck et al. 2009) (Fig. 1.1 and Table 1.1).

Table 1.1 Crop yield losses caused due to soil-borne virus (SBVs) around the world

Genus	Species	Yield loss (%)	Reference
Bymovirus	*Barley yellow mosaic virus* (BaYMV)	50%	Huth and Lesemann (1978)
	Barley mild mosaic virus (BaMMV)	>50%	Ketta et al. (2011) Cox et al. (2014)
	Wheat spindle streak mosaic virus (WSSMV)	Up to 80%	Drumm-Myers et al. (1993)
	Wheat yellow mosaic virus (WYMV)	20–44%	Palmer and Brakke (1975)
Furovirus	*Oat mosaic virus* (OMV) *Oat golden stripe virus* (OGSV)	>50% or even 100%	Walker et al. (1998)
Nepovirus	*Grapevine fanleaf virus* (GFLV)	~80%	Martelli and Savino (1991)

1.2 Transmission of Soil-Borne Viruses (SBVs)

Plant viruses require living organisms or viral vectors to carry them. The transmission of plant viruses to their host plants take place via many biotic vectors; however, abiotic transmission is also reported. The main vectors for SBVs are chytrids, plasmodiophorids, and nematodes. Member viruses of genus *Tombusvirus* are transmitted without a vector except *Cucumber necrosis virus* (CNV) which in nature is transmitted by zoospores of *Olpidium bornovanus* (Kakani et al. 2003). As far as abiotic transmission of SBV is concerned, the viruses are released into the soil along with root exudates, and from thereon they enter healthy roots through root injuries inflicted either via different arthropods or agricultural implements, e.g., *Cymbidium ringspot virus* (CymRSV), *Tomato bushy stunt virus*, and *Petunia asteroid mosaic virus* (PetAMV; genus *Tombusvirus*). Among genus *Carmovirus*, *Cucumber soil-borne mosaic virus* and *Galinsoga mosaic virus* (GMV) also show non-vector transmission (Sarwar et al. 2020). Major biotic vectors of SBVs are discussed further in this chapter.

1.2.1 Chytrid Fungi

Olpidium spp. are the vectors of all fungi-transmitted viruses. They belong to division Chytridiomycota also commonly called as chytrids and are true fungi-producing flagellated zoospores. Members of this fungal group are symptomless obligate intracellular root parasites, but *Olpidium bornovanus* has been shown to independently cause root disease (Stanghellini et al. 2010). The major families of SBVs are *Ophioviridae* and *Tombusviridae* and one phylogenetically unassigned virus. Out of all fungi-transmitted plant viruses, *Tobacco necrosis virus* (TNV genus *Necrovirus*) causing diseases in tobacco and many other crop species worldwide,

viz., bean stipple streak disease and Tulip augusta disease in tulips, is transmitted by *Olpidium brassicae* and holds paramount importance (Roberts 2014).

1.2.1.1 Mechanism of Transmission.

There are two modes by which virus transmission can take place.

(a) **In vitro transmission**: Here, the zoospores acquire the virus from the aqueous medium present outside the root system of plants.
(b) **In vivo transmission**: In this mode, zoospores acquire the virus during co-infection of the plant roots by both fungus and SBVs. The viruses involved in this type of transmission are a nuisance to control as the virus is present in the resting spores of the fungus and it can retain infectivity for decades, e.g., transmission of *Lettuce big vein virus* (LBVV) by *O. brassicae* (Campbell 1996; Rochon et al. 2004; Rochon 2009) (Table 1.2).

Table 1.2 Soil-borne viruses (SBVs) transmitted by *Olpidium* spp.

Family	Genus	Virus species	Vector
Ophioviridae	*Ophiovirus*	· *Freesia sneak virus* (FreSV) · *Lettuce ring necrosis ophiovirus* (LRNVOO) · *Mirafiori lettuce big-vein virus* (MLBVV) · *Tulip mild mottle mosaic virus* (TMMV)	*Olpidium*
Tombusviridae	*Carmovirus*	· *Melon necrotic spot virus* (MNSV)	*Olpidium*
	Dianthovirus	· *Carnation ringspot virus* (CRSV)	*Olpidium*
	Tombusvirus	· *Cucumber necrosis virus* (CNV) · *Cymbidium ringspot virus* (CyRSV) · *Petunia asteroid mosaic virus* (PetAMV) · *Tomato bushy stunt virus* (TBSV)	*Olpidium* and abiotic means of transfer
	Necrovirus	· *Beet black scorch virus* (BBSV) · *Chenopodium necrosis virus* (ChNV) · *Tobacco necrosis virus A* (TNV-A) · *Tobacco necrosis virus D* (TNV-D)	*Olpidium*
	Varicosavirus	· *Lettuce big-vein associated virus* (LBVaV)	*Olpidium*

1.2.2 Plasmodiophorids

The plasmodiophorids are placed in order Plasmodiophorales under family Plasmodiophoraceae and compose of microorganisms that are intracellular parasites of algae, oomycetes, and higher organisms. The nuclei of these parasites undergo the peculiar "cruciform" kind of nuclear division and further give rise to a plasmodium or multinucleate protoplast, hence named as plasmodiophorids (Sarwar et al. 2020). They are known to cause growth deformities in the root region, e.g., *Plasmodiophora brassicae* (soil-borne protist pathogen), inciting "club-root disease" of crucifers as well as transmit plant viruses. The different members of class Plasmodiophoromycetes, viz., *Polymyxa betae*, *P. graminis*, and *Spongospora subterranean* are known to transmit a number of viruses on temperate as well as tropical crops (Maraite 1991). Once categorized as fungi, now they are classified in the *Rhizaria* in phylum Cercozoa. Their affinity with the protozoans can be proved by studying the structure of zoospores, synaptonemal complex using electron microscopes, and rDNA sequence data for some species (Neuhauser et al. 2010).

1.2.2.1 Life Cycle

Plasmodiophorids exhibit complex life cycle and lacks complete understanding. It is composed of the following stages:

1. Zoosporic stage.
2. Plasmodia formation inside host cells.
3. Formation of resting spore.

1.2.2.2 Plasmodiophorid-Vectored Viruses

Approximately 20 species of 5 genera of SBVs, viz., *Benyvirus* (family unassigned) and *Bymovirus* (family *Potyviridae*), *Furovirus*, *Pecluvirus*, and *Pomovirus* (family *Virgaviridae*), are transmitted by plasmodiophorid fungi (Adams et al. 2009). The plasmodiophorid-transmitted viruses contain multi-segmented (2–5 RNA components) positive sense single-stranded RNA (ssRNA) genome. Except for bymoviruses which have flexuous filamentous geometry, other genera are rod shaped (Sarwar et al. 2020).

The genera *Polymyxa* and *Spongospora* are reported to transmit SBVs. The genus *Polymyxa* has worldwide prevalence including all those organisms which are obligate intracellular parasites of *Poaceae* (*P. graminis*) and *Chenopodiaceae* (*P. betae*) family. These 2 genera vector 15 most economically important SBVs (Roberts 2014). The different *Polymyxa* species can be further classified into different ribotypes (based on the rDNA sequence data) which differ in terms of host specificity and vector specificity. *Barley yellow mosaic virus* (BaYMV; genus *Bymovirus*) and *Barley mild mosaic virus* (BaMMV; genus *Bymovirus*) vectored by *P. graminis* are widely prevalent SBVs in Japan and whole of Europe causing yield losses of 70% or more. The shift to autumn-sown barley from spring-sown can be a reason of outbreaks of BaYMV and BaMMV in Western Europe, as the early-sown autumn barley seeds before emergence remain in the soil for longer time, which makes them

more susceptible to SBV infection. *P. graminis* is capable of transmitting *Benyvirus*, *Rice stripe necrosis virus* (RSNV), bymoviruses, furoviruses, and pecluviruses, while the remaining benyviruses, *Beet soilborne virus* (BSBV), and Beet virus Q (BVQ) are transmitted by *P. betae* (Sarwar et al. 2020). Morphologically, *P. graminis* and *P. betae* are indistinguishable but we can differentiate between them at molecular level. Twelve different viruses in the genera *Benyvirus*, *Bymovirus*, *Furovirus*, and *Pecluvirus* are reported to be transmitted by both *P. graminis* and *P. betae* (Mayo and Pringle 1998). Unlike *Polymyxa*, the genus *Spongospora* includes the species which are both vectors and plant pathogen, e.g., *S. subterranea*, which causes powdery scab disease in potato and also act as vector of *Potato mop-top virus* (PMTV; (genus *Pomovirus*; Jones and Harrison 1969) inciting "Spraing disease" in potatoes. Two member species of the genus are both plant pathogens and "fungal" virus vectors: *S. subterranea* f. sp. *subterranea* and *S. subterranean* f. sp. *nasturtii* (Merz et al. 2005). PMTV was first detected in the USA in 2003 and has spread to cooler areas of Europe (northern and central), Andean region of South America, Israel, Canada, and Japan (Jacobi et al. 1995; Lambert et al. 2003). The increasing incidence and spread of powdery scab disease is believed to be a reason for increased prevalence of PMTV worldwide. *S. subterranean* f. sp. *nasturtii* transmits *Watercress yellow spot virus* (WYSV) which holds great economic importance in France and England (Arnold et al. 1995; Walsh et al. 1988) (Table 1.3).

1.3 Nematodes

Nematodes are roundworms belonging to phylum Nematoda. Plant-parasitic nematodes belong to two major families *Longidoridae* and *Trichodoridae*, involved in transmitting viruses from genera *Nepovirus* and *Tobravirus*, respectively. Both the families include migratory ecto-parasitic nematodes of the root system. The name "*Nepovirus*" is deduced from "Nematode-transmitted polyhedral viruses," these viruses are polyhedral or isometric in shape while "tobraviruses" are straight tubular rod shaped. There are 22 longidorids (10 *Longidorus*, 1 *Paralongidorus*, and 11 *Xiphinema* species) and 14 trichodorids (5 *Trichodorus*, 9 *Paratrichodorus* spp.) known to vector plant viruses (Sarwar et al. 2020).

1.3.1 Transmission of Viruses by Nematodes

The nematodes can obtain the virus while feeding on infected plants, e.g., *Xiphinema index* can acquire the *Grapevine fanleaf virus* (GFLV) within 5–15 min of feeding on infected vines while for other nematodes an acquisition feeding period of 24 h might be needed. Following acquisition, the nematodes can immediately transmit the virus onto healthy plants (roots) without any latent period (Schellenberger et al. 2010). The nematodes can lose the virus within the first few months post acquisition; however, they can stay viruliferous for up to 1 year provided the nematodes are

Table 1.3 Plasmodiophorid-transmitted SBVs

Family	Genus	Species	Vector
Potyviridae	*Bymovirus*	· *Barley mild mosaic virus* (BaMMV) · *Barley yellow mosaic virus* (BaYMV) · *Oat mosaic virus* (OMV) · *Rice necrosis mosaic virus* (RNMV) · *Wheat spindle streak virus* (WSSV) · *Wheat yellow mosaic virus* (WYMV)	*Polymyxa*
Virgaviridae	*Furovirus*	· *Chinese wheat mosaic virus* (CWMV) · *Japanese soil-borne wheat mosaic virus* (JSBWMV) · *Oat golden stripe virus* (OGSV) · *Soil-borne cereal mosaic virus* (SBCMV) · *Soil-borne wheat mosaic virus* (SBWMV) · *Sorghum chlorotic spot virus* (SrCSV)	*Polymyxa*
	Pecluvirus	· *Peanut clump virus* (PCV) · *Indian peanut clump virus* (IPCV)	*Polymyxa*
	Pomovirus	· *Beet soil-borne virus* (BSBV) · *Beet virus Q* (BVQ)	*Polymyxa*
		· *Broad bean necrosis virus* (BBNV) · *Potato mop-top virus* (PMTV)	*Spongospora*
Unassigned	*Benyviridae*	· *Tobacco rattle virus* (TRV) · *Beet necrotic yellow vein virus* (BNYVV) · *Beet soil-borne mosaic virus* (BSBMV) · *Rice stripe necrosis virus* (RSNV)	*Polymyxa*
	Unassigned	· *Watercress yellow spot virus* (WYSV) · *Watercress chlorotic leaf spot virus* (WCLSV)	*Spongospora*
		· *Aubian wheat mosaic virus* (AWMV)	*Polymyxa*

stored (in vitro) at low temperatures without their host. Nematode-transmitted viruses are neither transstadial nor transovarian in nature (Sarwar et al. 2020).

1.3.1.1 Trichodorid-Transmitted Viruses

The genera *Trichodorus* and *Paratrichodorus* belonging to family Trichodoridae are mainly involved in transmission of SBVs. Both the nematodes are short in size ranging from 0.5 to 1.5 mm in length. Out of the total 75 species, only 14 species are reported as vectors of tobraviruses (Ploeg et al. 1992; Ploeg and Decraemer 1997). Only the didelphic trichodorid genera, i.e., having two ovaries, possess virus vector species. Trichodorids are present worldwide in the freely draining fields having usually sandy soils. *Trichodorus* genus is mainly found in the temperate region, whereas *Paratrichodorus* predominates the tropical and sub-tropical region. They contain a non-axial ventrally curved mural tooth or onchiostyle that can only pierce up to the epidermal cells of the root tip (Siddiqi 2002; Sarwar et al. 2020). They are plant root feeders which aggregate 1–3 mm behind the apical meristem around the zone of elongation. The feeding leads to necrosis and stunting of the roots manifested in the form of stubby roots followed by exhibition of other symptoms,

Table 1.4 Tobraviruses and their trichodorid vectors

Virus species	Nematode vector
Pea early browning virus (PEBV)	· *Paratrichodorus anemones* · *P. teres* · *P. pachydermus* · *Trichodorus primitivus* · *T. viruliferous*
Pepper ringspot virus (PepRSV)	· *P. minor*
Tobacco rattle virus (TRV)	· *P. allius* · *P. teres* · *P. anemones* · *P. hispanus* · *P. pachydermus* · *P. tunisiensis* · *P. minor* · *P. namus* · *T. primitivus* · *T. viruliferous* · *T. similis* · *T. cylindricus*

viz., wilting and chlorosis of the foliage. The secretions of the nematodes injected into the meristem during feeding may also lead to stubby root condition (Yeates et al. 1993; Oliveira et al. 2004). Among the viruses transmitted by this group, *Tobacco rattle virus* (TRV) has the widest host range (Roberts 2014). This virus is responsible for "corky ringspot" disease of potato tubers (Table 1.4).

1.3.1.2 Longidorid-Transmitted Viruses

Among *Longidoridae* family genera, viz., *Xiphinema*, *Paralongidorus*, and *Longidorus*, are reported to be virus vectors. Longidorids are larger in size reaching length of 2–12 mm (*Longidorus* and *Paralongidorus*) or even up to 1.6–6.0 mm (*Xiphinema*) in their adult stage (Sarwar et al. 2020). Compared to trichodorids, they are less restricted by soil type and are usually found in sandy and loamy soils. The members of this family bear a long hollow spear known as stylet which helps in penetrating and feeding on the plant roots. They feed at or behind the plant root tips exclusively and inject their secretions leading to galling or hyperplasia in the root region. The effects of their feeding are visible on the aerial parts of the plant as well (Griffin and Epstein 1964). On underground or roots of plants, necrosis and discoloration of the meristematic as well as cortical tissue is also evident. With few exceptions like *Cherry rasp leaf virus* (CRLV) and *Strawberry latent ringspot virus* (SLRSV) (formerly placed under *Nepovirus* now categorized in *Cheravirus* genus), they have been proven to transmit 13 out of 38 known nepoviruses (Roberts 2014). Among these, seven are transmitted by *Longidorus* species, one by *Paralongidorus*, and nine by *Xiphinema* (Roberts 2014; Sarwar et al. 2020). Many viruses are transmitted by longidorids, i.e., *Tomato ringspot virus* (ToRSV), *Tobacco ring spot virus* (TRSV), *Peach rosette mosaic virus* (PRMV), *Cherry*

Table 1.5 Transmission of plant viruses by longidorid vectors

Genus	Species	Nematode vector
Cheravirus	*Cherry rasp leaf virus*	*Xiphinema*
Unknown	*Strawberry latent ringspot virus*	*Xiphinema*
Nepovirus	*Arabis mosaic virus* *Artichoke Italian latent virus* *Beet ringspot virus* *Cherry leaf roll virus* *Cherry rosette virus* *Grapevine fanleaf virus* *Mulberry ringspot virus* *Peach rosette mosaic virus* *Raspberry ringspot virus* *Tobacco ringspot virus* *Tomato black ring virus* *Tomato ringspot virus*	*Xiphinema* and *Longidorus*

leafroll virus (CLRV), *Cherry rasp leaf virus* (CRLV), *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Tomato black ringspot virus* (TBRSV), and *Raspberry ringspot virus* (RRSV) (Table 1.5).

1.4 Detection of Soil-Borne Viruses (SBVs)

For timely and effective management of soil-borne diseases, there is a need for fast and accurate detection tools (DeShields et al. 2018). Diagnosis of soil-borne diseases is limited and hugely hindered because of the vast soil environment as compared to plant mass, and factors like nutrient and moisture status of the soil can also influence the diagnosis (Panth et al. 2020). The field of plant disease diagnostics has seen a dramatic change from visual inspection and identification of plant disease which relied on signs and symptoms to robust serological techniques like enzyme-linked immunosorbent assay (ELISA) and molecular methods like polymerase chain reaction (PCR) (Balodi et al. 2017). Among molecular detection methods, PCR and particularly the RT-PCR (real-time PCR)-based methods form the basic protocol of many diagnostic laboratories across the world owing to their accuracy and sensitivity (DeShields et al. 2018). Fomitcheva and Kühne (2019) were successful in developing a sensitive serological, i.e., Western Blot analysis and duplex RT-PCR-based method to detect and differentiate between the sugarbeet SBVs, viz., BNYVV and BSBMV. Simultaneous detection of three SBVs in wheat mainly CWMV, JSBWMV, and WYMV using reverse transcription loop-mediated isothermal amplification reaction (RT-LAMP) was reported by Fukuta et al. (2013). However, costly equipment and skilled personnel are needed to employ these laboratory techniques. On the other hand, on-site testing tools give results at the farmers' field and can be performed by the grower himself. Lateral flow devices (LFDs) like the Immunostrip and pocket diagnostic are the leading methods with respect to on-site pathogen detection as they are simple and one-step assays but they are not completely reliable.

LAMP is another such cheap method which involves simple colorimetric analysis (DeShields et al. 2018). DeShields et al. (2018) outlined protocol for on-site detection of potato soil-borne pathogens which involved the following steps:

1. Magnetic bead-based nucleic acid extraction.
2. Portable real-time PCR (fluorogenic probe-based assay).
3. Quantitative data analysis using a laptop/computer.

This protocol enabled the detection of even as less as 100 copies of pathogen's DNA. A CRISPR-Cas12a-based detection system has recently been developed for detecting BNYVV in sugarbeet roots by Ramachandran et al. (2021). In this approach, viral RNA amplification is achieved by single-step isothermal RT-recombinase polymerase amplification (RPA) method followed by confirmation of the RT-RPA amplicon sequence identity with BNYVV sequence. Afterward, the RT-RPA reaction products are diluted ten-fold serially and 5 μL from each dilution is used further as template in the CRISPR-Cas12a reaction containing fluorescently labeled ssDNA reporter. The reaction is incubated at 37 °C. A strong fluorescence signal was deducted from infected roots than healthy roots which decreased linearly in reactions having increased levels of serial dilutions (Ramachandran et al. 2021).

1.5 Management of SBVs

Soil-borne bymoviruses are agronomically important in barley and wheat crops responsible for huge amount of yield losses annually (Campbell 1996; Kühne 2009; Kanyuka et al. 2003). Continuous efforts are being made to find resistance genes against these pathogens in the breeding programs of several countries (Takahashi et al. 1973; Ruan et al. 1984; Zhou and Cao 1985; Götz and Friedt 1993; Ordon and Friedt 1993). So far, a total of 18 resistance genes have been identified in barley against BaMMV and BaYMV. Resistance (R) gene *rym3* is identified from mutant cultivar 'Ishuku Shirazu' or 'Ea 52' which is derived from cultivar 'Chikurin Ibaraki 1' via mutagenesis (Saeki et al. 1999; Ordon et al. 2005, 2009; Kai et al. 2012). However, resistance conferred by these genes except $Rym14^{Hb}$, $Rym16^{Hb}$, Rym17, rym18, and $eIF4E_{HOR3298}$ is short lived and overcome by new races of the viruses, e.g., demise of *rym4* gene in European winter barley varieties by BaYMV-2 race (Kühne et al. 2003; Kanyuka et al. 2005; Habekuß et al. 2008; Kim et al. 2011; Arai et al. 2018). This has led to a search for durable resistance sources. One such way is pyramiding of resistance genes like *rym5* and *rym1/11-d* present in landrace 'Mokusekko 3', which offers complete resistant to all the reported isolates of BaMMV and BaYMV (Kanyuka et al. 2005; Habekuß et al. 2008; Kim et al. 2011; Arai et al. 2018; Shi et al. 2019). Rupp et al. (2019) reported that silencing of *TaeIF(iso)4E* and *TaeIF4G* genes provide resistance to WSMV, TriMV (*Triticum mosaic virus*), and SbWMV in wheat lines. These genes code for the Eukaryotic initiation factors (eIFs) which are required by the RNA viruses for

Table 1.6 List of some of the R genes/QTLs against cereal SBVs

S. No.	Resistance gene/QTL	Crop	Donor	SBV	Reference
1.	*YmYF*	Wheat	Yangfu 9311 (China)	WYMV	Liu et al. (2005)
2.	*Ymlb*	Wheat	Ibis (Netherland)	WYMV	Nishio et al. (2010)
3.	*Qssm-mtpsa-7BS*	Wheat	Dic2 (Emmer wheat)	WSSMV	Holtz et al. (2017)
4.	*rym1*	Barley	Mokusekko 3 (China)	BaMMV/ BaYMV	Okada et al. (2003) Yang et al. (2014)
5.	$rym^{b}_{HOR4224}$	Barley	HOR4224 (Japan)	BaMMV	Perovic et al. (2014)
6.	$Rym14^{Hb}$	Barley	*Hordeum bulbosum* (wild relative)	BaMMV/ BaYMV	Ruge et al. (2003)

Table 1.7 Cultural and physical measures of SBVs control

S. No.	Type of control	Strategy	Reference
1.	Cultural control	· Rogueing of diseased plants · Selection of less-susceptible cultivars · Culture on heavy types of soil, e.g., done in tulips · Change of planting date, e.g., late planting is done for autumn planted bulbs · Soil disinfection against TRV in gladiolus	Asjes (1974)
2.	Physical control		Luvisi et al. (2015)
	(a) Heat treatment	· *Potato virus Y* can be managed by steam treatment and soil solarization using ethylene-vinylacetate or high efficiency infrared films up to 20 cm of soil depth	
	(b) Air pressure	· Maintenance of matric potential of -40 kPa in field obstructs the movement of *P. graminis* zoospores vectoring SBVs within the soil	Cadle-Davidson et al. (2003)

movement between cells, replication of their genome, and production of viral proteins (Diaz-Pendon et al. 2004) (Tables 1.6 and 1.7).

1.6 Conclusion

Plant diseases have a significant impact on sustainable crop production, and over the years, even after improved chemical control, resistance development, and improvements in technology have been introduced to protect crop plants, plant diseases continue to cause severe reductions in crop yield and quality. There are a number of pests and diseases that affect crop plants. The soil-borne virus group is a particularly important pathogen that causes severe crop yield losses. Typically, these

soil-borne viruses are transmitted by soil-inhabiting fungi, fungi-like plasmodiophorids, and nematodes, which are worldwide in distribution and primarily multiply in crop roots. The management of soil-borne viruses is crucial because they cause high economic losses to crops, particularly cereals. The only way to prevent severe losses in quality and yield of crop plants has been to use virus-resistant cultivars. Resistance breeding thus has made substantial progress as a means of management of soil-borne viruses.

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An In Silico Outlook for the Detection and Surveillance of Evolving and Persistent Plant Pathogens

2

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Abstract

The diversity and social behaviour of plants are astounding. They developed a wide variety of molecular mechanisms to react to a complex network of environmental signals, multiple pathways activated by various responsive genes, abiotic stresses, and diseases brought on by bacteria, fungus, nematodes, and viruses that affect plant growth and crop yield. The molecular foundation of plant responses has thus been the subject of substantial research. The advancing omics technologies offer vital tactics for fostering molecular research and cutting-edge methods for omics-assisted crop improvement. Bioinformatics has aided in genome sequencing of various plant species, gene identification, phylogenetic profiling of plant species, detection of transcription factor binding sites of the genes, and the discovery of different sites where protein interactions can take place. To help to understand biology at the system level, bioinformatics begins to show promise in unravelling genetic networks. Plant life plays important and diverse roles in our society, our economy, and our global environment. Feeding the increasing world population is a challenge for the modern plant biotechnology. Crop yields have increased during the last century and will continue to improve by novel strategies and technologies. The transition from expressed

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sequence tags and microarray-based techniques to more potent strategies like RNA sequencing and related technologies has been encouraged by the next-generation sequencing, which has largely favoured deeper insights in plant genome organizations and on-functional responses to variable environmental parameters. Simultaneously, the development in the proteomics techniques that is 2D gel coupled to mass spectrometry, of high-throughput shotgun approaches, and of more robust LC-MS and GC-MS and metabonomic technologies are able to unravel fluctuations of non-volatiles and volatiles, paving the way to a better understanding of the effects of plant biological processes and investigations.

Keywords

Pathogen detection · Surveillance · Persistent plant pathogens · Agrigenomics · DNA microarray · Artificial intelligence

2.1 Introduction

Numerous microorganisms coexist alongside plants, which thrive above ground in the phyllosphere and underground in the rhizosphere (Vorholt 2012; Bulgarelli et al. 2015. They may be endophytes that live inside the plants, epiphytes that are found on plant surfaces, or hyphae that are found in the soil near the roots. These microorganisms can show beneficial, neutral, and harmful effects on plant health and development (Newton et al. 2010). Pathogens are organism that causes disease in the plants and reduces plant growth and productivity. The plant pathogen includes viruses, bacteria, fungi, nematode, and parasitic plants. They cause diseases in leaf, stem, vascular system, and fruits of plants. For example, *Pseudomonas syringae* is a bacterium, responsible for production of less fruits by tomato plants. Worldwide insect pests cause estimated loss of 14%, weeds caused a 13% loss, and plant diseases caused a 13% loss. Worldwide trade and movement of plant material is the reason for spread of plant pathogens to new areas. And these pathogens can have negative impact on local plants. It has been estimated that there is a loss of one quarter of global product due to pre- and post-harvest pathogens (Lichtenberg and Olson 2018). The key for prevention of spread of plant pathogens is rapid and accurate detection. So, it is very necessary to study microbial communities, including bacteria, viruses, archaea, and fungi (Baker et al. 1985; Mehetre et al. 2018). To improve detection sensitivity, serological methods were frequently used with electron microscopy. Pathogen diagnostics primarily employs a number of IEM versions, such as solid-phase immune electron microscopy (SPIEM) and immunosorbent electron microscopy (ISEM) (Bhardwaj and Kulshrestha 2020). However, a number of conventional molecular diagnostics are now widely utilized globally to specifically identify plant pathogenic pathogens. These methods typically involve the amplification and sequencing of several genes of pathogens. But most of these procedures cannot be applied in the field or require high prices, and these method use some enzymes, reagents that have short half-life. And these methods are greatly

dependent on the expertise of the analyst. Different approaches can be used to simultaneously detect multiple pathogens based on biochemical properties that include:

(a) Polyvalent Polymerase chain reaction.
(b) DNA microarray techniques.
(c) Next-generation sequencing.

The most accurate approaches for detecting and identifying diseases nowadays use molecular diagnostic techniques. A DNA microarray is an array of microscopic DNA patches, each of which contains thousands of copies of a particular DNA sequence known as a probe. To determine the relative abundance of transcripts in the target sample, these are employed to hybridize a cDNA/RNA sample. The field of microbiology has undergone a revolution with the introduction of next-generation sequencing (NGS) technology, and plant virus diagnostics is no exception (Maree et al. 2018; Villamor et al. 2016), because NGS does not require any previous information of viral sequences (Stobbe and Roossinck 2014). For diagnostics, there are different sequencing platforms, such as second-generation and third-generation sequencing. However, second-generation platforms are not very cost-effective due to their high sample turnover rates and need for capital inputs (Theuns et al. 2018). Nanopore sequencing is among the systems that are most widely used. By monitoring voltage changes when DNA flows through a membrane-based protein nanopore, it allows for direct DNA sequencing. Due to advances in genomes and proteomics, researchers have accumulated enormous amounts of data. However, we are now living in the era of machine learning, a group of analytical techniques that automate the process of developing models and learn from data to generate insights. As technology developed, several image-based diagnosis methodologies—including artificial intelligence—were also applied. With the least amount of resource utilization, artificial intelligence produces greater yields and higher-quality produce. A pathogen's interaction with or infection of a plant triggers a complicated chain of plant defence reactions. Different defence mechanisms begin as a result, including the creation of reactive oxygen species (ROS), strengthening of the plant cell wall, and the manufacturing of numerous defensive enzymes through diverse routes (Saunders et al. 2012). A high-throughput method is needed to identify genes responsible for resistance to pathogen. By applying machine learning, Pal et al. showed that support vector machine (SVM) was used to predict plant resistance proteins (R proteins) produced after plant-pathogen interaction, by 10,270 features obtained from sequencing of proteins, which achieved 9.11% on the test datasets (Pal et al. 2016). Unlike genomics data, platforms with sensors and highly automated ground and aerial robots are utilized in plant disease research to acquire real-time data from agricultural fields (Pena et al. 2015).

2.2 Agrigenomics

Agrigenomics comprises Agri+genomics; Agri means agriculture, and genomics is the study of an organism's entire genetic makeup. Agrigenomics is the application of genomics in agriculture, which speed up the process of developing crops, higher production value, stress tolerance, disease resistance, and sustainability (Bevan et al. 2017). The health of plants is affected by some biotic and abiotic variables. Invasive microbial infections and soil-borne pathogens, for instance, can result in the loss of thousands of trees due to their ability to spread over the planet and alter the composition and ecology of environments (Rizzo et al. 2002). Ecological invasions and change in climate can change microbes and their environment. For instance, microbial diseases are evolving quickly, and environmental stress from changing temperatures and weather patterns over time can weaken plant hosts (Verma et al. 2021). Therefore, it is essential to adapt to and mitigate these effects if we want to maintain healthy ecosystems and effective agricultural systems (Fig. 2.1).

There are many classical methods for detecting plant pathogens, e.g. microscopic and biochemical methods. Many times, serological techniques are combined with electron microscopy that increases detection sensitivity. But there are vast numbers of viruses or bacteria from same species having lots of similarity. Microbial genomics emphasize on the structure, function, evolution, mapping, and editing of microorganisms. The first genome sequenced was microbial: the bacterium *Haemophilus influenzae*, which is the first free-living organism whose whole genome was sequenced via shot gun sequencing (Fleischmann et al. 1995). The

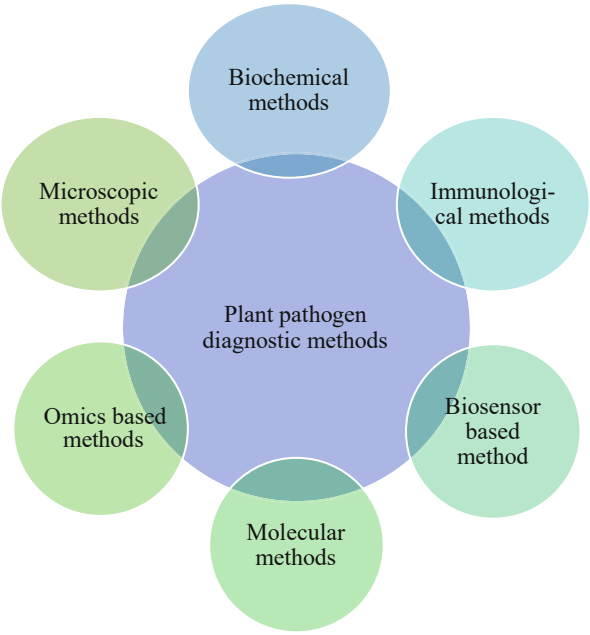

Fig. 2.1 Plant pathogen diagnostic methods

first eukaryote whose whole genome was sequenced is the fungus *Saccharomyces cerevisiae* (Goffeau et al. 1996). Thanks to recent developments in DNA sequencing technology, we can now sequence either a piece or the entire genome of a microorganism to find out more about the species, composition, structure, phylogenetic relatedness, and function of microbial communities (Caporaso et al. 2012).

Nucleic acid-based methods have three major steps:

(a) The isolation of the nucleic acid (DNA/RNA).
(b) Amplification.
(c) Product analysis.

The final steps immediately display the outcome (Zhang et al. 2020). We'll talk about the most significant methods created for plant pathogen analysis in the part that follows.

2.2.1 Polymerase Chain Reaction (PCR)

PCR is one of the most important methods for molecular detection of various pathogens. It detects the DNA or RNA of the viruses with the help of molecular primers by amplifying a particular region of the viral genetic material (Li et al. 2008; Zhang et al. 2008). Nateqi and coworker identified Iris severe mosaic virus (ISMV) using specific primers with the help of RT-PCR (Nateqi et al. 2015). Significant advancements in molecular techniques utilize multiplex detection techniques, allowing for the more effective detection of numerous viruses. One of the most popular post-amplification detection is DNA microarray techniques. A DNA microarray is a set of small DNA patches that are strategically positioned on a solid surface, typically glass. A cDNA sample or target is hybridized using thousands of distinct DNA sequences known as probes present in the tiny spots. If the sample contains multiple pathogens, microarray offers a reliable and effective method for pathogen diagnosis (Zhang et al. 2008). A DNA microarray was used to identify 61 species of young vine decline fungal infections that cause significant mortality in young vineyards (Úrbez-Torres et al. 2015). Major drawbacks of microarray are that it can detect only those sequences, for which the array is designed to identify (Bumgarner 2013) (Table 2.1).

2.2.2 Next-Generation Sequencing-Based Methods

Prior to the development of next-generation sequencing methods, sanger sequencing, or first-generation sequencing, dominated the scientific community. NGS systems' capacity to produce vast amounts of data, along with their quick turnaround and low cost, made this method popular in a variety of biological research domains (Mehetre et al. 2021). The term "omics" refers to a number of biological fields, including metagenomics, genomics, proteomics, and others that, as a result of the

Table 2.1 List of some plant viruses detected with the help of PCR techniques

S. no.	Virus name	Host plants	Technique	Reference
1.	*Pepino mosaic virus* (PepMv)	Tomato	RT-PCR	Ling (2007)
2.	*Tomato chlorosis virus*	Tomato	RT-PCR	Liu et al. (2019)
3.	*Potato virus M* (PVM)	Pepino	Multiplex RT-PCR	Ge et al. (2013)
4.	*Cucumber mosaic virus* (CMV)	Lily	Multiplex RT-PCR	Kimathi et al. (2020)
5.	*Lily mottle virus* (LMoV)	Lily	Multiplex RT-PCR	Kimathi et al. (2020)
6.	*Tobacco mosaic virus* (TMV)	Tomato, bell paper	RT-PCR and duplex RT-PCR	Vinayarani et al. (2011)
7.	*Grapevine fan leaf virus* (GFLV)	Grapes	IC-RT-PCR	Koolivand et al. (2014)
8.	*Begomovirus Potyvirus Cucumber mosaic virus* (CMV)	Clove basil	RT-PCR	Sinha and Samad (2019)

development of NGS technologies, aid in the investigation of numerous cellular molecules. Nowadays, metagenomics is mostly employed to categorize and describe microbial populations. Meta means "beyond", which describes how metagenomics goes beyond conventional genomic approaches to detect bacteria' genetic diversity and function (Solden et al. 2016). This method starts with the collection and processing of DNA from the field, followed by the bioinformatics pre-processing of DNA sequence reads, the identification of taxonomic profile and any other relevant functional or genomic elements, statistical analysis, data validation, and finally the visualization and communication of the findings (Quince et al. 2017). Because viral metagenomics does not require virus-specific primers or prior knowledge of the virus, it has been used to objectively detect novel viruses in plants (Zhao et al. 2019). The outcome of the metagenomic functional prediction can be determined by the bioinformatics tools (Table 2.2).

2.2.3 Second-Generation Technologies

Technologies of the second generation have high-throughput sequencing capabilities and can produce data quickly and cheaply, for instance, the commercially available GS FLX by 454 Life Sciences; HiSeq, MiSeq, and NextSeq by Illumina, Inc.; and SoLiD by ABI (Hadidi 2019). The most promising method for detecting newly and reemerging viruses is now second-generation sequencing technologies (Barzon et al. 2013). Plants infected with viruses create specific RNA molecules called short interfering RNAs (siRNA). A cell's defence mechanism known as RNA silencing (RNAi) uses siRNAs as a guide to identify and eliminate ssRNA and dsRNA molecules that are identical to the inducer (Voinnet 2001). In plant samples with

Table 2.2 Bioinformatics tools for metagenomic analysis of plant pathogens

Sr. no.	Bioinformatics tools	Uses	References
1.	BLAST, MAPSeq, QIIME, SINTAX, and IDTAXA	Used as classifiers, for alignment of target sequence with reference sequences	Murali et al. (2018)
2.	SILVA, RDP, Greengenes, and NCBI	For bacterial sequence analysis	Balvočiūtė et al. (2017)
3.	UNITE and WARCUP	Fungal sequence analysis	Xu (2016)
4.	MetaPhlAn HUMan pipeline	Taxonomic profiling	Segata et al. (2012) Franzosa et al. (2018)
5.	PICRUSt2 Tax4Fun2	Predict functional potential of the microbiome	Douglas et al. (2020) Nguyen et al. (2021)

virus infection, NGS finds a large number of siRNA sequences (Loconsole et al. 2012). A potyvirus (Moroccan watermelon mosaic virus), a carlavirus, and two more putative carlaviruses linked to cucumber vein-clearing viruses were identified by Mumo and colleagues utilizing illuminate Miseq sequencing from papaya leaves in Kenyan farms (Mumo et al. 2020) (Table 2.3).

2.2.4 Third-Generation Sequencing

Comparing third-generation sequencing methods to second-generation methods reveals significant advantages. For example, they can eliminate the need to create contigs from scratch using short sequence reads because they can generate noticeably longer reads from individual RNA or DNA samples. Currently, Pac Biosciences (USA) and Oxford Technologies' MinION nanopore sequencing are two examples of third-generation sequencing platforms (ONT, UK) (Ambardar et al. 2016). Additionally, the genomes of species of *Begomovirus* known to cause economically significant infections in significant crop plants have been identified and sequenced using nanopore sequencing (Leiva et al. 2020). Leiva and colleagues used nanopore sequencing to decode the entire genome of the primary emerging pathogen in Southeast Asia, the single-stranded DNA Sri Lankan cassava mosaic virus (SLCMV) (Leiva et al. 2020) (Table 2.4).

2.3 Applications of Nanopore Sequencing for Plant Pathogen Detection

Nanopore refers to nanohole that is distributed across semipermeable membrane and serves as channel to detect the potential change when the analyte DNA/RNA passes through the hole (Xue et al. 2020). Nucleic acid DNA/RNA is an important genetic

Table 2.3 Second-generation sequencing platform-based identification of plant viruses causing diseases of various crops

S. no.	Viruses	Disease	Host plant	Platform used	References
1.	*Papaya mottle-associated virus* (PaMV)	Papaya ringspot disease	Papaya	Illumina MiSeq	Mumo et al. (2020)
2.	*Grapevine leafroll-associated virus 1 and 3*	Leafroll disease	Grapevine	Illumina HiSeq	Zhao et al. (2019)
3.	*Begomovirus*, *Potyvirus*, and *Crinivirus*	Sweet potato virus disease	Sweet potato	Illumina MiSeq	Nhlapo et al. (2018)
4.	*Grapevine fan leaf virus* (GFLV)	Virome of grapevine	Grapevine	Illumina HiSeq	Vigne et al. (2018)
5.	*Norovirus* (NoV)	Norovirus gastroenteritis	Strawberry	Illumine HiSeq	Bartsch et al. (2018)
6.	*Aphid lethal paralysis virus* (ALPV)	Aphid lethal paralysis virus	Cucumber	Illumina MiSeq	Maina et al. (2017)
7.	*Cucumber mosaic virus* (CMV) *Potato virus Y* (PVY) *Tobacco mosaic virus* (TMV) *Pepper mottle virus* (PMV) *Brassica yellow virus* (BYV)	Different viral diseases	Tobacco	Illumina HiSeq	Akinyemi et al. (2016)

Table 2.4 Third-generation sequencing-based identification of causative agents of viral diseases of various crops

S. no.	Virus name	Host plant	Platform	Reference
1.	*Potato virus Y* (PVY)	Potato plant	MinION Nanopore	Della Bartola et al. (2020)
2.	*Tomato severe rugose virus* (ToSRV)	Tomato weed	MinION Nanopore	Duarte et al. (2020)
3.	*Wheat streak mosaic virus* (WSMV)	Wheat plant	MinION Nanopore	Fellers et al. (2019)
4.	*Cassava mosaic disease* (CMV)	Cassava plant	MinION Nanopore	Leiva et al. (2020)
5.	*Tomato yellow leaf curl virus* (TYLCV)	Tomato plant	MinION Nanopore	Chalupowicz et al. (2019)
6.	*Plum pox virus* (PPV)	Prunus plant	MinION Nanopore	Bronzato Badial et al. (2018)
7.	*Zucchini yellow mosaic virus* (ZYMV)	Butternut squash	MinION Nanopore	Chalupowicz et al. (2019)
8.	*Arabis mosaic virus* (ArMV)	Potato plant	MinION Nanopore	Monger et al. (2020)
9.	*Sowthistle yellow vein virus* (SYVV)	Sowthistle plant	MinION Nanopore	Steinberg et al. (2017)

material, and accurate sequencing is very important to know the genome information of the organisms. The sequencing of whole genome of most organisms cannot happen at once; the genome is broken into smaller fragment. After the sequencing of each fragment, small pieces of DNA sequences are generated. These reads can be analysed by two different approaches:

1. **Read mapping**: - It is based on alignment of the reads against the reference genome to detect variations in the sequenced genome.
2. **De novo assembly**: -To construct the original sequences, reads were combined, when a reference genome does not exist (Steinberg et al. 2017).

They are relieved on short read technologies. Since nanopore sequencing can occur in very long reads (more than 2 Mb) and does not need to occur in amplified DNA (Payne et al. 2019). Pacific Biosciences (PacBio, CA, USA) and Oxford Nanopore Technologies (ONT, Oxford, UK) introduced third-generation sequencing in 2011. SMRT sequencing and nanopore sequencing employing MinION Oxford Nanopore Technologies are now the most appealing sequencing techniques for metagenomics-based pathogen identification (ONT) (Heather and Chain 2016).

Single-molecule real-time (SMRT) sequencing is done using zero-mode waveguide detection (ZMW). ZMW is a 10-nm-diameter nanopore that offers little room for DNA polymerization. The bottom of a ZMW contains immobilized DNA polymerase. Ten kilobase (kb) single-stranded pieces of the template DNA are created. A reaction cell is introduced along with four separate fluorescent dyes that are connected to each of the four blocked 3′OH DNA bases. Upon the onset of nucleotide polymerization, a fluorescent signal is produced. The generated fluorescent signal is amplified to a detectable level, detector detect fluorescent signal and transmitted to nanopore- external space.

2.4 DNA Microarray for Detection of Plant Viruses

Microarray technique was originally used to investigate differences in messenger RNA accumulation. As a result, an array's layout usually consists of gene-specific DNA fragments bonded to a solid substrate in a spatially separated manner (Li et al. 2005). The fluorescent nucleic acid is hybridized to the array after the RNA sample has been fluorescently tagged in an enzymic procedure. The fluorescence enables the identification of hybridization events on the solid support, and the identity of the gene is determined from the array position (Loy et al. 2002). DNA may be attached to a solid support in incredibly small areas, allowing researchers to study the expression of many different genes at the same time in a highly parallel manner. Numerous variations in array methodology have been described in the literature, including various solid supports; techniques for immobilizing capture probes; sources for capture probes; methods for designing them; methods for labelling and, if necessary, amplifying the target nucleic acid; hybridization, washing, scanning of arrays; and analytical techniques (Hoen et al. 2003).

2.4.1 Methods for Detecting Plant Viruses

2.4.1.1 Array Fabrication on Solid Supports

Glass microscope slides are the most common solid supports for microarrays because they have low inherent fluorescence, are chemically inert, and can be manufactured and handled easily (Barbulovic-Nad et al. 2006). In comparison to substrates like nylon membranes, which are frequently used for low-density hybridization investigations, glass as a microarray substrate allows for the deposition of capture probes in extremely small areas, enabling a higher density of capture probes (Egeland and Southern 2005).

2.4.1.2 Capture Probes

PCR products produced from cDNA libraries or genomic DNA were initially employed as capture probes on microarrays (Bodrossy et al. 2003). Making cDNA capture probes takes time, necessitates amplifying and purifying each probe individually, and is prone to errors and cross-contamination, especially as the number of probes increases. Furthermore, the poor specificity of cDNA probes results from their ability to tolerate sequence mismatches (Chou et al. 2006).

2.4.1.3 Array Spotting

There are several methods for depositing capture probes on the surface of planar arrays; the easiest and most popular one involves physically applying very small amounts of capture probe to the surface. While low-density arrays can be created by hand, robots often perform spotting (Hegde et al. 2000).

2.4.1.4 Capture Probe Design

The design of oligonucleotide capture probes is based on the sequence's distinctiveness as well as a variety of other factors (Ratushna et al. 2005). Numerous software programmes are available that employ design criteria including melting temperature, GC content, secondary structure produced by self-annealing, and hybridization free energy for the selection of oligonucleotide probes. Another element that can affect a capture probe's success or failure is its attachment to the target nucleic acid's secondary structure; however probe design algorithms do not yet take this into account (Peplies et al. 2003).

2.4.1.5 Target Preparation

The most popular labels for labelling target nucleic acids are the cyanine dyes Cy3 and Cy5 (GE Healthcare, Little Chalfont, England), which have excitation wavelengths of 635 nm and 532 nm, respectively. Two-colour labelling is used in gene expression research to enable the hybridization of two samples (such as two distinct tissues or treated and untreated samples) to the same array and the comparison of their gene expression profiles (Staal et al. 2005).

2.4.1.6 Signal Amplification

For detecting huge numbers of target nucleic acids, such as a high-titre virus in infected material, reverse transcription labelling is the most effective method. Signal amplification is required because this method has been demonstrated to be insensitive enough to miss targets at low levels (Xiang et al. 2002).

2.4.1.7 Hybridization, Washing, and Scanning of Arrays

The tedious process of target nucleic acid hybridization in solution to immobilized capture probes might take up to 24 h to complete. To achieve the best sensitivity (maximum signal strengths), specificity (suppression of nonspecific binding and background signals), and reproducibility across experiments, the hybridization temperature, salt concentration, pH of the hybridization buffer, and rigour of the following washing steps must all be optimized (Han et al. 2006).

2.4.2 Novel Formats and Nonplanar Arrays

While planar substrates such as glass are now the most extensively used format for DNA microarrays, their high prices and low throughput may make them unsuitable for disease detection applications. The different array platforms available have been thoroughly explored elsewhere; some of the platforms that may be particularly appealing for plant virus detection are covered here (Call 2005) (Fig. 2.2).

The SMRT PacBio RSII platform offers sufficient throughput and cost effectiveness. The SMRT PacBio and MinION sequencing is similar in terms of read length 10–15 kb and a throughput of 0.5–1.0 Gb per run (van Dijk et al. 2018) (Fig. 2.3).

Due to its portability and simplicity, MinION nanopore sequencing is quickly gaining favour with scientists and is the preferred platform for sequencing. The obstruction of a nanopore causes base-specific variations in MinION, which are then converted into DNA sequence data (Lu et al. 2016). MinION sequencing is based on DNA electrophoresis, in which a-haemolysin nanopores are embedded across a semipermeable membrane which serve as channels for electrophoresis. The membrane separates two chambers into cis and trans which is filled with electrolyte solution. When a voltage is applied, the movement of ions through the pore starts and creates an electric field. An analyte can be captured in the pore or transported across the pore. When the analyte enters into the pore it alters the ionic current by

1. Producing a change in electric field within the pore.
2. Binding of ions to the traversing analyte causes the reduction of ionic current.

Each analyte's present modification has a distinct duration and magnitude. In MinIon sequencing, first the template DNA is separated by using Covaris g-TUBES. Then cyclodextrins covalently bind to the nanopore that increases interaction of nucleotide channel. To determine the sequence of the template DNA, the ion current is monitored (Garalde et al. 2018).

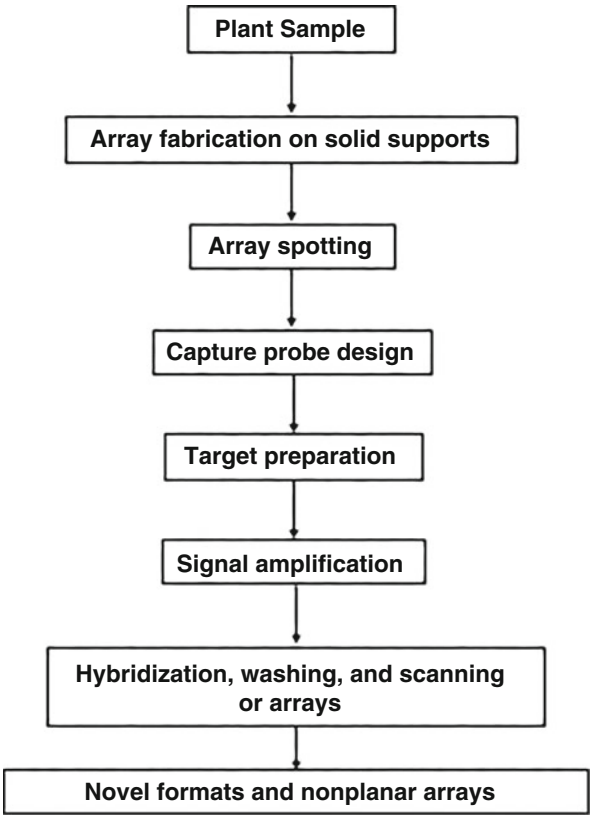

Fig. 2.2 Schematic representation of microarray method for detection of pathogens from plants

In nanopore sequencing, naturally occurring protein pores are manufactured and solid-state nanopores are used (Xue et al. 2020). There is a vast repertoire of biological pores. There are various natural nanometre-sized pores that act as ion channels, porins, aquaporins, pore-forming toxins (PFTs), and viral pores (Gilbert et al. 2017). Nanopore sensor PFTs are a-haemolysin (a-HL) or cytolysin A (Cly A) that are expressed in *E. coli* as soluble monomers and isolated by using affinity chromatography (Fig. 2.4). Other channel types are *Mycobacterium smegmatis* porin A (Msp A). Leiva and colleagues sequenced the complete genome of single-stranded DNA *Sri Lankan cassava mosaic virus* (SLCMV) by nanopore sequencing, which was a major emerging pathogen in Southeast Asia (Leiva et al. 2020). Due to its portability, the MinION platform is developing into a potent tool for on-site sample sequencing. This helps with quick microbial identification in a variety of conditions, such as microbial paleomats in the Antarctic (Johnson et al. 2017). Hu et al. diagnosed fungal wheat disease caused by *Zymoseptoria tritici*, *Puccinia striiformis* f. sp. *tritici*, and *Pyrenophora tritici repentis* by nanopore sequencing (Hu et al. 2019).

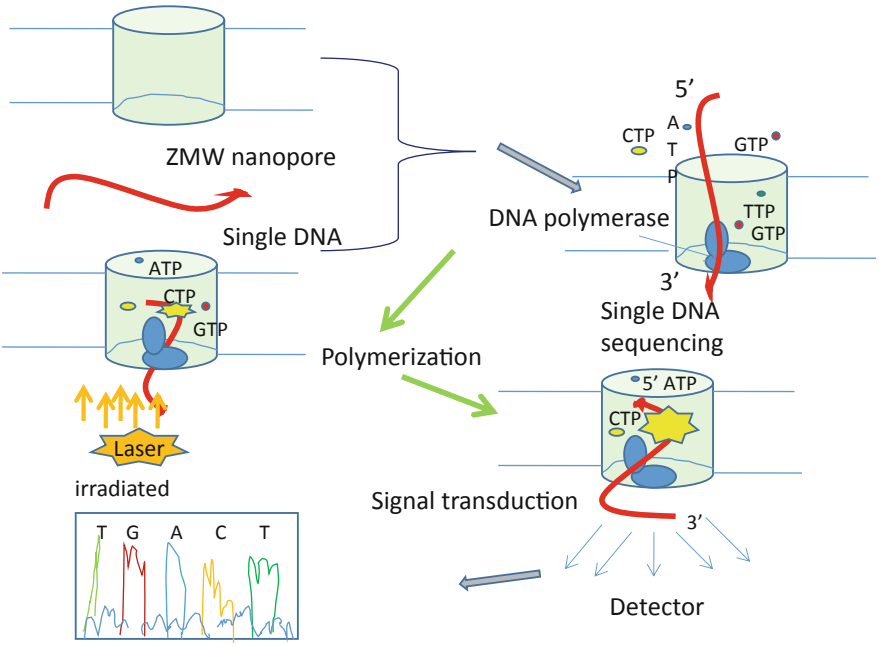

Fig. 2.3 Representation of working of SMRT sequencing technique

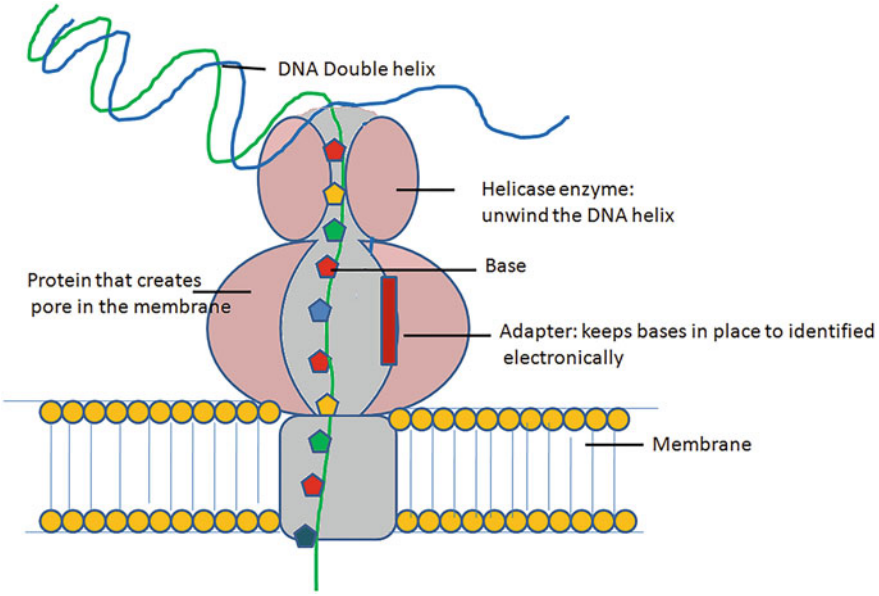

Fig. 2.4 Basics scheme of nanopore sequencing

2.5 Precision Metabolism for Plant Pathogens

Pathogens have created a variety of techniques for invading, feeding on, and reproducing in plants. Fungi, bacteria, oomycetes, and viruses are examples of plant pathogens. Biotrophic diseases need living tissue to develop and proliferate, yet the tissue frequently perishes in the late stages of infection (hemi-biotrophic pathogens). On the other hand, necrotrophic pathogens consume the dead tissue at the beginning of the infection and kill the host tissue. In general, viruses need living tissue to survive, but bacteria and fungus can both use biotrophic and necrotrophic strategies to survive. There are striking similarities between the mechanisms used by plants to defend themselves from both necrotrophic fungi and bacteria and biotrophic fungi and bacteria (Lucas 1998). The salicylic acid-dependent responses are more efficient against biotrophic infections, but the jasmonate/ethylene route is crucial in preventing necrotrophic diseases. Additionally, pathogens are divided into groups according to the tissues they infect and the environments they like. One of the most common categories is that the pathogen primarily targets above-ground and below-ground tissues (Zipfel 2008). Above-ground tissue examples that are green, assimilate producing, or assimilate importing include source leaves and flowers (Fig. 2.5). Pathogens infecting source tissue, such as roots, flowers, and sink leaves, would experience different conditions than pathogens attacking sink or assimilate-producing tissue, such as roots, flowers, and sink leaves, in terms of basic metabolism and defence responses. The majority of microorganisms cannot harm plants because of the defence mechanisms that have been developed and performed by plants. Recognizing the presence of microorganisms is the first step in triggering defence responses. It has been shown that both plants and animals can recognize

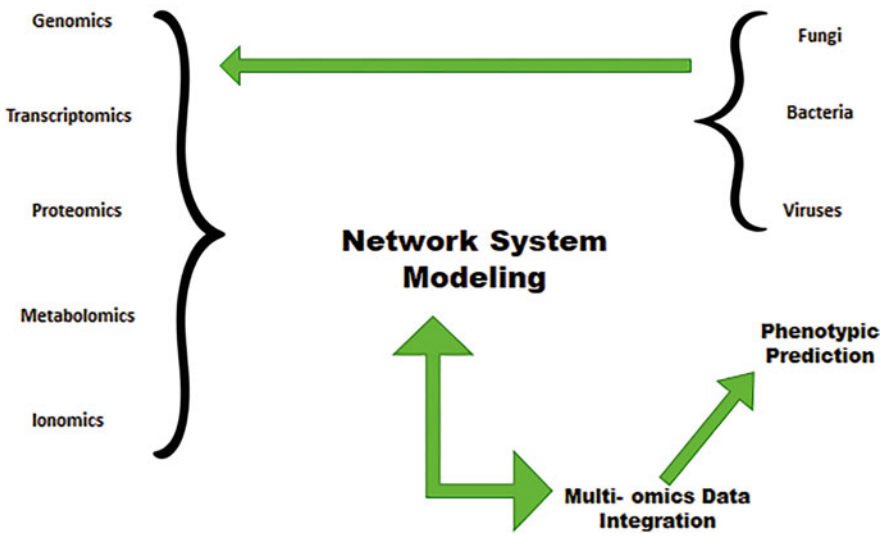

Fig. 2.5 Plant-pathogen interaction

pathogen-associated molecular patterns (PAMPs), and the discovery of elicitors produced by microorganisms marks the start of basal resistance (Lodha and Basak 2012).

Gene expression is controlled, which activates defence mechanisms such cell wall strengthening, the accumulation of phytoalexins, and pathogenesis-related (PR) proteins. The idea is that some microbes developed virulence-enhancing effector chemicals that rendered them toxic and undermined plant defences (Segarra et al. 2007). In response to these advantageous interactions, the virulent disease can spread throughout the weak plant. Innate immunity is a defence mechanism used by plants. Defence systems that have been pre-programmed and activated lead to innate immunity. Structures such as the cell wall and cytoskeleton, as well as antimicrobial compounds, are used in defence responses to ward off diseases and pests. Induced defences are activated by identifying proteins (effectors) that the pathogen has translocated to the host cell or by spotting pathogen-associated molecular patterns (PAMPs) on the pathogen surface (Dodds 2010; Mur et al. 2008).

For many years, it has been assumed that basic metabolism supports cellular energy requirements for plant defensive responses during plant-pathogen interactions (Snijesh and Singh 2014). Due to the production of hundreds of genes from numerous defensive pathways, energy is essential during the execution of plant defence responses (Zulak et al. 2009). Furthermore, defence responses appear to have a fitness cost; *Arabidopsis* mutant plants that produce defence responses constitutively are stunted and have lower fertility, whereas mutant plants that lack defence signaling pathways are taller (Valcu et al. 2009).

Chlorophyll fluorescence imaging has revealed changes in photosynthesis at the infection site and the tissue around it in various plant-microbe interactions. The decline in photosynthesis was quicker and more pronounced after inoculation with an avirulent strain. Since light reactions during photosynthesis result in chloroplast ROS, which can be employed for defence responses, photosynthesis downregulation is paradoxical (Jones and Sasser Jr 2001). Nonetheless, two alternative pathways have been proposed:

1. Pathogen effector-induced reduction of photosynthesis.
2. Sugar signal-mediated feedback control.

Downregulation of photosynthesis, regardless of the method, reduces the energy cost associated with overexpression of other energy-producing processes. Energy can be produced, for instance, by boosting the activities of respiratory metabolism, cell wall invertase, and carbohydrate transporters. This metabolic switch from source to sink may further boost the expression of defence-related genes and the synthesis of secondary metabolites like phytoalexins (Dangl and Jones 2001). While primary metabolism's significance as a source of energy is undeniable, the analysis's attention is on how it controls plant defensive responses in the presence of potential pathogens or pathogen-derived elicitors (Bonfig et al. 2006).

2.6 Artificial Intelligence-Based Methods for Plant Disease Detection

The agricultural cycle begins with seed sowing and ends with harvesting. Malady invasion, the board of capacity, pesticide control, recognizable proof of weed and the executives of weed, lack of suitable soil and water, and so on are among the significant difficulties impacting the general production of the yield. Artificial intelligence (AI) and machine learning (ML) have entered different classifications. Artificial intelligence developments are based on previous learning experiences. Back proliferation, artificial neural networks, and convolutional neural networks are examples of machine learning (ML) processes that are being used to computerize machine tasks and create cutting-edge breakthroughs. The sole goal of machine learning is to maintain a coherent model (machine) with quantifiable data from previous encounters to make precise and correct decisions. ML is a numerical method for creating intelligent machines (Kothari 2018).

AI assists in the prediction of infection and its treatment based on data identified with water pressure, supplement content, harvest photos, atmosphere, and soil dampness content. Plant disease is a big threat to food security since it has a real impact on harvest output and, as a result, reduces the nature of yield. The ability to diagnose field disease accurately and appropriately is a test. Human intervention is required for the regular arrangement of plant disease distinguishing proof. Plant illnesses are identified through visual study of plants. Wrong decisions and procrastination in making the best decision have a negative impact on profitability. Human interventions, on the other hand, have now been combined and in some cases, supplanted by various advancements.

With advancements in technology and a reduction in the cost of picture acquisition, a range of image-based diagnosis methodologies have emerged. In any case, an image encases a large amount of data, making it difficult for the PC framework to deal with it directly. With its quick scientific growth and vast application area, artificial intelligence (AI) is one of the most important areas of research in software engineering. The core concept of AI in agriculture is its adaptability, speed, precision, and cost-effectiveness.

In agriculture, artificial intelligence not only helps farmers use their natural agricultural skills but also shifts to direct farming to produce higher yields and better quality with fewer resources. On farms, AI sensors can detect and identify weeds, as well as diagnose plant illnesses, pests, and malnutrition (Goswami et al. 2018).

The methodologies that have been used to identify disease, segment the affected area, and classify diseases. Artificial intelligence (AI) can provide a practical and effective answer to the problem, and machine learning (ML) and deep learning (DL) have been introduced. Using machine learning to train enormous datasets made publicly available gives us a clear technique to detect disease in plants on a massive scale (Soni 2018).

Machine learning-based technologies for detecting and categorizing diseases on agricultural items such as plants, fruits, and vegetables will be used. A robot is

supplied that uses image processing and machine learning to identify the leaf illness (Amara et al. 2017).

In machine learning and pattern recognition, an artificial neural network is a computational model. A proposed method for plant disease recognition utilizing a feed forward back-propagation algorithm was evaluated, and it worked well with a precision of roughly 93%. The treatment was tested on plant diseases such as early scorch, cottony mould, late scorch, and small whitening. A model was designed to improve the accuracy in identifying two forms of fungus-caused diseases in cucumber plants: Downy Mildew and Powdery Mildew (Zhang et al. 1999).

Using a back-propagation algorithm, a system was developed to recognize and categorize illnesses such as leaf spot, bacterial blight, fruit spot, and fruit rot in pomegranate plants, with an experimental result of around high accuracy. Using the neural network, back-propagation approach, a work on identifying the groundnut plant disease Cercospora leaf spot was proposed. The experimental results and observations demonstrate that they correctly recognized 4 types of diseases out of 100 sample diseased leaf photos with a 97.41% accuracy rate (Hilbert and Ostendorf 2001).

Artificial intelligence is the ability to learn without being explicitly configured, which is basically like how a human works. If the presentation of the assignment improves with more knowledge, the computer learns from previous encounters (which are taken care of in information) concerning a few kinds of errands. Learning can be classified as:

2.6.1 Supervised Learning

For model preparation, supervised learning refers to a named dataset that includes both input and output boundaries. When creating a model, the ratio of preparing and testing data is preserved at 80:20. Classification and regression are two other terms for supervised learning. The setup is based on the supervised way of learning errands, in which the output is a discrete value. This discrete worth could be multi-classed or parallel. Reach is a supervised learning model that produces persistent worth, whereas relapse is not. The goal of the relapse is to anticipate a value that is more in line with production esteem (Jagga and Gupta 2014) (Fig. 2.6).

2.6.2 Unsupervised Learning

Targets are not supplied to display to be produced in unsupervised learning; therefore the model only has input bounds and no output boundaries. Unsupervised learning is divided into two types: bunching and association. Information organized as gatherings made by different examples distinguished by the machine model is clustered. Association is a standard-based strategy for sorting out relationships among the boundaries of a large informative collection (Pothuganti 2013).

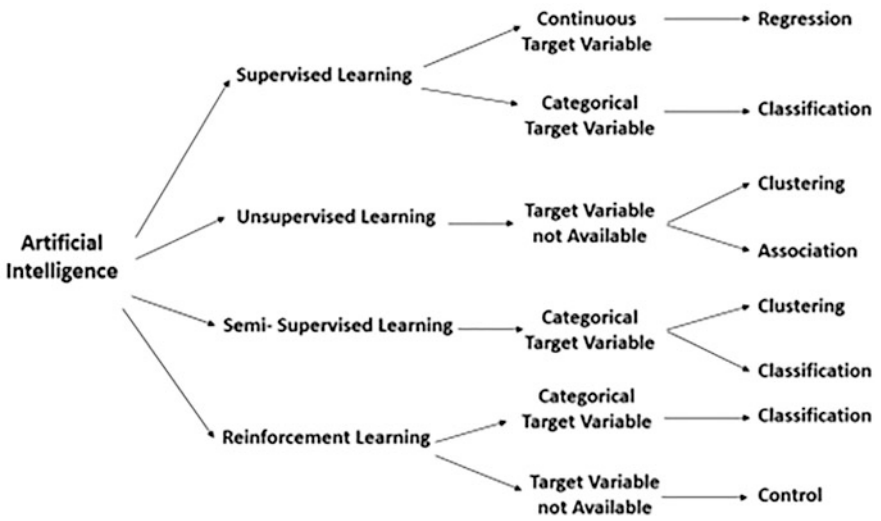

Fig. 2.6 Flowchart on different approaches involved in machine learning

2.6.3 Semi-Supervised Learning

Semi-supervised learning operates in the same area as the previously mentioned processes. This method of learning is used when dealing with material that is partially labelled and partially unlabelled. To compute marks, an unsupervised technique is used, and then these determined qualities are passed on to supervised learning strategies. This approach is more well-known in image datasets with many unnamed images (Huda et al. 2017).

2.6.4 Reinforcement Learning

With criticism to learn instances and conduct, the model's execution continues to improve. When information is taken care of, it is discovered and added to the information that is being prepared. As a result, the more it learns, the more it will be prepared and experienced. Temporal difference, Q-learning, and deep adversarial networks are reinforcement learning algorithms (Wang et al. 2021).

2.7 Conclusion

Natural resources are diminishing and the human population is outpacing them in the modern era, which necessitates the development of novel renewable and nonrenewable resource alternatives. A more reliable option is emerging: plant metabolic engineering. Plant metabolic engineering can close the gap between supply and

demand for plant-based foods, natural medicines, biofuels, complex organic compounds like flavonoids, and crops with improved nutrition. Site-directed mutagenesis, CRISPER/Cas9, RNAi, cell-free synthesis, and the overexpression of genes that are important for metabolism allow us to control and change plant metabolism for the benefit of humans. Although there are many uses for this discipline, producing sustainable and high-yielding crops is primarily concerned with problems like low productivity, the development of undesirable products, and plant pathogen resistance. Plant metabolic engineering has a lot of potential as long as we pay close attention to how metabolic pathways work, how they behave, and how to control them to meet our needs.

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Emergent Tools and Techniques in Diagnosis of Soil-Borne Phytopathogens

3

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Abstract

Higher plants are infected by a large number of plant pathogens. Their effects range from minor symptoms to catastrophic events that result in the destruction of large areas of food crops. Catastrophic plant disease exacerbates the current food supply deficit, which has left at least 800 million people hungry. Plant pathogens' populations are difficult to control because they vary in time, space, and genotype. Most insidiously, they evolve, often overcoming resistance that is the plant breeder's hard-won achievement. It is very necessary to define the problem and explore solutions in order to avoid the losses they cause. The major genera and species of disease-causing organisms can now be quickly and reliably identified, credit goes to recent advances in plant pathogen detection based on immunological and nucleic acid-based techniques. Monoclonal antibodies or polymerase chain reaction (PCR)-based methods, OMICS techniques, protein-based approaches, and nucleic acid-based approaches are highly sensitive and specific and have the potential to replace traditional technologies.

Keywords

Plant pathogens · DNA/RNA · Polymerase chain reaction (PCR) · OMICS techniques

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3.1 Introduction

Plant diseases caused by various emerging pathogens cause serious economic losses in plant. It causes losses of around 10% (Strange and Scott 2005). A large number of plant pathogens cause diseases, viz. fungi, bacteria, virus, and nematodes in agricultural fields (Kalsoom et al. 2020). The global food supply shortage is compounded by devastating plant diseases (Rehman et al. 2020). Plant pathogens are difficult to track because their distributions are complex in terms of time, space, and genotype. As they grow quickly and are always defeating, re-emerging, and chronic/endemic pathogens; it is a difficult task for plant pathologists to manage. To overcome the losses they cause, it is necessary to define the problem and explore the solutions. Plant pathogen detection is a point of concern both within and outside the plant pathology community, given the economic, social, and environmental consequences of plant diseases (Brownlie et al. 2006). On a scientific level, rapid and accurate identification of the causative organism, precise estimation of the severity of the disease and its impact on production, and identification of its virulence mechanisms are required. Inaccurate plant disease diagnosis can result in the failure of management strategies, resulting in huge crop losses and overall trade losses. Inadequate sanitary and phytosanitary (SPS) measures exacerbate the loss of trade (Miller et al. 2009). The advent of sensitive and specific molecular methods has revolutionized the detection of disease-causing organisms in recent years. Diagnostics have seen rapid and exciting advancements, and all working plant pathologists will be exposed to these advancements in the near future. Immunological assays and nucleic acid-based assays, in particular, are now available for a wide range of microorganisms. Traditional techniques like baiting, culturing, and microscopy are still widely used and are the mainstay of plant health diagnosticians, but molecular approaches are becoming more widely available.

A wide range of molecular methods have been used to detect, identify, and quantify a wide range of plant pathogens that live in soil (Sharma et al. 2015). Molecular techniques have also been used to investigate the genetic variability of pathogen populations, as well as to describe new fungal species (Judova et al. 2012). In general, these methods are much faster, more specific, sensitive, and accurate, and they can be performed and interpreted by people with no prior knowledge of taxonomy (Fig. 3.1). Molecular techniques can also distinguish closely related organisms at different taxonomic levels due to their high degree of specificity (Ghosh et al. 2017). Here, in this chapter the most important tools for detection of various soil-borne plant pathogens and its implementation in disease diagnosis are properly illustrated.

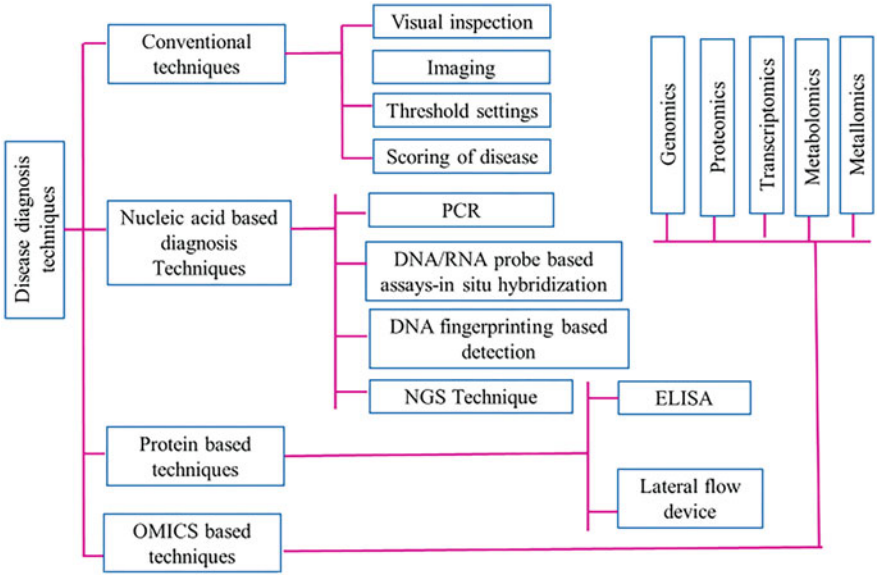

Fig. 3.1 Various diagnostic techniques for soil-borne plant pathogen

3.2 Advanced Techniques for Diagnosis of Soil-Borne Phytopathogens

3.2.1 Nucleic Acid-Based Diagnostic Techniques

3.2.1.1 Multiplex PCR

It is used to detect coincident and careful detection of the different DNA or RNA targets from a single reaction. Moreover, it can also be mapped out to verify the occurrence of more than one pathogen in a given plant sample by recognizing more than one common specific sequences. There is a constant need for sensitive detection for the proper propagation of pathogen-free plant material, and therefore this technique is useful in plant pathology as different soil-borne pathogens constantly infects a single host. Some of the examples of simultaneous detection of different pathogens by multiplex PCR in one attempt includes hosts like turf grass, wheat (Sun et al. 2018), strawberry (Li et al. 2011), etc. This technique is mainly sensitive towards the number of targets to be detected, due to the presence of different primers instead of the total amount of primer occurring in a mix.

3.2.1.2 Real-Time PCR

Quantification and identification or detection of pathogen is a very important requirement for plant disease management. Real-time PCR is mainly used for identification and detection of various plant pathogenic fungi, bacteria, nematodes,

oomycetes (water moulds), viruses, and biocontrol agents. Therefore, the development of real-time PCR holds significance as quantification based on traditional methodology of culturing is contemplated as somewhat imprecise and also in some instances unreliable (Tarafdar et al. 2018). Real-time PCR is different from the classical endpoint PCR from the point of quantification of the amplified PCR outcome at each PCR cycle. Real-time PCR gives exact template quantification because the development of the exponential phase is observed. Recent developments have been done in detection techniques based on real-time PCR to diagnose and identify a number of phytopathogenic fungi (Schena et al. 2004; Lievens et al. 2005). These techniques are more sensitive than a conventional PCR as these allows pathogenic fungi to be detected by a determined increase of fluorescence during PCR amplification (Lees et al. 2002); this decreases the risk of false-positive results and assists in quantitative and multiplex analyses.

3.2.1.3 Magnetic Capture Hybridization PCR

This PCR assay was mainly established to deal with PCR inhibitors in plant extracts during DNA isolation steps. Magnetic capture hybridization PCR (MCH-PCR) is a technique that uses DNA isolation along with a purification period that consists of hybridization with the use of single-stranded DNA (ssDNA) probe on magnetic beads that is followed by the PCR amplification of target DNA sequences (Jacobsen 1995). The hybridization of double-stranded DNA (dsDNA) and magnetic beads allow separation of the complex from inhibitors (Capote et al. 2012). The magnetic beads are covered in biotinylated oligonucleotides specific to a particular DNA region of interest related to fungal pathogen (Walcott et al. 2004). Real-time-based PCR assays and MCH were done for two cucurbit seed pathogens, viz. *Acidovorax avenae* subsp. *citrulli* and *Didymella bryoniae*, which causes bacterial fruit blotch and gummy stem blight, respectively, and were evaluated accordingly. This assay provided simultaneous identification of both tested pathogen in cucurbit seed samples (Ha et al. 2009). Process of creating a MCH-PCR capture probe includes the selection of oligonucleotide probe sequence from extremely conserved regions of fungal pathogens (Langrell and Barbara 2001). The specificity of the chosen sequence can be examined through BLAST. The 5′ end of probe is biotinylated (Chen and Griffiths 2001) to attach it to streptavidin-covered magnetic beads (Johnson et al. 2013). MCH-PCR decreases the total detection time, increases PCR sensitivity, and removes the inhibitors of the amplification reaction and additional non-target DNA (Amagliani et al. 2006).

3.2.1.4 End-Point PCR

Emergence of PCR reformed the precise identification of different plant pathogens in disease management that also includes fungi (Ma and Michailides 2007). This is an in vitro technique that takes a piece of DNA template which is amplified exponentially (Caetano-Anolles 2013) through repeating denaturation cycles, annealing, extension, and final extension and finally holds reactions at varied temperatures using specified primers, deoxyribonucleotide triphosphates (dNTPs), and a thermostable Taq DNA polymerase in buffer solution (Griffiths 2014). This PCR

framework is contemplated as a cost-effective choice in comparison to other existing molecular diagnostic choices for fungal plant pathogens. End-point PCR allows accurate detection of fungal plant pathogens by developing either universal primers to amplify numerous pathogens or definitive oligonucleotides that will target certain fungal species followed by sequencing. By using Basic Local Alignment Search Tool (BLAST) analysis, comparison against ex-type cultures available in the NCBI GenBank database is done and for every set of nucleotide sequences of fungal isolates, identification of each isolate can be discerned. The existence of targeted phytopathogenic fungi was confirmed by presence of a target unveiled in agarose gel electrophoresis (Mirmajlessi et al. 2015). However, end-point PCR assays are still considered time-consuming as it is difficult to design primer sets that characterize closely related fungal pathogens. *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* (which cause speck rot and Sphaeropsis rot diseases in apple, respectively) were diagnosed using end-point PCR and real-time PCR analysis but it was found that quantitative approach of a real-time PCR was more sensitive and rapid than the end-point PCR (Sikdar et al. 2014).

3.2.1.5 Nested PCR

It is also known as modified version of end-point PCR and uses two sets of primers employed at two rounds of PCR cycles to intensify sensitivity and specificity. Nesting helps in significantly low usage of non-specific PCR primers in the initial cycle of PCR for the amplification of various pathogens, and then the pathogen-specific primers are used in the next cycle (Bhat and Browne 2010). *Pilidiella granati* causes emerging diseases in pomegranate cultivation, namely, twig blight and crown rot, and the nested PCR assay improved both detection and sensitivity of *P. granati* and made it possible to detect the causative agent when the sample contained DNA as low as 10 pg of *P. granati* (Yang et al. 2017a, b). Great yam disease caused by *Colletotrichum gloeosporioides* (Raj et al. 2013) and eucalyptus dieback disease caused by *Cylindrocladium scoparium* (Qiao et al. 2016) were also detected by this technique. By using this PCR technique, the sensitivity of detection can be increased from 10- to 1000-fold over an end-point PCR assay (Ippolito et al. 2002; Silvar et al. 2005). But the risk of cross-contamination due to the manipulation of previously amplified samples is high which can give false-positive outcomes, and the nested PCR assays are also time-consuming (Raj et al. 2013). Therefore, nested PCR and end-point PCR methods that may produce amplicon contamination are not recommended to be used as reliable diagnostic methods.

3.2.1.6 BIO-PCR

BIO-PCR assay is a modified version of end-point PCR technique that includes a pre-assay incubation step of a diseased sample to increase the biomass of the causal agent. This approach is basically utilized to condense target pathogens by propagating the target pathogen in a growing media that prevents the growth of non-target microorganisms to improve detection (Schaad et al. 1995), and this has been essentially applied to detect seed-borne fungal pathogens (Kumar et al. 2020). Lupin anthracnose disease caused by *Colletotrichum lupine* was identified using the

BIO-PCR method. It was done by incubating the seeds with altered Yeast Malt Broth to enrich *C. lupine* biomass and a species-specific primer set was designed based on rDNA IGS sequence. This standardized protocol helped in the detection of *C. lupine* in *Lupinus* spp. (Pecchia et al. 2019). The seed-borne fungal pathogens like *Alternaria alternata*, *A. radicina*, and *A. dauci* were identified with the help of specific primers of ITS in rDNA using deep-freeze blotter procedure during the BIO-PCR assay (Konstantinova et al. 2002). There are several advantages of this technique over end-point PCR, viz. high sensitivity, detection of living cells to avoid false positives, and elimination of PCR inhibitors (Marcinkowska 2002; Fatmi et al. 2005). Some of the limitations of this technique are that it is time-consuming and costs are incurred when selective media is utilized for the detection (Schena et al. 2004; Mancini et al. 2016).

3.3 DNA/RNA Probe-Based Assays-In Situ Hybridization

3.3.1 Fluorescent In Situ Hybridization

Fluorescent in situ hybridization (FISH) is a technique used for identifying and locating a specific DNA sequence on a chromosome. It is a comparatively recent and innovative technology in plant disease diagnostics. It's a combination of specificity in DNA sequences with the sensitivity of detection systems based on fluorochromes (Hijri 2009; Cui et al. 2016). This technique involves the use of DNA or RNA probes that are directly or indirectly labelled with fluorochromes to detect DNA or RNA sequences in cells or tissues (Shakoori 2017). In normal FISH methods, fluorescently mono-labelled oligonucleotide probes are hybridized to the ribosomal RNA (rRNA) of microbial cells, and the stained cells are then visualized by wide field epifluorescence or confocal laser scanning microscopy (Lukumbuzya et al. 2019). When a plant is infected with pathogen, then pathogen-specific rRNA sequences will be present in those plants, and this specific information conferred by RNA can be detected by FISH (Fang and Ramasamy 2015). Southern blight in tomato is caused by a soil-borne pathogen, *Sclerotium rolfsii*, and the soil smears having DNA isolation with 0.06 pg μL^{-1} of; this pathogen was effectively detected by FISH assay that used an oligonucleotide probe labelled with cyanine dyes Cy3 and Cy5 (Milner et al. 2019). It is seen that reproducibility, specificity, sensitivity, precision, and speed are the best features of FISH (Bozorg-Ghalati et al. 2019). Amongst mixed species specimens, this technique can also deliver information about resolution, morphology, and identification of main pathogens (Frickmann et al. 2017). False-positive results with auto-fluorescence materials are usual drawbacks that reduce specificity during this assay (Moter and Göbel 2000).

3.4 Next-Generation Sequencing

Next-generation sequencing (NGS) or high-throughput sequencing (HTS) is a technology used to determine the arrangement of nucleotides in targeted regions of DNA or RNA or entire genomes and is a new approach for diagnostics. The development of this technique has fuelled innovative schemes for detection and identification of phytopathogens (Chalupowicz et al. 2019). Some of the major steps involved in DNA-based NGS are isolation and fragmentation of DNA, library preparation, massive parallel sequencing, bioinformatics analysis, and variant/mutation annotation and interpretation (Qin 2019). Commonly available advanced sequencing methods in HTS includes massively parallel signature sequencing, pyrosequencing, colony sequencing, and sequencing by oligonucleotide ligation detection (SOLID) (Rajesh and Jaya 2017). RNA-sequencing (RNA-Seq) deals with advanced coverage and greater resolution of the dynamic nature of the transcriptome. The Illumina HiSeq platform is the most universally functional NGS platform for RNA-Seq and has established the standard for NGS; also, this platform recently has released a desktop sequencer that goes by the name MiSeq (Kukurba and Montgomery 2015). RNA-Seq-based NGS can be utilized in the rapid identification of fungal plant pathogens inducing novel diseases. A whole genome sequencing protocol was established by using Illumina MiSeq to detect a novel fungal pathogen that causes Sarcococca blight in ornamental plants, namely, *Calonectria pseudonaviculata*. A 51.4 Mb genome of the two host isolates was identified with a unique single nucleotide polymorphism and were both identified as *C. pseudonaviculata* (Malapi-Wight et al. 2016). Datasets built on NGS from population genomics can be exploited to regain variations including single nucleotide polymorphisms (SNPs), insertions and deletions (INDELS), and structural variations (Potgieter et al. 2020). *Puccinia striiformis* f. sp. *tritici* (PST) is an emerging or re-emerging plant infecting fungus that causes yellow (stripe) rust in wheat and triticale. Field pathogenomics was carried out by using RNA-Seq based on NGS of wheat leaves infested with PST to get knowledge about emergent pathogen populations. The results showed that there was a considerable amount of shift in the PST population in the UK, which is likely because of a latest introduction of divergent and unfamiliar PST lineages (Hubbard et al. 2015). RNA- and DNA-based NGS approach was conducted to develop molecular diagnostics for the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Comparative genomics using RNA-Seq of close relative species *P. humuli* discovered seven specific regions in *P. cubensis* that allowed for the development of diagnostic markers (Withers et al. 2016).

3.5 DNA Fingerprinting

DNA fingerprinting is a technique that simultaneously detects lots of minisatellites in the genome to construct a pattern unique to an individual. Fingerprinting methods allow the screening of random areas of the pathogen genome so that species-specific sequences can be recognized when the conserved genes do not show much deviation

to successfully identify species or strains (Patil 2018). Usually, the fingerprinting techniques are used to analyse phylogenetic arrangement of fungal populations. However, these methods are also utilized to distinguish specific sequences used for the identification of pathogen at very low taxonomic level and can also be used to point out the different strains of the same species with virulence, different host range, and compatibility group.

3.5.1 PCR-Restriction Fragment Length Polymorphism (RFLP)

Specific and sensitive detection methods have been developed, mainly based on polymerase chain reaction (PCR), and a considerable amount of progress has been made when it comes to using DNA-based methods for detection, identification, and classification of soil-borne plant pathogens. Nuclear ribosomal DNA (rDNA) amplified through PCR helps to characterize, distinguish, and classify soil-borne pathogens on the basis of phylogeny, employing the restriction fragment length polymorphism (RFLP). For instance, 10 *Phytophthora* species infecting different crops through extensive RFLP of PCR-amplified rDNA were detected and differentiated, thus permitting selective detection of these *Phytophthora* spp. (Camele et al. 2005). Amplification and digestion were done through PCR primers specific to the genus *Phytophthora* and the resultant amplicons had a specific restriction pattern of 27 different *Phytophthora* species (Drenth et al. 2006). When analysis of the ITS region was done through PCR-RFLP, presence of different anastomosis group within isolates of *Rhizoctonia solani* was shown. Distinction between pathogenic and non-pathogenic strains of *Pythium myriotylum* was also allowed (Gómez-Alpízar et al. 2011). Genetic polymorphism within populations of *M. phaseolina* isolated from chickpea targeting PCR-amplified rDNA was also revealed (Sharma et al. 2012).

3.5.2 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) technology is a simple, rapid, and inexpensive technique that uses short synthetic oligonucleotides of random sequences as primers to amplify small amount of total genomic DNA under low annealing temperatures by PCR. RAPD mechanism has been used in genetic mapping, molecular taxonomy, evolutionary studies, and diagnosis of several fungal species (Nasir and Hoppe 1991). The analysis of DNA products generated through RAPD has provided information on disparity and exclusion of genetic traits amongst strains. In the process, on sorting out the resultant PCR outcome, a semi-distinctive profile pattern is noticed. Characterization of the amplified DNA depends on the nucleotide sequence homology amongst the template DNA and oligonucleotide primer present at the end of each amplified product. As a result, more robust polymorphic amplification products in every experiment are generated rather than other marker systems, and on top of that their application does not need any prior

sequence information. Therefore RAPD markers are found to be more suitable for studies on the genetic structure of fungal populations (Nasir and Hoppe 1991). Also, even the most minute changes can be analysed using this marker. RAPD have several advantages that proved to be significant in studying formae speciales and races of *Fusarium oxysporum* (Belabid et al. 2004). Also, characterization of many strains of *Fusarium*, *Alternaria*, and *Rhizoctonia* spp. were also done with the help of RAPD analysis (Kini et al. 2002).

3.5.3 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a technique based on PCR that is used in genetic research, DNA fingerprinting, and also genetic engineering. The process of AFLP includes cutting of target DNA into fragments using two restriction enzymes from the total genomic DNA, and the resultant strands are then ligated with double-stranded nucleotide adapters. A section of restriction fragments is then selected for the process of amplification. In order to amplify the fragments, specific primers that consist of a restriction site sequence and additional nucleotides at the 3′ end are used as selective complementary agents to the adapter. And the upper hand of this AFLP technique is that it requires very little amounts of DNA templates as compared to other fingerprinting methods such as RAPD and inter-simple sequence repeats. Application of this technique does not require any prior sequence information and results in markedly more robust polymorphic amplification products in each experiment than other marker systems, and therefore, for this very reason AFLP markers are perceived to be very suitable for studies on the genetic assembly of fungal populations (Gargouri et al. 2006; Sharma et al. 2012). Major applications of AFLP makers include analyses of genetic variation below the species level, basically in examination and differentiation of population structure including estimation of FST analogs and genetic variation within populations (Sharma et al. 2012). AFLP markers possess a potential of delivering vital information under the extreme time constraints frequently needed by pending conservation decisions, and therefore, such analyses are critical for conservation genetics. Apart from problems of population structure and variation, AFLP markers have also been used to evaluate gene flow and dispersal. The high resolution of AFLP markers allows testing for clonal identity amongst individuals (i.e. absence of recombination) and thus permits inference about sexual versus asexual modes of reproduction (Majer et al. 1998).

3.5.4 Simple Sequence Repeats (SSR)

Also known as microsatellites or short tandem repeats (STRs), simple sequence repeats (SSRs) are monograms of one to six nucleotides repeated several times in all of the eukaryotic genomes. These nucleotide units can vary in number of repetitions between individuals, and their distribution in the genome is nearly random. Using locus-specific flanking primers, variations in the tandemly repetitive units having a

high polymorphic banding pattern can be identified through PCR, and the resultant PCR products of diverse lengths can be obtained. For identification of genetic alterations between or within the closely linked species in soil-borne pathogens, these are known as the best and ideal markers. Several thousand potentially polymorphic markers are available but the advantages of SSRs are that they are highly polymorphic, co-dominant, and multi-allelic. Applications of SSR markers also include genome analysis and genetic mapping and is being widely used for the study of the genetic diversity of soil-borne plant pathogenic fungi within species, e.g. *Macrophomina phaseolina* (Reznikov et al. 2018), *Ceratocystis fimbriata* (Steimel et al. 2004), *Puccinia triticina* (Szabo and Kolmer 2007), *S. sclerotiorum*, and *Sclerotinia subarctica* (Winton et al. 2007); and in genetic map construction, e.g. a genetic map of *Magnaporthe grisea* containing of 176 SSR markers was constructed (Zheng et al. 2008). In another testing, microsatellite markers specific to *Phytophthora ramorum* were used to distinguish between A1 and A2 mating types isolates of this pathogen amongst two different geographic origins (Prospero et al. 2004).

3.6 Protein-Based Approach

3.6.1 Elisa

The enzyme-linked immunosorbent assay (ELISA) is another technique for identification of soil-borne pathogen that is based on antibodies and colour change in the analysis. The target antigens from the bacteria, viruses, and fungi are made to specifically bind with antibodies joined together in a pair to an enzyme. Based on the colour change because of the interaction between the substrate and the immobilized enzyme, detection can be visualized. Specific monoclonal antibodies have been used in ELISA to get lower limits of detection, viz. in the order of 10^5–10^6 CFU/mL (López et al. 2003). With the application of specified monoclonal and recombinant antibodies, available commercially, the performance of ELISA can be improved. Lateral flow devices and tissue print-ELISA that helps in detection have been fabricated for on-site detection of plant diseases. However, the sensitivity for bacteria is very low (10^5–10^6 CFU/mL) making it hard for the identification of plant diseases and only allows confirmation after visual symptoms appear but not for early detection before disease symptoms occur (López et al. 2003).

3.6.2 Lateral Flow Devices

Lateral flow devices (LFDs) are known as one of the most readily available farmer-friendly diagnostic tool. They are simple to use and the results are also quick, usually in less than 10 min. They are readily used for the diagnosis of plant viral diseases, and these LFDs are commercially available. LFDs are constructed based on serological specificity of monoclonal or polyclonal antibodies to particular targeted

pathogens, and their sensitivity of LFDs varies with type and target of antibody used. An LFD-based test using an IgM monoclonal antibody to detect *Rhizoctonia solani* can detect even a little amount as 3 ng mL^{-1} of antigen that equals to the sensitivity of standard ELISA procedures (Thornton 2008). This case study was especially interesting because the target was a soil-borne plant pathogenic fungus and not a bacterial pathogen for which specific antibodies are generally widely available, and most commercial LFD-based tests target plant viruses only. Making species-specific antibodies to fungi has always been a challenge but, as noted above, is successfully achieved for some targets.

3.7 Multi-omics Approaches for Plant Disease Diagnosis

The term 'Omics' was derived from a Greek word and added as a suffix to a type of studied molecule, and it means the study of all those molecules. Currently, omics is a distinct term that refers to a set of techniques, protocols, and methods for studying the entire molecular content of a cell, organ, or organism. The omics is divided into several levels: genomics (study of DNA), epigenomics (study of non-genetic DNA modifications), transcriptomics (study of RNA content), proteomics (study of protein content), metabolomics (study of metabolites content), and other omics derivatives (Guillemina et al. 2016). A relatively large number of studies, including gene expression, genetic diversity, phylogeny, comparative genomics, epigenetics (Sabir et al. 2014), genetic improvement of crops, biodiversity conservation, and imparting disease resistance in crops for further targeted breeding, exemplify these techniques (Rubiales et al. 2015). When compared to traditional single omics studies, multi-omics studies allow for a deeper understanding of cells, organisms, and microbial communities, with a focus on mechanisms involved in growth, adaptation, development, and disease progression (Sirangelo 2019). Furthermore, proper multi-omics data integration allows for a more comprehensive investigation of biological pathways. Abiotic stress factors are abnormal climatic or soil conditions (lack or excess of water, mineral nutrients, or salt). They can cause changes in cellular metabolism that affect plant growth, and, as a result, the plant is attacked by a variety of pathogens and insect-pests. Transcriptomics has been used successfully to identify gene interactions that play an important role in stress tolerance/susceptibility, highlighting relevant findings by grouping genes with similar expression profiles. Metabolome analysis is important because it allows for the verification of the true effects caused by transcriptomic and proteomic variations (Sirangelo 2019). Several studies have been conducted over the past decades to investigate the interactions between plant immune response and pathogens (fungal, bacterial, and viral agents). During plant-pathogen interactions, a complex cascade of defence responses is induced: signals from these microorganisms are detected by plant immune systems via various mechanisms. Pathogen-produced molecules (elicitors) in particular are recognized, resulting in activation of the plant's basal immune system, which prevents further colonization by incompatible pathogens and limits the spread of compatible pathogens (Schadt et al. 2005; Bittel and Robatzek

2007). As a result, several defence reactions are activated, including the production of reactive oxygen species, the strengthening of the plant cell wall, and the synthesis of specific enzymes. Identification of plant resistance genes is a top priority in the study of plant-pathogen interactions. Because there are so many genes involved, high-throughput methods are required to identify them and thus combat pathogens. The advancements in genomics and proteomics technologies were significant, allowing for significant results in this area of research. Machine learning programmes, which are a collection of analytic methods that automate the process of model generation and iteratively learning from data, are now being developed to provide effective tools for identifying genes/proteins involved in host-pathogen interaction (Xin and Tingwei 2017). Despite the fact that machine learning techniques have been used in a variety of fields, only a few studies have been conducted to predict plant pathogens using these datasets. More progress in this direction is critical for accurate detection and diagnosis of plant pathogens.

3.7.1 Genomic Approaches

The study of DNA structure (sequence and variations) or the complete genetic makeup of organisms and functions is referred to as genomics (information carried). Genomics seeks to establish a link between how DNA influences traits and phenotypic expression in organisms. Although the term 'genomics' is relatively new, its origins can be traced back to the early 1900s, when Johannsen introduced the concept of the gene, and later in 1920, when Hans Winkler coined the term genome (McKusick and Ruddle 1987). The first genomes to be sequenced were microbial, ushering in an era of tool development and exponential generation of whole genome sequences; the bacterium *Haemophilus influenzae*, the first free-living organism to have its whole genome sequenced via shotgun sequencing (Fleischmann et al. 1995); and the fungus *Saccharomyces cerevisiae*, the first eukaryote to have its whole genome sequenced (elegans Sequencing Consortium 1998). It is possible to understand how DNA regions encode some features and traits by studying DNA differences between species and within a species. A genetic marker is a DNA variation that can be easily identified. The understanding of these markers and their possible associations with traits and phenotype variation is at the heart of genomics research. The advancement of next-generation sequencing (NGS) technologies has drastically reduced sequencing costs while also hastening the availability of whole genome sequences, de novo sequence assemblies, and resequencing of multiple strains of a single species. Microbial genomics is an interdisciplinary field concerned with the structure, function, evolution, mapping, and editing of genomes in bacteria, fungi, archaea, viruses, and other microscopic organisms. The integration of genomics questions and tools can help with ecological questions, particularly those involving environmental change. For example, it is critical to understand the genome's evolutionary history in order to determine whether certain elements may change rapidly in response to temperature changes (rapid evolution). To understand microbial functions in related species, it may be

necessary to draw comparisons across genomes or to dwell into transcriptomics and other -omics. Functional genomics, in conjunction with transcriptomics and proteomics, studies gene and protein expression and function on a genome-wide or system-wide scale using genomic data. The combination of genomics and transcriptomics has resulted in a better understanding of pathogen's biology, plant-pathogen interactions, and plant health management (Lindeberg 2012; Sundin et al. 2016).

3.7.2 Transcriptomics Approaches

Transcriptomic analysis is a living link between the genome, proteome, and cellular phenotype. While proteins are the end products of gene expression, analysing and quantifying mRNA levels is a useful molecular tool (Aharoni and Vorst 2001). It is possible to obtain gene expression profiles from hosts (plants) or gene expression changes of host-associated pathogens using high-throughput technologies such as microarrays or RNA-Seq techniques. This information is may be useful in gaining a comprehensive understanding of a plant's response to a treatment (e.g. pathogen attack) and in providing new insights into biological processes. Furthermore, the function of novel and/or several uncharacterized genes from both organisms can be investigated. However, it is sometimes difficult to achieve success with a single gene expression strategy, and it becomes critical to understand the entire metabolic networks between genes, transcripts, proteins, and metabolites in biological systems (Oksman-Caldentey and Saito 2005). In this regard, it is necessary to conduct a comprehensive analysis using functional genomics technologies such as transcriptomics, proteomics, and metabolomics for the characterization of plant-pathogen interactions in order to better understand the genetic and metabolic adaptations of a specific plant species to infection. It is critical to design better plants that have a sustained response to the attack of specific pathogenic organisms (Gomez-Casati et al. 2016). Plants can detect the presence of pathogens such as bacteria, viruses, or fungi by recognizing specific molecules released by the pathogens during the infection process (Martin et al. 2003). Plants, in this sense, have evolved an immune system to defend themselves against pathogens. Pathogenic organisms target different proteins in plant cells and disrupt the immune response, resulting in disease development. Many plants, on the other hand, express a variety of resistance proteins that can detect the presence of specific effectors and activate a defence mechanism. To better understand the molecular basis of this response, Pombo et al. (2014) used RNA-Seq technology to identify genes involved in specific immune responses to *Pseudomonas syringae* in tomato plants (Pombo et al. 2014). Other proteins involved in pathogen defence, such as nucleotide-binding leucine-rich repeat receptors (NB-LRR), were also described (Bernoux et al. 2011). Several attempts have been made to engineer disease resistance in economically important crop plants, but many have failed. However, it has been demonstrated that overexpression of a serine/threonine kinase (Pto) in tomato induces gene expression changes that result in an increased immune response against *P. syringae*, conferring disease protection (Mysore et al. 2003). Furthermore, in

Malus × *domestica*, overexpression of *NPR1*, a gene involved in systemic acquired resistance in plants, results in increased disease resistance (Malnoy et al. 2007). This gene had no negative effects on plant growth and development, and it has been proposed that it could be used for non-specific resistance genetic engineering in plants (Cao et al. 1998).

Several genes that respond to virus attack have also been discovered using transcriptomic approaches. Furthermore, RNA appears to be another sequence-specific plant defence mechanism against virus invasion. It has been reported that virus replication is associated with the accumulation of small RNAs involved in the specific cleavage of viral transcripts; however, this could be suppressed by virus proteins that inhibit the host defence response (Czosnek et al. 2013). This mechanism, known as virus induced gene silencing (VIGS), has been routinely investigated in *Nicotiana benthamiana* or *A. thaliana*, as well as in some *Solanum* species, to assess the functions of candidate genes and to discover new genes required for diverse pathways (Brigneti et al. 2004). A geminivirus that infects tomatoes is one of the most studied tomato pathogens (*Tomato yellow leaf curl virus*: TYLCV). The production of genetically engineered plants to resist infection by the TYLCV by the expression of viral proteins or gene silencing strategies has been described, but to date, breeding remains the preferred method of obtaining plants resistant to the TYLCV (Czosnek et al. 2013; Shepherd et al. 2009). Sade and colleagues recently reported the alteration of several tomato cultivars' genes and metabolites in response to the TYLCV using comparative transcriptomic and metabolomic analyses. Many amino acids, polyamines, and phenolic and indolic metabolites had altered levels, all of which led to the synthesis of defence compounds. Furthermore, they reported the induction of a hexose transporter gene (*LeHT1*) following TYLCV infection (Sade et al. 2013). When this virus infects a tomato cultivar, several changes in sugar metabolism occur, including a decrease in photosynthesis, an increase in invertase expression, and the release of hexoses, which causes the defence response to be activated. When this mechanism fails, it promotes virus replication and disease establishment. It is possible that increased levels of internal hexoses activate phytohormone-mediated responses, regulate cell homeostasis, and efficiently activate plant defence responses (Sade et al. 2013). Thus, overexpression of *LeHT1* could be a promising strategy for obtaining virus-resistant tomato plants.

3.7.3 Proteomic Approaches

Proteomics in conjunction with genomics has significantly contributed to the large-scale functional assignment of candidate proteins, and several antimicrobial proteins expressed during phytopathogenic interaction have been identified using this approach (Mehta et al. 2008). Many of these antimicrobial peptides are listed in the PhytAMP database (www.phytamp.pfba-lab-tun.org) (Hammami et al. 2009) as well as other databases such as CAMP (Collection of Antimicrobial Peptides, www.camp.bicnirrh.res.in) (Thomas et al. 2010). The collection of such data in databases

would thus facilitate the investigation of the potential of several peptides as alternatives in response to increasing antibiotic resistance or for increasing plant resistance to pathogens through genetic engineering. Many filamentous fungi, such as *Trichoderma*, the most widely used biocontrol fungus, on the other hand, have been extensively studied using genomics, transcriptomics, proteomics, metabolomics, and secretomics. Anti-microbial peptides and several genes from these organisms have been identified and transferred to plants to improve tolerance to biotic and abiotic stress (Nicolas et al. 2014). The transgenic lines demonstrated increased resistance to pathogens such as *Alternaria alternata*, *Alternaria solani*, *Botrytis cinerea*, and *Rhizoctonia solani* (Lorito et al. 1998). Similar results were obtained in apple using an endochitinase or exochitinase gene, demonstrating that these genes can be used to control diseases in plants. Other antimicrobial peptides, such as thanatin(s), confer a broad spectrum of antimicrobial activity when expressed. *Arabidopsis* plants were transformed with this gene and tested for pathogen resistance in order to investigate the effect of thanatin. Transgenic plants have increased antifungal and antibacterial activity against *Botrytis cinerea* and powdery mildew, as well as antifungal and antibacterial activity against *Pseudomonas syringae* pv. *tomato* (Wu et al. 2013). As a result, it was proposed that thanatin (s) could be an ideal candidate for the development of transgenic crops with broad-spectrum resistance to phytopathogens (Wu et al. 2013).

The challenge of applying omics to pathogen attack and plant defence is to identify changes in biochemical pathways and metabolic networks that may correlate with a cell, tissue, or organism's physiological and developmental phenotype. As a result, we can identify changes in metabolite levels that are induced after infection and can develop different strategies to obtain transgenic plants with increased levels of these metabolites, which could potentially confer greater disease resistance. Plant genetic engineering to increase phytoalexin compounds for disease resistance necessitates the manipulation of a single or a few genes directly involved in their biosynthetic pathways or signaling/regulatory pathways (Jeandet et al. 2013). The most common examples are associated with the synthesis of resveratrol, one of the most abundant phytoalexins in plants. Stilbene synthase (STS) generates it via the phenylpropanoid acid pathway, using phenylalanine or tyrosine as precursors. The introduction of two STS genes from grapevine into tobacco conferred resistance to *Botrytis cinerea* infection, which was the first approach to increase resveratrol levels in plants (Hain et al. 1993). Similarly, it has been reported that *Phoma medicaginis* resistance in *Medicago sativa* transformed with an STS gene (Hipskind and Paiva 2000). Using similar approaches, the introduction of different STS genes conferred pathogen resistance to several crops, including rice (*Pyricularia oryzae* resistance), barley, and wheat (*Botrytis cinerea* resistance) (Jeandet et al. 2013).

3.7.4 Metabolomic Approaches

Many plant metabolites influence the phenotypic properties of plant tissues and play a role in stress and pathogen responses. To understand the dynamics of the

metabolome, analyse fluxes in metabolic pathways, and decipher the role of each metabolite in response to various stimuli, metabolites must be identified and quantified at the same time (Gomez-Casati et al. 2013). The challenge of applying omics to pathogen attack and plant defence is to identify changes in biochemical pathways and metabolic networks that may correlate with a cell's, tissue's, or organism's physiological and developmental phenotype. As a result, we can identify changes in metabolite levels that are induced after infection and develop different strategies to obtain transgenic plants with increased levels of these metabolites, which could potentially confer greater disease resistance. Most plants change their metabolism to increase the concentration of defence compounds that protect them from pathogen attack. Many plant pathogens, on the other hand, manipulate the host metabolism to counteract defence responses, thereby inducing favourable nutritional conditions. Metabolomics advances have resulted in the generation of large metabolic profiles that have been shown to be specific to each plant tissue during pathogen infection. Cereals, such as maize, rice, wheat, sorghum, and barley, are one group of economically important plants that are constantly attacked by pathogens such as viruses, bacteria, and fungi. Until the year 2000, the majority of cereal metabolomic studies were based on the assessment of various compounds such as vitamins, sterols, phenolic and volatile compounds, and a few metabolites related to biotic or abiotic stresses (Khakimov et al. 2014). Recent advancements in integrated transcriptomics and metabolomics technologies allow for the screening of a wide range of cereals for pathogen-resistant genotypes as well as biochemical phenotypes (Langridge and Fleury 2011). Bollina et al. discovered nearly 500 metabolites in barley cultivars resistant to *Fusarium* head blight (FHB), one of the most serious diseases affecting cereal crops such as maize, barley, and wheat (Bollina et al. 2010). Surprisingly, the majority of the metabolites are produced by the phenylpropanoid, flavonoid, fatty acid, and terpenoid metabolic pathways. Other maize studies revealed that benzoxazinones (BX) play a role in resistance to the fungus *Setosphaeria turcica* (Ahmad et al. 2011). Plants also produce secondary metabolites in response to nematodes and pest herbivores, such as benzoxazinoids; flavonoids, such as C-glycosyl flavones, which have been identified as an effective protectant against corn earworm (Lee et al. 1998); and *Pratylenchus* and *Heterodera*, two cereal nematodes.

Given recent advances in cereal metabolomics in disease resistance, targeting metabolic pathways appears to be a promising strategy for obtaining transgenic cereals with increased pathogen resistance. It was recently reported that rice lines with high levels of momilactone have been developed, and their effectiveness in protecting against *Magnaporthe grisea* and *Xanthomonas oryzae* has been demonstrated (Kurusu et al. 2010). Thus, using integrated metabolomics and transcriptomics data, it may be possible to manipulate several biosynthetic pathways to design different crop improvement strategies. A novel approach, on the other hand, is the modulation of the synthesis of compounds such as phytoalexins to protect plants from infections. Phytoalexins are low molecular weight metabolites with antimicrobial properties that are toxic to prokaryotic and eukaryotic organisms and are synthesized de novo during biotic stress (Ahuja et al. 2012). Plant genetic

engineering to increase phytoalexin compounds for disease resistance necessitates the manipulation of a single or a few genes directly involved in their biosynthetic pathways or signaling/regulatory pathways (Jeandet et al. 2013). The most common examples are associated with the synthesis of resveratrol, one of the most abundant phytoalexins in plants. Stilbene synthase (STS) generates it via the phenylpropanoid acid pathway, using phenylalanine or tyrosine as precursors. The introduction of two STS genes from grapevine into tobacco conferred resistance to *Botrytis cinerea* infection, which was the first approach to increasing resveratrol levels in plants (Hain et al. 1993).

3.7.5 Metallomic Approaches

The study of metalloproteins or any other metal-containing biomolecule, as well as the entire metal and metalloid species within a cell or tissue type, is referred to as metallomics. As a result, metallomics can be considered a subfield of metabolomics, despite the fact that metals are not typically thought of as metabolites. However, because of the interactions and functional connections of metal ions and their species with genes and proteins, metallomics is linked to genomics and proteomics, resulting in a multidisciplinary research field (Mounicou et al. 2009). Metals are necessary for the majority of living organisms, but when present in excess, they become toxic. Metal availability and toxicity can influence disease outcome in the context of plant-pathogen interactions. Metals have profound effects at multiple levels, including plant health, plant defence signaling, and changes in the environment that the pathogen encounters in plants. A lack of minerals is generally associated with an increase in plant disease. Furthermore, they have an impact on the pathogen's mineral nutrition, virulence gene expression regulation, and toxicity. ROS play an important role in plant defence mechanisms as both potent antimicrobials generated in situ in response to pathogenic proteins and signaling molecules that induce systemic responses (Reczek and Chandel 2015). An intriguing finding from comparative physiological and transcriptomic analyses of hyper-accumulators and related non-hyper-accumulators is that most key steps of hyper-accumulation rely on different regulation and expression of genes found in both types of plants (constitutive overexpression of genes encoding transmembrane transporters, such as members of the ZIP, HMA, MATE, YSL, and MTP families) (Rascioa and Navari-Izzo 2011). This could imply that by simply increasing the expression of a few endogenous candidate genes, it is possible to increase metal accumulation in economically important plants, thereby strengthening their disease resistance.

3.7.6 Databases and Software Tools for Multi-omics Study

A recent study reported on several tools for multi-omics data integration (Biswapriya et al. 2019). A plethora of free resources, databases, software tools, and approaches to assist researchers in integrating multi-omics data are available. Gene and protein

resources for multiple species, such as GenBank and UniProt (Benson et al. 2013; UniProt Consortium 2018), are also available. Similarly, context-specific curated databases and software tools for various biological areas, including selected plant species, are described. Data from various species' genomes, transcriptomes, proteomes, and metabolomes are stored in such databases. Plant Metabolic Network, or PMN, is a good example for plants (Schlapfer et al. 2017) (Table 3.1).

Several bioinformatics resources have different formats, and many of them are incompatible with the most widely used standards. Furthermore, different software packages may require non-standard input data and produce outputs that are incompatible with others. Another important goal of multi-omics integration research is the creation and visualization of network models. Multi-layer networks, which have recently been developed, allow for the interpretation of specific interactions between different omics layers (Kivelä et al. 2014). All of the tools mentioned above, however, still require improvements, such as the availability of pathway databases that provide links between genes, proteins, and metabolites. The few existing resources (Fabregat et al. 2018) focus on metabolic pathways, but other types of pathways, such as protein and metabolite signaling, gene activation, and many others, are also important in plant science. Existing commercial tools include some of these pathways, but their incompatibility with many other bioinformatics software packages makes them difficult to integrate into multi-omics open-access pipelines (Pinu et al. 2019). The real ability of tools to uncover multi-omics data relationships in order to analyse pathway cross-talk remains limited. As a result, new methodological approaches and software tools for pathway analysis that integrate multi-omics datasets would be required. Model-based inference of multi-omics data for

Table 3.1 List of some software packages and databases which are used for multi-omics studies

Sl. no.	Software Packages	Function	References
1.	KaPPA-View	This method maps pathways by combining transcriptomics and metabolomics data from plants	Tokimatsu et al. (2005)
2.	MapMan	This was first used in *Arabidopsis*, but it is now used in many other species as well. It integrates data from metagenomics, transcriptomics, and metabolomics, handles KEGG and KOG clustering, and maps expression responses	Usadel et al. (2005)
3.	VitisNet	This software is used to manage data from metagenomics, transcriptomics, proteomics, and metabolomics	Grimplet et al. (2009)
4.	MADMAX	This is a database for managing and analysing multiple omics experiments. It combines data from metagenomics, transcriptomics, and metabolomics, as well as statistical analysis and pathway mapping	Lin et al. (2011)
5.	MetaboAnalyst	This is used to manipulate data from genomics, transcriptomics, proteomics, and metabolomics, as well as to run data processing and statistical analysis and to develop pathway analysis	Chong et al. (2018)

pathway analysis also necessitates the use of machine learning approaches (Chaudhary et al. 2018).

3.8 Conclusion

From visual assessment of disease signs and symptoms to pathogen identification at the molecular level, the science of plant disease diagnostics has developed through technical breakthroughs. In order to control disease and preventing pathogen spread to previously uninfected regions, precise plant pathogen identification is crucial. In this context, recent technological breakthroughs in the field of plant pathology, which is coupled with bioinformatics, biotechnology, and molecular biology, have certainly paved a way towards the early detection and diagnosis of the new emerging, previously undescribed, and re-emerging plant diseases. These molecular techniques have helped in successful identification and diagnosis of a wide range of culturable and non-culturable plant pathogens, in sole and co-infections of agriculturally important crops, horticultural fields, floricultural systems, ornamental species, and various forest species. Conventional methods of pathogen detection such as blotter method, towel paper test, and other procedures may give the information about the presence or the viability of the pathogen but at the same time these methods prove to be time-consuming and labour-intensive. Also, these types of methods have limited specificity and are having less sensitivity. Molecular techniques such as PCR, quantitative PCR with serological methods, and flow cytometry on the other hand are more sensitive, are less time- and labour-consuming, and thereby can prove to be an alternative to the conventional methods. The approaches discussed in this paper have helped to produce accurate, sensitive, and specific pathogen detection for a variety of applications.

3.9 Future Prospective

It is indeed fascinating to think about the future of plant disease diagnosis and how it can be used for the disease management. Traditional techniques are being modified or integrated with modern nucleic acid-based approaches for considerable advances in sensitivity, and inventive changes are making complicated procedures easier and allowing for the examination of a large number of samples. Some of the technologies described are difficult to understand for anyone who may not be experts in the field of molecular biology. As a result, it is critical that specialists, extension officers, and consultants carry out or guide these assays while new user-friendly methodologies are need to be created. Furthermore, consistent standards are required for the approach to be accepted as a standard protocol across the world.

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Diagnosis and Detection of Soil-Borne Fungal Phytopathogens in Major Crops

4

Ravindra Kumar, Seweta Srivastava, Sudheer Kumar, Udai B. Singh, Tsewang Chosdon, Manoj Kumar Yadav, Ankush Kumar, Aradhana Sagwal, Ratan Tiwari, and G. P. Singh

Abstract

Phytopathogenic soil-borne fungal species can inflict huge economic disturbances in the global agricultural sector. Soil-borne diseases, incited by fungal pathogens, e.g. root rot, stem rot, crown rot, damping-off, blights, vascular wilts, etc., inflict significant economic losses in agricultural and horticultural crops' yields and quality, globally. To achieve effective disease control, precise and quick detection or identification of plant infecting fungi is required. For accurate plant disease diagnosis, DNA-based approaches have become widespread. Recent breakthroughs in the field of fungal detection and differentiation; various polymerase chain reaction (PCR) assays such as nested, multiplex, quantitative, bio, and magnetic-capture hybridisation PCR techniques; post and isothermal amplification methods; DNA and RNA-based probe development; and next-generation sequencing have resulted in novel molecular diagnostic tools. Symptomatic and asymptomatic diseases caused by culturable and non-culturable fungal pathogens can be detected using these molecular-based detection approaches in both single-infection and co-infection conditions. Plant disease diagnostics require molecular techniques that are more reliable, quicker, and

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easier to use than traditional procedures. The present chapter highlights molecular diagnostic tools that have come a long way including rapid developments in recent past. However, it requires further firming up before becoming integral part of efficient plant disease management.

Keywords

Agricultural crops · Disease diagnosis · Molecular detection · Phytopathogens · Soil-borne fungi

4.1 Introduction

Plant diseases are very important as they have posed historical impacts on the human civilisation globally, and even in recent era, they are capable enough to cause great economic losses and can raise concerns for food safety around the world (Kumar and Gupta 2020). To the convenience of the study, plant pathologists have classified plant diseases into three major distinct groups: (a) seed-borne diseases, (b) soil-borne diseases, and (c) air-borne diseases. There are no clear dividing lines between these three groups, and a disease may use one or more mechanisms to spread or survive. For instance, the loose smut disease of wheat incited by *Ustilago segetum* sp. *tritici* is an entirely seed-borne and seed-transmitted disease (Kumar et al. 2020). The dormant mycelium of the pathogen remains deep seated in the seed embryo. The mycelium becomes activated and grows alongside the host plant with no visible symptoms, when these contaminated seeds are sown in the field. The pathogen expresses itself after ear emergence only, and instead of healthy spikelets, smutted ones with mass of millions of teliospores arise. These teliospores are blown away by the wind after sometime, allowing them to infect new plants (Gupta and Kumar 2020b). As a result, we can observe that, although being seed-borne, the ailment needs the support of air to complete its life cycle. The nature of the disease is determined by the primary commencement of disease transmission. There are also some diseases where the primary source of infection might come from a variety of sources. The bakanae, or paddy foot rot disease, is an example. Although the pathogen of this disease, *Fusarium moniliforme*, is thought to be primarily seed-borne, inoculum of *Fusarium moniliforme* present in the soil is capable of infecting rice plants with bakanae disease (Gupta et al. 2015; Gupta and Kumar 2020a). Similarly, the Karnal bunt of wheat relies on all three pathways for survival and spread: soil-borne, air-borne, and seed-borne (seed co-contaminant) (Kumar and Gupta 2020; Kumar et al. 2020).

Soil-borne diseases caused by diverse soil-dwelling microbes are among the most challenging threats to agriculture production worldwide. These diseases are very difficult to manage due to the complexity in delivery of the pesticides efficiently at target pathogen's site in the soil. Moreover, the symptoms produced on aerial and underground parts are very similar in case of many soil-borne diseases. Hence, timely and efficient detection of these diseases and their inciting pathogens is

prerequisite for effective disease management (Kumar et al. 2008). The current chapter discusses recent advances in the development and utilisation of molecular approaches for identification of established and emerging soil-borne plant pathogenic fungi.

4.2 Soil-Borne Plant Pathogens Produce a Variety of Symptoms

4.2.1 Rotten Roots

A wide array of fungus and associated organisms cause soil-borne diseases. *Pythium* and *Phytophthora*, *Rhizoctonia*, *Cylindrocladium*, and *Armillaria* are the most common genera that cause root rots. The symptoms of these diseases are the breakdown of the actual root system; certain pathogens are exclusive to the juvenile roots, while others can affect the older root system. Wilting, leaf death and fall, branch and limb death, and, in severe situations, the death of the entire plant are all apparent indications. The following are some examples of these disorders:

4.2.2 *Rhizoctonia* Root Rot Disease

The words "damping-off", "wire stem", "head rot", and "crown rot" all refer to the same issue. The fungus only infects the outer cortical tissues of older seedlings, causing a lesion that is elongated and tans to reddish-brown in colour. The zone may widen and lengthen until it encircles the stem; when this happens, the plant will die.

4.2.3 Stem, Collar, and Head Rots

These diseases are caused by a variety of pathogens, including *Phytophthora*, *Sclerotium*, *Rhizoctonia*, *Sclerotinia*, *Fusarium*, and *Aspergillus niger*. The most evident sign of these diseases is the degeneration of the stem at ground level. Wilting symptoms, leaf death, and plant death are all common consequences of this degradation. The following are some examples of these disorders. *Phytophthora* spp. can cause various diseases including pineapple heart rot, potato and tomato blight, and numerous fruit rots in these conditions. In damp, warm conditions, *Rhizoctonia* spp. can cause maize leaf blight and cabbage head rot.

4.2.4 Wilts

Fusarium oxysporum and *Verticillium* spp. are the two most frequent fungi that cause these infections. This disease results in internal necrosis of the vascular tissue

in the plant's stem and wilting of the foliage. Similar to how some bacterial species can lead to the same.

4.2.5 Blights on Seedlings and Damping-Off Diseases

Seedling diseases are known by a variety of names, including seedling blight and damping-off. *Pythium*, *Phytophthora*, *Rhizoctonia*, *Sclerotium rolfsii*, and *Fusarium* spp. are the most frequent fungus that kill seedlings. Several fungi can infect seedlings during the germination, pre-emergence, or post-emergence stages of seedling establishment. *Pythium*, *Rhizoctonia*, and *Sclerotium rolfsii* are frequently linked to seedling death in vegetables like beans, tomatoes, cucurbits, and other cruciferous plants.

4.2.6 *Pythium* Damping-Off Disease

Pythium debaryanum, *Pythium ultimum*, *Pythium aphanidermatum*, and *Pythium graminicola* are the most common species found. The disease frequently manifests itself in a nearly circular pattern. This is due to fungi's proclivity for rapidly spreading from their source, which is one of the field markers used to distinguish illnesses from other causes that produce similar symptoms.

4.2.7 Damping-Off *Phytophthora*

The *Pythiaceae* family includes *Phytophthora* species, which are classed as *Oomycetes.* Low stem rot, or damping, is caused by *P. cactorum*, *P. fragariae*, *P. palmivora*, and *P. syringae* on vegetables, forest trees, and ornamentals. *Phytophthora* is more active than *Pythium* in warmer soil temperatures (15–23 °C), although it still thrives in a cold environment. Flooding and hot temperatures are the order of the day. At first, the injured tissue develops a mushy, watery brown rot. The plant parts that have been damaged may dry out in a few days.

4.2.8 Major Soil-Borne Disease Caused by Fungal Pathogens

The agents that induce soil-borne diseases make up a diverse group. Fungi, which are multicellular microorganisms, are considered as major soil-borne pathogens causing diseases in cereals, pulse, oilseed, fruit, vegetables, crops, etc. Some important soil-borne diseases of cultivated crops are mentioned in Table 4.1 and Fig. 4.1 along with some pathogenic fungi in Fig. 4.2.

Table 4.1 Some important soil-borne fungal diseases and their phytopathogens in various agricultural crops

S. no.	Crop	Disease name	Fungal pathogen	Reference
1.	*Alliums*	Damping-off	*Pythium* spp., *Rhizoctonia* spp.	Sharma et al. (2022)
		Basal rot	*Fusarium oxysporum* f. sp. *cepae*	Le et al. (2021)
		Pink rot	*Phoma terrestris*	Mishra et al. (2012)
		White rot	*Sclerotium cepivorum*	Zewide et al. (2007)
2.	Banana	Panama wilt	*Fusarium oxysporum* f. sp. *cubense*	Aguilar-Hawod (2020)
3.	Bean	Ashy stem blight	*Macrophomina phaseolina*	Díaz-Díaz et al. (2022)
4.	Brinjal	Collar rot	*Sclerotium rolfsii*	Jadon (2009)
5.	Carrot	Cavity spot	*Pythium violae*	Lyons and White (2008)
		Cottony rot	*Sclerotinia sclerotiorum*	Kora et al. (2003)
		Crown rot	*Rhizoctonia solani*	Marcou et al. (2021)
		Southern blight	*Sclerotium rolfsii*	Rubayet et al. (2020)
		Phytophthora root rot	*Phytophthora* spp.	Williamson-Benavides and Dhingra (2021)
		Root die back	*Pythium* spp.	Kalu et al. (1976)
6.	Celery	Crater spot	*Rhizoctonia solani*	Houston and Kendrick (1949)
		Fusarium yellows	*Fusarium oxysporum* f. sp. *apii*	Epstein et al. (2017)
		Pink rot	*Sclerotinia sclerotiorum*	Bolton et al. (2005)
7.	Chickpea	Collar rot	*Sclerotium rolfsii*	Javaid and Khan (2016)
8.	Chilli	Foot rot	*Sclerotium rolfsii*	Sultana (2012)
		Southern blight	*Sclerotium rolfsii*	Sharf et al. (2021)
9.	Cole crops	Bottom rot and wire stem	*Rhizoctonia solani*	Keinath (2019)
		Club root	*Plasmodiophora brassicae*	Yu et al. (2022)
		Fusarium yellows	*Fusarium oxysporum* f. sp. *conglutinans*	Yu et al. (2020)
		Root rot	*Phytophthora megasperma*	Williamson-Benavides and Dhingra (2021)

(continued)

Table 4.1 (continued)

S. no.	Crop	Disease name	Fungal pathogen	Reference
		Verticillium wilt	*Verticillium dahliae*	Kowalska (2021)
		White mould	*Sclerotinia sclerotiorum*, *S. minor*	Faraghati et al. (2022)
		White rust	*Albugo candida*	Asif et al. (2017)
10.	Cucumber, melons, squash	Charcoal rot	*Macrophomina phaseolina*	Marquez et al. (2021)
		Damping-off	*Pythium* spp., *Rhizoctonia solani*	Lamichhane et al. (2017)
		Fusarium wilt	*F. oxysporum* f. sp. *melonis* (muskmelon); *F. oxysporum* f. sp. *niveum* (watermelon); *F. oxysporum* f. sp. *cucumerinum* (cucumber)	Egel and Martyn (2007)
11.	Finger millet	Foot rot	*Sclerotium rolfsii*	Manu et al. (2012)
12.	Groundnut	Stem rot	*Sclerotium rolfsii*	Jacob et al. (2018)
13.	Guava	Wilt	*Fusarium oxysporum* f. sp. *psidii*	Srivastava et al. (2011), Singh et al. (2021)
14.	Indian mustard	Sclerotinia rot	*Sclerotinia sclerotiorum*	Singh et al. (2020)
15.	Lentil	Foot/root rot	*Sclerotium rolfsii*	Khalequzzaman (2016)
16.	Lettuce	Bottom rot	*Rhizoctonia solani*	Wallon et al. (2021)
		Lettuce drop disease	*Sclerotinia sclerotiorum* and *S. minor*	Mihajlović et al. (2022)
		Wilt	*Fusarium oxysporum* f. sp. *lactucum*	Egel and Martyn (2007)
17.	Maize	Stalk rot	*Fusarium moniliforme*	Jiskani et al. (2021)
		Stem rot	*Sclerotium rolfsii*	Soytong (1991)
18.	Pea	Damping-off	*Pythium* spp., *Rhizoctonia solani*	Lamichhane et al. (2017)
		Fusarium root rot	*F. solani* f. sp. *phaseoli*	Wu et al. (2022)
19.	Pepper	Damping-off	*Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*	Lamichhane et al. (2017)
		Root rot	*Phytophthora capsici*	Lozada et al. (2021)
		Verticillium wilt	*Verticillium dahliae*	Kowalska (2021)
20.	Potato	Black dot	*Colletotrichum atramentarium*	Lees and Hilton (2003)

(continued)

Table 4.1 (continued)

S. no.	Crop	Disease name	Fungal pathogen	Reference
		Black scurf	*Rhizoctonia solani*	Tjimune et al. (2021)
		Charcoal rot	*Macrophomina phaseolina*	Marquez et al. (2021)
		Fusarium dry rot	*Fusarium sambucinum*	Erper et al. (2022)
		Leak	*Pythium* spp.	Çakır et al. (2020)
		Pink rot	*Phytophthora erythroseptica*	Çakır et al. (2020)
		Powdery scab	*Spongospora subterranea*	Tsror et al. (2020)
		Silver scurf	*Helminthosporium solani*	Tiwari et al. (2022)
		Verticillium wilt	*Verticillium dahliae*	Kowalska (2021)
		White mould	*Sclerotinia sclerotiorum*	Ojaghian (2018)
21.	Rice	Bakanae	*Fusarium fujikuroi*	Jiang et al. (2021)
		Sheath blight	*Rhizoctonia solani*	Senapati et al. (2022)
		Stem rot	*Sclerotium oryzae*	Ghosh et al. (2020)
22.	Soybean	Collar/foot/ root rot	*Sclerotium rolfsii*	Borah and Gogoi (2020)
		Charcoal rot	*Macrophomina phaseolina*	Bradley and Río (2003)
23.	Spinach	Damping-off	*Fusarium oxysporum*, *Pythium* spp., *Rhizoctonia solani*	Sharma et al. (2022)
24.	Sugar beet	Collar/root rot	*Sclerotium rolfsii*	Rasu et al. (2013)
25.	Sugarcane	Pokkahboeng	*Fusarium moniliforme*	Srivastava et al. (2020b)
26.	Sunflower	Charcoal rot	*Macrophomina phaseolina*	Weems et al. (2011)
		Collar/root rot	*Sclerotium rolfsii*	Rasu et al. (2013)
27.	Strawberry	Crown rot	*Macrophomina phaseolina*	Mertely et al. (2005)
28.	Tomato	Wilt	*Fusarium oxysporum* f. sp. *lycopersici*	Katyayani et al. (2019), Manda et al. (2021)
		Damping-off	*Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*	Sharma et al. (2022)

(continued)

Table 4.1 (continued)

S. no.	Crop	Disease name	Fungal pathogen	Reference
		Foot rot	*Fusarium solani*	Ribeiro et al. (2022)
		Verticillium wilt	*Verticillium dahliae*	Mazzotta et al. (2022)
29.	Wheat	Root rot	*Sclerotium rolfsii*	Elad et al. (1980)
		Foot rot	*Rhizoctonia solani*	Ophel-Keller et al. (2008)
		Dwarf bunt	*Tilletia controversa*	Yuan et al. (2009)
		Take-all disease	*Gaeumannomyces graminis* var. *tritici*	Ophel-Keller et al. (2008)
		crown rot	*Fusarium pseudograminearum* and *F. culmorum*	Ophel-Keller et al. (2008)
		Root rot, crown rot, and spot blotch	*Bipolaris sorokiniana*	Ophel-Keller et al. (2008)

4.3 Detection Methods of Soil-Borne Plant Pathogenic Fungal Species

4.3.1 Traditional Methods

Identifying disease indications, direct isolation in artificial conditions, and laboratory identification by morphological or biochemical assays have all been used in the past. These methods required an experienced and competent laboratory staff to perform them since they could result in problems with identification, erroneous findings interpretation, improper disease diagnosis, and, ultimately, incorrect disease therapy (Atkins and Clark 2004; Martinelli et al. 2015). Furthermore, these methods are time-consuming, non-quantitative, and prone to contamination and mistakes and result in major delays in plant treatment. Although molecular technologies are becoming more readily available, conventional procedures are still frequently employed and are the mainstay of plant pathologists.

Traditional approaches for identifying soil-borne infections, such as baiting and the use of selective media, such as Botrytis Selective Media (BSM) for *Botrytis cinerea*, because they are inexpensive and not technically demanding, are used extensively (Horner and Wilcox 1995, 1996; Pryor et al. 1998; Edwards and Seddon 2001). They are, however, often time-consuming, error-prone, and occasionally erroneous, and they necessitate a thorough understanding of classical taxonomy as well as a high level of competence for interpretation and analysis. They are not well suited to large-scale sample analysis or rapid diagnosis, and producers must rely on specialised diagnostic facilities. Other drawbacks include the inability to precisely

Fig. 4.1 Some major diseases of agricultural crops having soil-borne phase in their disease cycle; false smut (**a**), bakanae (**b**), sheath blight (**c**) of rice; flag smut (**d**), Karnal bunt (**e**), spot blotch (**f**) of wheat; spot blotch of barley (**g**); late blight of potato (**h**), late blight of tomato (**i**), *Sclerotinia* stem rot of mustard (**j**); smut (**k**), ergot (**l**), and downy mildew or green ear disease (**m**) of pearl millet

identify infections and the difficulties of culturing some species in vitro (Ghosh et al. 2015; Sharma et al. 2015). Using a combination of traditional pathogen knowledge and molecular detection technologies, these constraints could be overcome with greater precision and reliability (Ghosh et al. 2019). Therefore, the focus of this chapter is on emerging molecular approaches that are increasingly being employed for the detection and identification of diseases which spread through soil.

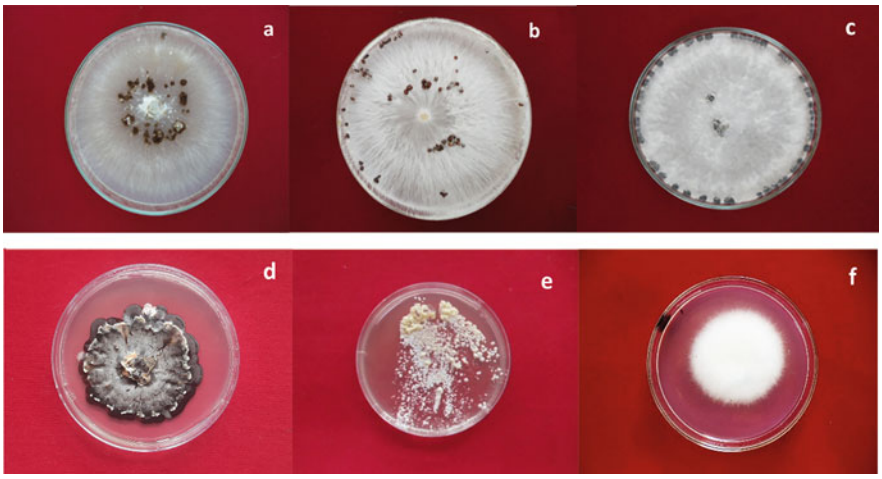

Fig. 4.2 Some important soil-borne phytopathogens; *Rhizoctonia solani* (**a**), *Sclerotium rolfsii* (**b**), *Sclerotinia sclerotiorum* (**c**), *Bipolaris sorokiniana* (**d**), *Tilletia indica* (**e**), and *Fusarium moniliforme* (**f**)

4.3.2 Recent Detection Techniques for Soil-Borne Fungal Phytopathogens

Since the traceable early history of detection and diagnosis of plant diseases, the methodologies employed for the detection and identification of the pathogens were knowingly or unknowingly being judged on the certain criteria such as ease of performance, reliability, scientific reasoning, etc., before validation and widespread adoption of these techniques (Srivastava et al. 2020a). Ball and Reeves (1991) devised six main requirements for selection of detection techniques in case of seed-borne pathogens, which may be applicable to other kinds of the phytopathogens. The technology to be employed for detection of phytopathogens must have to fulfil six main requirements (Ball and Reeves 1991) with some modification in case of soil-borne fungal pathogens as given below:

(a) **Specificity**—a target organism's ability to be distinguished from others found on tested samples.
(b) **Sensitivity**—the capacity to find organisms in samples with low occurrence.
(c) **Speed**—little time is needed, allowing for quick action against the target pathogen(s).
(d) **Simplicity**—reduction of several testing phases to lower error and allow testing by a team that isn't always extremely competent.
(e) **Cost-effectiveness**—costs should determine acceptance to the test.
(f) **Reliability**—regardless of who conducts the test, techniques must be sufficiently reliable to produce reproducible results both within and across samples of the same stock.

The molecular techniques employed for detection of soil-borne fungal phytopathogens includes several techniques, viz. conventional PCR, real-time PCR, end-point PCR, bio-PCR, nested-PCR, multiplex-PCR, RPA, LAMP, RCA, NASBA, FISH, etc. For the species-specific detection of fungal phytopathogens, technologies with a high level of sensitivity and specificity are employed. With the help of these procedures, diseases of different crops can be found using incredibly little samples or tissues. Due to their specificity, sensitivity, speed, simplicity, and reliability, molecular detection methods have recently taken the lead in the detection, identification, and quantification of soil-borne fungal pathogens. To some extent, these methods are also cost-effective, especially when samples need to be diagnosed in bulk. Therefore, we can say in recent times use of molecular techniques must be preferred over any other available conventional techniques for better understanding, interpretation, and accuracy. Some important techniques are as follows:

4.3.2.1 PCR-Based Approaches

Conventional PCR

PCR is a strong technology for amplification of DNA sequences exponentially. A PCR process requires a pair of primers that are complementary to the sequence of interest. The DNA polymerase extends the primers. The amplicons, or copies created after the extension, are re-amplified with the same primers, resulting in exponential amplification of the DNA molecules. The amplified PCR products are next analysed using gel electrophoresis, which makes conventional PCR time-consuming because the reaction must end before the post-PCR analysis can begin. Real-time PCR tackles this problem by detecting the amount of PCR product while the reaction is still in the exponential phase, thanks to its ability to quantify PCR amplicons as they accumulate in a "Real Time Detection" mode (qPCR).

Real-Time PCR

Real-time polymerase chain reaction (real-time PCR), commonly referred to as quantitative polymerase chain reaction (qPCR), is a molecular biology laboratory technique. Instead of waiting until the end, like in conventional PCR, it monitors the amplification of a particular DNA molecule during the PCR (in real time). This method is an upgraded version of traditional PCR in which the DNA may be quantified while the amplification is taking place (Mackay 2004). The proportional number of copies of the target DNA and RNA sequences can be calculated by extrapolating the Ct (cycle threshold) value of the fungal samples using sequence-specific primers (Balodi et al. 2017). The use of fluorescent dyes like SYBR Green I or sequence-specific fluorescence-labelled probes like the TaqMan probe has allowed for monitoring of reactions during amplification steps (Badali and Nabili 2012). Fluorescent signal is produced when the fluorescent dye intercalates with DNA. After each cycle of amplification, this signal grows as the amount of targeted DNA grows (McCartney et al. 2003; Alemu 2014). The fluorescent dye is less expensive as a monitoring agent; however, it has limitations due to its non-specific character. Intercalating dye binding to all existing DNA might, in fact, provide

erroneous findings in the form of primer dimer. Then, because of their great specificity, fluorogenic probes became popular (Atkins and Clark 2004; Bu et al. 2005). Two types of fluorescent dyes are attached to these probes: one is a reporter dye that attaches to the 5′ end, and the other is a quencher dye that attaches to the 3′ end. The emission of fluorescence is prevented by the close proximity of the reporter and the quenching dye. Taq polymerase's exonuclease activity causes the reporter dye to detach from the quenching dye and fluoresce (Dasmahapatra and Mallet 2006). The disease-causing fungus *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Stachybotrys chartarum*, and *Alternaria alternata* have all been identified and quantified using qPCR (Black 2009).

The sensitivity of real-time PCR appears to be higher than that of conventional PCR. With real-time PCR, amplification of *Rhizoctonia solani* target DNA isolated from soil was achieved at 900 bp, but not with traditional PCR (Lees et al. 2002). Similarly, a TaqMan-based PCR yielded the same level of sensitivity for specific identification of *Helminthosporium solani* in soil and tubers (Cullen et al. 2001). A further boost in sensitivity can be reached by combining two consecutive amplifications with conventional (first amplification) and labelled primers (second amplification) without sacrificing the benefits of real-time PCR. *Rosellinia necatrix* (Schena et al. 2002; Schena and Ippolito 2003), *Verticillium dahliae* (Nigro et al. 2002), *Phytophthora nicotianae*, and *P. citrophthora* (Ippolito et al. 2000) were detected using this method (nested Scorpion-PCR) on different substrates (soils, roots, bark, and/or woody tissues) that are naturally infected. Nested Scorpion-PCR produced higher levels of sensitivity and took substantially less time than traditional detection procedures (Schena et al. 2004). A real-time PCR-based marker for the detection of *Tilletia indica* teliospores in soil was recently created (Gurjar et al. 2017).

Cryphonectria parasitica is a hypervirulent and emerging fungal plant pathogen that produces blight, deadly cankers on bark, dieback, and wilting in chestnut trees, *Castanea dentata*, and *C. sativa* (Murolo et al. 2018; Jain et al. 2019). With the aid of rDNA ITS sequences, qPCR was able to identify *C. parasitica* with a sensitivity of 2 fg of genomic DNA, which is equal to a single spore of the disease (Chandelier et al. 2019). *Ramularia collo-cygni*, a newly discovered fungal pathogen, causes little dark patches on leaves, sheaths, and awns, making it tough to analyse the disease by using traditional methods (Havis et al. 2015). The first report on the molecular identification of *Ramularia collo-cygni* in barley seed was developed and submitted using a qPCR assay (Havis et al. 2014). Another novel fungal pathogen identified by qPCR is a fast-growing and aggressive British *Verticillium longisporum* (Depotter et al. 2017). The fungi that produce *Phomopsis* stem canker in sunflowers, *Diaporthe helianthi* and *Diaporthe gulyae*, were discovered and quantified using qPCR. The assay was used to successfully screen these causal compounds from the same genus (Elverson et al. 2020). *Pyrenophora tritici-repentis* and *Parastagonospora nodorum* co-infect wheat and have similar physiognomies, making traditional disease identification difficult. To execute a duplex qPCR test, two dual-labelled probes with unique fluorogenic reporters were custom built (permitting parallel but independent amplification of DNA sequences from

P. tritici-repentis and *Pa. nodorum*), and the results were precise and suitable for simultaneous variation, as well as for high-throughput screening of several diseases (Abdullah et al. 2018). This method is rapid and accurate (Sikdar et al. 2014), and it can provide precise pathogen load information (Garrido et al. 2009), as well as high-throughput detection of target DNA in biological domains (Schena et al. 2013). Additionally, the TaqMan probe adds another degree of specificity (Shuey et al. 2014). Nevertheless, qPCR necessitates the use of a specialised equipment, which can be costly both in terms of the device and the probe (Abdullah et al. 2018).

End-Point PCR

The use of PCR revolutionised the reliable detection of many plant pathogens, including fungi a prerequisite for disease control (Ma and Michailides 2007). A fragment of DNA template is exponentially amplified in this in vitro process (Caetano-Anolles 2013) using specified primers, deoxyribonucleotide triphosphates (dNTPs), and a thermostable Taq DNA polymerase in buffer solution, through several cycles of denaturation, annealing, extension, final extension, and final hold reactions at varied temperatures (Griffiths 2014). By creating either specialised oligonucleotides that target certain fungal species or universal primers that amplify a variety of pathogens accompanied by sequencing, end-point PCR enables the precise diagnosis of fungal plant diseases. Nucleotide sequences can be compared to ex-type cultures recorded in the NCBI GenBank database utilising the Basic Local Alignment Search Tool (BLAST) analysis to identify each fungal isolate. The existence of a target shown by agarose gel electrophoresis guarantees the prevalence of known plant pathogenic fungi (Mirmajlessi et al. 2015).

The end-point PCR assay for *Phymatotrichopsis omnivora* detection, as well as a SYBR Green qPCR with a primer set PO2F/PO2R was highly sensitive (1 fg) in screening infected plants (Arif et al. 2013). The soil-borne fungus *Phymatotrichopsis omnivora* is responsible for root rots in important crops such cotton, alfalfa, soybeans, vegetable crops, and fruit and nut orchards. These assays may be used to predict the likelihood of disease in a field, assess the pathogen's survival during crop rotations with nonhosts, and examine fungal growth on resistant germplasm used in breeding programmes, among other things. The outlined assays may potentially be used in agricultural biosecurity regulations and microbial forensics (Arif et al. 2014).

Nested PCR

Nested PCR uses two sets of primer pairs for two rounds of PCR amplification to increase specificity and sensitivity. This technique also facilitates the use of general PCR primers in the initial round of PCR for amplification of several pathogens, accompanied by pathogen-specific primers in the second round (Bhat and Browne 2010). *Pilidiella granati* is responsible for pomegranate twig blight and crown rot, both of which are new to the pomegranate business. *P. granati* sensitivity and detection were improved by a nested PCR assay, which allowed for the determination of the causative agent even when only 10 pg of *P. granati* DNA was present in the sample (Yang et al. 2017). Great yam disease is caused by *Colletotrichum*

gloeosporioides, and eucalyptus dieback is caused by *Cylindrocladium scoparium* (Raj et al. 2013; Qiao et al. 2016), wherein this method was employed for detection. The sensitivity of detection with nested PCR could be raised by a factor of 10–1000 when compared to an end-point PCR experiment (Ippolito et al. 2002; Silvar et al. 2005). On the other side, because previously amplified samples are manipulated, nested PCR tests take a bit longer and have a higher chance of cross-contamination, which might also result in false-positive results (Raj et al. 2013). Actually, the use of nested PCR and end-point PCR as diagnostic tools is not advised due to the possibility of amplicon contamination.

Multiplex PCR

A multiplex PCR assay employs a single reaction mixture with multiple primer pairs to amplify multiple pathogens at the same time (Sint et al. 2012). Electrophoresis can then be used to separate and visualise the produced amplicons. Designing primers for the multiplex assay is essential for successful amplification, and particular sets of primers must have comparable annealing temperatures (Zhao et al. 2014). Using the multiplex PCR approach, a contemporaneous diagnostic assay has been developed to detect 12 fungi related with cranberry fruit rot. The ITS-LSU and TEF-1 gene sections were used to successfully identify the fungal infections *Allantophomopsis cytisporea*, *A. lycopodina*, *Phyllosticta elongata*, *Coleophoma empetri*, *Colletotrichum fioriniae*, *C. fructivorum*, *Fusicoccum putrefaciens*, *Monilinia oxycocci*, *Phomopsis vaccinii* (Conti et al. 2019), *Fusarium oxysporum*, *Bipolaris cactivora, Phytophthora nicotianae*, and *Phytophthora cactorum* are pathogenic fungi that threaten the cactus industry's export sector. This issue was resolved by using multiplex PCR assays. These quarantine fungal infections in grafted cactus were found to be detectable and identifiable using the diagnostic technique (Cho et al. 2016). Despite the fact that multiplex PCR assays are speedy and reliable, they can be costly and resource-intensive, and they have a lower sensitivity than other methods (Pallás et al. 2018).

4.3.2.2 Isothermal Amplification-Based Methods

A variety of methods, usually including the use of enzymes to take on the denaturing function at higher temperatures, enable DNA amplification to occur at a single, constant (isothermal) temperature. As opposed to PCR, which alternates between high temperatures for DNA denaturation and low temperatures for primer annealing and DNA synthesis, this does not require this. For instance, recombinase polymerase amplification (RPA) is comparatively new isothermal amplification technique (Piepenburg et al. 2006). RPA uses two primers, operates at 37–42 °C, and lasts for 10–30 min. Exponential amplification is produced by the process' cyclical repeating (Ereku et al. 2018).

Going beyond the laboratory has turn out to be a reality for molecular diagnostics, thanks to the development of isothermal amplification technologies, which allow nucleic acids to be amplified at a specific temperature without the use of thermocyclic equipment. Time and instruments no longer limit the amplification stage. Finding adequate ways for speedy and user-friendly plant preparations and detection

Table 4.2 List of the main isothermal amplification methods applied for fungal plant pathogen detection

Method	Target	Advantages	Disadvantages	References
Loop-mediated isothermal amplification (LAMP)	DNA/RNA	Rapid, isothermal, extremely sensitive, and relatively inexpensive	Designing primers can be challenging	Ammour et al. (2017), Aglietti et al. (2019), Wilisiani et al. (2019)
Recombinase polymerase amplification (RPA)	DNA/RNA	There is no need for an initial denaturation stage because the process is quick and isothermal	Long primers are required, sensitivity and specificity may differ	Ahmed et al. (2018), Gaige et al. (2018), Burkhardt et al. (2019)
Rolling circle amplification (RCA)	DNA/RNA	Isothermal, highly specific, and sensitive	Costly, and detection could be complicated	Rezk et al. (2019)
Strand displacement amplification (SDA)	DNA/RNA	Rapid and isothermal	Amplification of lengthy transcripts is inefficient	Song et al. (2018), Venzac et al. (2018)
Helicase-dependent amplification (HDA)	DNA	There is no need for an initial denaturation stage because the process is speedy and isothermal	High-level optimisation is required	Schwenkbier et al. (2015a, b), Wu et al. (2016)
Nucleic acid sequence-based amplification (NASBA)	RNA	Rapid and isothermal	The procedure is costly	Tsaloglou et al. (2011), Dobnik et al. (2014)

of amplicons following amplification are among the challenges to be solved. A summary of methodologies for in-field phytopathogen diagnostics based on several forms of isothermal amplification, as well as their benefits and drawbacks, are available (Table 4.2).

Recombinase Polymerase Amplification (RPA)

Isothermal RPA, first described in 2006 (Piepenburg et al. 2006), is a highly selective and sensitive isothermal amplification technology that operates at 37–42 °C, requires minimal sample preparation, and can amplify as few as 1–10 DNA target copies within 20 min. It has been used to amplify RNA, miRNA, ssDNA, and dsDNA from a wide range of organisms and materials. A growing number of papers describing the use of RPA are being published, and amplification has been done in solution phase, solid phase, and bridge amplification formats. RPA has also been effectively used with a variety of detection methods, including end-point lateral flow strips and real-time fluorescence detection (Lobato and O'Sullivan 2018). The recombinase-primer complexes are used in RPA reactions to scan double-stranded DNA and promote strand exchange at cognate locations, resulting in a better accuracy of recognition than PCR (Piepenburg et al. 2006). The

RPA produces a "single band" amplification product that is used for further molecular biology studies when contrasted to LAMP, another isothermal DNA amplification method (Iseki et al. 2007). As a result, the RPA assay might be used for routine field monitoring. In addition, RPA technology can be used in conjunction with a lateral flow dipstick to create a quick amplification and visual detection system.

A recombinase polymerase amplification (RPA) test was created to specifically detect *Bipolaris sorokiniana* based on the calmodulin gene sequences. Nineteen fungi related with wheat were used to test the RPA assay's specificity, and it was established that the detection limit for *B. sorokiniana* pure fungal DNA is 10 pg (Zhao et al. 2021). Several soil-borne fungal infections might be found immediately using the RPA test on artificially infected and field-collected plant tissues. These results imply that the RPA assay is a rapid and reliable technique for identifying soil-borne fungus.

Loop-Mediated Isothermal Amplification (LAMP)

Tsugunori et al. (2000) devised a nucleic acid amplification method. Because of its excellent specificity, simplicity, efficiency, and speed, this technique is widely employed. Isothermal amplification that relied on the precise design of four primers is referred to as LAMP (Notomi et al. 2000). To identify the six distinct sequences of the target DNA, LAMP employs two lengthy outside primers and two brief inner primers. The first inner primer, which has DNA sense and antisense sequences, will hybridise the target sequence, and DNA synthesis will start. The second inner and outer primers use the single-stranded DNA produced by the outer primer as a template to create a loop-structured DNA molecule. The outer primer also engages in strand displacement DNA synthesis. Two extra primers are annealed to these loops in modified LAMP. They speed up the reaction by up to 30 min by boosting it and producing additional DNA products (Nagamine et al. 2002).

This enables it a superior choice for plant pathogen diagnostics at the point of care in the field (Fukuta et al. 2013) and a different, trustworthy method for microbial pathogen detection and plant disease diagnosis (Ghosh et al. 2016, 2017). The LAMP assay's benefits and ease of use also include possibility of determining whether a reaction is positive or negative with the naked eye by spotting an elevation in turbidity or a change in colour, as well as the low cost of the equipment and chemicals needed for the reaction (Ghosh et al. 2017). At the same time, the lack of precision in primer designing and the large variety of primers to be chosen are the biggest roadblocks to this research gaining popularity. Nonspecific amplification and primer-dimer products result from using suboptimal primers and temperatures (Rolando et al. 2020). Complex multiplexing is another disadvantage of LAMP, which stems from the difficulty of designing two or more sets of primers. Nonetheless, a number of multiplexed LAMP (non-plant pathogen) systems have been created (Tanner et al. 2012).

Because this approach is less sensitive to inhibitors than PCR, it has been used to detect a variety of plant pathogens, including *Pythium aphanidermatum* from tomato roots (Fukuta et al. 2013), *Fusarium oxysporum* f. sp. *ciceris* (Ghosh et al. 2016) and *Rhizoctonia bataticola* (Ghosh et al. 2017) from disease-infested chickpea fields,

Didymella bryoniae from cucurbits (Tian et al. 2017a), and *Colletotrichum truncatum* from soybeans (Tian et al. 2017b). *Plasmodiophora brassicae* was detected in soil, roots, and seeds using a loop-mediated isothermal DNA amplification (LAMP) promising test with excellent sensitivity, precision, and simplicity. *P. brassicae* is a soil-borne protist pathogen that causes clubroot disease in cruciferous plants around the world (Yang et al. 2021). This method could detect *P. brassicae* in the soil with as little as 1 fg plasmid DNA or 10 resting spores. The LAMP was proved more sensitive than conventional PCR in detecting *P. brassicae* at lower levels in soil samples. Because resting spores of *P. brassicae* are the principal source of infection and can survive in soil for many years, the low level of detection allows forecasting models for clubroot prevalence (Yang et al. 2021).

Rolling Circle Amplification (RCA)

Using the isothermal amplification principle, rolling circle amplification amplifies circular DNA (RCA). By using a DNA polymerase with strand displacement activity (like 29 DNA polymerase), RCA implies spreading a single primer that has been annealed to a circular DNA template. The liberation of ssDNA is caused by the ability of newly synthesised DNA to displace already existing DNA through strand displacement activity. The long single-stranded DNA strand that comes from this enzymatic process of primer expansion and strand dislocation has a complementary sequence to the circular template.

For plant pathogen detection, rolling circle amplification has been frequently employed. Several approaches, such as RFLP and direct sequencing, have been employed in conjunction with RCA to efficiently identify and classify plant pathogens with substantially less work and cost than traditional technologies. By adding fluorescent dye to the reactions, naked eye visibility of the RCA product has been obtained for 40 *Fusarium* strains (Davari et al. 2012). For the detection of fungal infections, padlock probes have been ligated and then RCA has been established (Najafzadeh et al. 2011).

Nucleic Acid Sequence-Based Amplification (NASBA)

NASBA is an isothermal transcription-based amplification technique that is explicitly meant for single-stranded RNA or DNA sequence amplification. Compton (1991) was the first to introduce it, and it is conducted at 41 °C. The approach is highly suitable for RNAs such as mRNA, rRNA, tmRNA, or genomic RNA since reverse transcription activity is integrated into the amplification process (Deiman et al. 2002). NASBA, on the other hand, cannot amplify double-stranded DNAs that have not been denaturated (Yates et al. 2001). "Self-sustained sequence replication" (3SR) and transcription-mediated amplification (TMA) are other terms for it (Ghosh et al. 2019). The amplification power of NASBA is comparable to or better than that of real-time PCR tests, and it does not require a heat cycler (Loens et al. 2006). Additionally, because NASBA only requires brief reactions, has good sensitivity and tight control, and is unaffected by inhibitors, it is particularly appropriate for lab-on-a-chip systems (Honsvall and Robertson 2017). The usage in identifying fungal

infections in plants is extremely infrequent. It might, however, be used in the future to identify fungal diseases.

Helicase-Dependent Amplification (HDA)

HDA is probably the easiest techniques for isothermal nucleic acid amplification that closely resemble an in vivo DNA replication process by using a helicase to isothermally decompress DNA duplexes rather than heat to break away the nucleic acids, allowing labelled primers to anneal to the DNA template and lengthen under the activity of the polymerase, just like in conventional PCR. In 2004, Vincent et al. (2004) discovered this approach, which was later patented by Kong et al. (2007). Because of its simple reaction steps, helicase-dependent amplification has now become a common isothermal approach. Although it uses the same principle as PCR to amplify the target sequences with a pair of primers, the steps are simpler because there are no additional temperature cycling phases.

HDA paired with chip-based detection of *Phytophthora* species that are regulated has a lot of promise for on-site detection. Portable testing devices could be used in the field or at a place where a suspect plant needs to be evaluated with significant improvements. This can shorten the time between taking a sample of sick plants and getting a meaningful result by concentrating sampling, detection, and intervention. Isothermal nucleic acid amplification was developed to replace PCR, which requires a costly thermocycler, in order to achieve a potential field use. tHDA-based amplification and on-chip detection may be carried out in small and portable devices, allowing for on-site operation. Thermal cycling and time-consuming technical requirements are not required for the tHDA performance. Furthermore, the development of disposable, low-cost chips may hasten the availability of portable devices for chip-based DNA analytics in the near future.

4.3.2.3 Post Amplification Techniques

DNA Microarray

Schena et al. at Stanford University in California, USA, first introduced DNA microarrays in 1995 (Schena et al. 1995). A DNA microarray (DNA chip, gene chip, or biochip) is a collection of tiny DNA patches glued to a solid surface (typically glass) in predetermined positions (Bhatia and Dahiya 2015). It is a great tool for genetic study since it can display the expression of thousands of genes at the same time. It may apply thousands of nucleotides to a surface in an ordered array, allowing for simultaneous probing of thousands of distinct sequences (Hadidi et al. 2004; Barba and Hadidi 2008; Guigó 2013).

High performance and multiple diagnosis of diverse plant pathogens such as viruses, viroids, bacteria, and fungi have been made possible because of advancements in DNA microarray technology (Tiberini and Barba 2012; Musser et al. 2014; Nam et al. 2014; Krawczyk et al. 2017). Fungal pathogens are targeted with PCR primers and fluorescent probes like *Spongospora subterranea* (ITS region), *Rhizoctonia solani*, *Fusarium* sp. (TEF-1α), *Alternaria solani*, *A. alternata* (Alt_a1 gene), and *Colletotrichum coccodes* (TUB2) were used with

qPCR microarray technology in 48-well silicon microarrays (Nikitin et al. 2018). A unique microarray test (ArrayTube) that comprised marker genes ITS, TEF-1, and 16S rDNA with effective probes was used to find multiple sugar beet root rot pathogens such as *Aphanomyces cochlioides*, *Botrytis cinerea*, and *Penicillium expansum* (Liebe et al. 2016). On standard microscope slides, batch-based DNA microarrays can be produced quickly, easily, consistently, and affordably (Wöhrle et al. 2020).

DNA Macroarray

To make DNA macroarrays, on a nylon or nitrocellulose membrane, species-specific probes (15–30 bases of oligonucleotides) are arranged on well plates. Afterwards, probe hybridisation with PCR-generated and tagged target DNA sequences can be detected (Clark et al. 1999; Zhang et al. 2008). The manufacturing of membrane-based macroarrays requires simply a pin-tool. A 96-well microtitre plate-size membrane can hold over 1000 distinct detector oligonucleotides, and individually array can be cleaned and reused several times, albeit having a lesser throughput than a microarray (Zhang et al. 2008).

For the identification and detection of fungal and oomycete pathogens in agriculture, a range of macroarrays have already been created. The accuracy and sensitivity of these detecting systems have been demonstrated (Zhang et al. 2007). Over a hundred *Pythium* spp. can be detected using one of the most thorough DNA arrays (Tambong et al. 2006). Nevertheless, plenty of the macroarray investigations that have been published to date have only a few detector oligonucleotides for a specific set of pathogens. An apple disease detection array included 5 controls and 21 oligonucleotides specific for 7 fungal taxa and 1 bacterial target, whereas a tomato vascular wilt pathogen detection array had 3 controls and 10 oligonucleotides specific for 5 taxa (Sholberg et al. 2005). The array detection's slightly elevated capacity has yet to be realised. New vine decline (YVD), a complicated disease in grapevine induced by 51 fungal species and accountable for high mortality in young vineyards around the world, has been detected using DNA microarray (Table 4.3). This DNA array demonstrated to be a quick and specific approach for detecting and identifying the majority of YVD fungus in a single test, with the ability to be utilised in commercialised diagnostics (Úrbez-Torres et al. 2015).

4.3.2.4 DNA or RNA Probe-Based Assays

Because DNA-RNA probe assays are speedier and more sensitive than traditional diagnostics for plant diseases that require microbes culturing, molecular probe assays are rapidly replacing them. Molecular probe assays can be completed in a matter of hours or minutes, whereas culture procedures can take days/weeks. DNA and RNA probes are the most common types of molecular probes; however, cDNA probes and synthetic oligonucleotide probes can also be utilised for a variety of applications. There are four different types of probes that can be used in in situ hybridisation. Table 4.4 lists the probe types and their characteristics.

Table 4.3 Microarray vs macroarray—a overview

Feature	Microarray	Macroarray
Array platform	The glass slide	The nylon or nitrocellulose membrane
Size of the sample spots	Microarray sample spots are generally fewer than 200 μm in diameter, and these arrays can have thousands of dots	Sample spot sizes of 300 μm or larger are found in macroarrays
Advantage	The identity of the clone is revealed right away	Outcomes furnished in full-length clones
	Commercial arrays are available to buy	Obtaining clones in expression plasmids is simple
	Representation of rare genes can be more complete	Can create bespoke libraries to meet your specific requirements There is no need to know the order ahead of time
	Many companies are offering screening and data analysis services, as well as a simple screening process	Non-biased gene coverage on the array
	Array quality (particularly commercial arrays) is somewhat more stable	Filters are reusable
	To produce probes, you might start with total or mRNA	Screening techniques that are adaptable
	To compare two populations, a single hybridisation is used	
Disadvantage	For further research, full-length clones are required	Rigorously laborious
	Only one array can be utilised at a time	Each clone must be sequenced
	To accomplish hybridisation, you'll need a fluidic station and a reader	The quality of libraries and filters can differ
	Custom arrays are more costly than regular arrays	Rare transcripts may not be completely covered
	Gene coverage varies by company and EST database utilised for design	The amount of DNA at each place can differ from one filter to the next
	Sequence information is required to generate the array	Typically, each probe should only be screened once
	The quality of "home-spotted" arrays varies significantly	To compare two populations, sequential hybridisation was used
		Filters have a limited lifespan
		PhosphoImage displays are costly

In Situ Hybridisation (ISH)

In situ hybridisation is also termed as hybridisation histochemistry. It's a gold mine of information for recognising and counting fungi (Aslam et al. 2017). ISH is a technique for detecting and localising nucleic acid sequences in anatomically intact cells or morphologically conserved tissue slices. Single-stranded RNA probes, also known as riboprobes, are utilised in this approach. 35S is used to mark these probes.

Table 4.4 The information of probe types

Probe types	Advantages	Disadvantages
Double-stranded DNA (dsDNA) probes	Steady, accessible, easier to obtain	Self-hybridise, less sensitive, need denaturation before hybridisation
Single-stranded DNA (ssDNA) probes	Reliable, easier to maintain, more selective, RNase resistant, advanced tissue penetration, and no self-hybridisation	Time-consuming and expensive
RNA probes (riboprobes)	RNase has improved temperature constancy, tissue penetration, and specificity while reducing background noise	Sensitive to RNases
Synthetic oligonucleotides	Inexpensive, robust, readily available, easily dealt, more specific, RNase resistant, greater tissue penetration, and repeatability	Acquire nucleotide sequence information

Northern blots and in situ hybridisation are very similar. Both of these rely on the hybridisation of tagged DNA/RNA probes to homologous mRNA sequences. The use of beginning material differs between these two procedures. Tissue digest is utilised as the starting material in northern blots, while histological sections are used in in situ hybridisation. Regardless of whether direct hybridisation is used or not, signal hybridisation identifications are most effective following fungal growth or biological amplification (Jensen 2014).

The radioactive isotopes 35S, 125I, and 32P are commonly used to label probes because they are extremely sensitive and easy to quantify for detection. Non-isotopic probes can be labelled using biotin, digoxigenin, tyramide, alkaline phosphatase, or bromodeoxyuridine. Signal detection techniques include photography, autoradiography along with X-ray film, liquid emulsion, and microscopic techniques (Corthell 2014). *Puccinia horiana* isolate PA-11, *Uromyces transversalis* isolate CA-07, and *Phakopsora pachyrhizi* isolate Taiwan 72-1, which infect *Chrysanthemum morifolium*, *Gladiolus hortulanus*, and *Glycine max*, were identified as rust pathogens using the ISH approach (Ellison et al. 2016). Several *Fusarium oxysporum* formae speciales were genetically engineered with two marker genes and stained with fluorochrome-labelled probes in in situ hybridising transcripts of the marker genes (Nonomura et al. 1996).

Fluorescence In Situ Hybridisation (FISH)

FISH is a type of ISH that uses fluorescent probes to connect with particular chromosomal regions in order to show sequence complementarity. FISH and all other in situ hybridisation techniques share the same fundamental principles; the only difference is that one uses a fluorescent probe to detect specific nucleotide sequences across cells and tissues (Hijri 2009). In plant disease diagnosis, fluorescent in situ hybridisation (FISH) is a relatively new and creative method. It integrates the selectivity of DNA sequences with the accuracy of fluorochrome-based detection techniques (Hijri 2009; Cui et al. 2016). To identify DNA or RNA sequences in cells

Table 4.5 Advantages and disadvantages of ISH and FISH

Feature	ISH	FISH
Advantage	On the same tissue, variety of new hybridisations can be performed. Tissue libraries can be made and preserved in the freezer for later use. The most specific and efficient method of probing is with riboprobes (Aslam et al. 2017)	FISH/s main strengths include reproducibility, sensitivity, specificity, accuracy, and rapidity (Bozorg-Ghalati et al. 2019). It also has the ability to provide data on resolution, morphology, and pathogen identification in combined species specimens (Frickmann et al. 2017)
Disadvantage	The expense and hazards of radioactive probes, as well as the complexity of identifying targets with low DNA and RNA quantities, are the major drawback of ISH (Jin and Lloyd 1997)	False-positive autofluorescence outcomes are a major stumbling block that lowers test specificity (Moter and Göbel 2000)

or tissues, FISH techniques employ DNA or RNA probes that are fluorochrome-labelled explicitly or implicitly (Shakoori 2017). Using wide field epifluorescence or confocal laser scanning *Sclerotium rolfsii* imaging, fluorescently mono-labelled oligonucleotide probes are hybridised to the ribosomal RNA (rRNA) of microbial cells in classical FISH (Lukumbuzya et al. 2019). Plants infected with a pathogen will have rRNA sequences peculiar to that pathogen. FISH allows for the accurate determination of the information that RNA provides (Fang and Ramasamy 2015). The soil-borne pathogen *Sclerotium rolfsii* causes southern blight, which damages tomatoes. FISH approach, which used an oligonucleotide probe stained with cyanine dyes Cy3 and Cy5, was efficient in detecting soil smears in a DNA isolation with 0.06 pg (Milner et al. 2019) (Table 4.5).

4.4 Use of Next-Generation Sequencing (NGS) in Plant Pathogen Detection

Due to its ability to target many unique signature loci of pathogens in a diseased plant metagenome, next-generation sequencing (NGS) has potential as a diagnostic tool. NGS holds a lot of promise for detecting key eukaryotic plant diseases (Espindola et al. 2015). NGS was first used for genome sequencing, supplementing and later substituting the classical genome sequencing method, which included cloning of DNA fragments, Sanger sequencing and genome walking to sequence individual clones, and compilation of the sequenced clones. New NGS platforms and versions have been created on an exponential scale as sequencing chemicals, computer hardware and software, as well as computational capability have advanced. Different NGS systems have their own set of benefits and drawbacks (Tsang et al. 2021).

The baseline genotypes, which might be used to learn the biology and evolution of some other species' genomes, have been sequenced. An instance of a

circumstance in which the target cannot be properly defined is the appearance of a novel pathogen. The full genome of the pathogenic organism can be sequenced using NGS without the requirement for specialised primer pairs or PCR amplification because it does not require prior knowledge of pathogen sequences (Hadidi et al. 2016; Malapi-Wight et al. 2016). Third-generation sequencing is a development in single-molecule sequencing technology, which also has advantages over second-generation sequencing techniques among NGS technologies (Schadt et al. 2010). The time needed to collect and analyse the massive volumes of sequence data is the largest drawback of NGS (Espindola et al. 2015). Inadequate RNA production and/or integrity, RNA stability, and contamination with DNA, salts, or chemicals are typically barriers to the development of next-generation technologies (Cortés-Maldonado et al. 2020). Despite how quickly and easily the sample can be gathered, NGS analysis requires bioinformatics and mycological skills; as a result, accurate bioinformatics analysis knowledge is essential to prevent misinterpretation.

4.5 Conclusion and Future Challenges

We glanced traditional methodologies as well as currently available advanced technologies for detecting and identifying fungal pathogens causing soil-borne diseases. The purpose of this chapter was to highlight the developments in the field of advanced detection technologies. Plant pathogen diagnostic techniques have made a substantial contribution to our capacity to detect and examine pathogens in the lab and, more subsequently, in the field. Existing molecular procedures provide consistent sensitivity and are generally faster than traditional techniques. Monitoring and the implementation of novel disease control measures enable a thorough understanding of pathogenicity variables, as well as fast and effective detection of fungal infections down to the species level. Furthermore, early detection of resistance levels in soil-borne fungus in a field would aid farmers in developing effective resistance management plans to combat disease.Nevertheless, because no single approach meets all, if not the majority, of the developing criterion for faster, more effective, repeatable, and sensitive outcomes, there is still a significant knowledge gap in this sector.

Quantitative PCR has been frequently utilised to quantify and separate causal agents when the sample load is too small to detect using other PCR-based methods. Amplification techniques are showing promise in the field of fungal disease detection, allowing for the identification of pathogens such as *Alternaria* spp., *Colletotrichum* spp., *Fusarium* spp., *Verticillium* spp., *Botrytis* spp., and others that cause a variety of devastating soil-borne plant diseases. The ability of NGS to sequence fungal genomes without prior knowledge of the pathogen's sequence makes it useful for discovering new and emerging illnesses. The molecular methods described in this chapter for diagnosing fungal plant diseases are precise, effective, lab-based, and require high-end equipment. On the other hand, mycology and bioinformatics knowledge are intended to prevent inaccurate portrayal of the outcomes of molecular biological study. By integrating molecular methodologies

with other novel technological advancements, point-of-care testing for fungal illness diagnosis should become commonplace. Scientists have been tasked with developing practical molecular diagnostics for crop diseases. We anticipate that this will start to alter in the upcoming years.

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Detection and Diagnosis of Important Soil-Borne Pathogens

5

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Abstract

The agricultural industry has serious economic concerns globally because soil-borne plant diseases can result in catastrophic losses in crop yields, both in terms of quantity and quality. If a suitable and precise management approach is to be optimized, early, quick, and reliable pathogen identification is crucial. Historically, the most popular techniques for diagnosing plant diseases have relied on labour-intensive, time-consuming colony-based morphological approaches. For precise disease diagnosis and detection, technologies based on nucleic acids are now often utilized. Innovative molecular tools for pathogen detection and differentiation have been made possible by current developments in standard and variable PCR methods, including nested, quantitative, magnetic capture hybridization (MCH); multiplex, biological, post, and isothermal amplification; development of DNA and RNA-based probes; and next-generation sequencing (NGS). These nucleic acid-based detection techniques are used to identify symptomatic and asymptomatic infections caused by culturable and non-culturable fungal pathogens. Even though molecular diagnostic methods have made

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significant strides recently, there is still more to be done regarding their development and use in plant diseases. Molecular methods that are more consistent, efficient, and user-friendly than conventional methods are needed for soil-borne pathogen diagnosis. These approaches have high significance because of their unique specificity in separating related species at various taxonomic levels. Scientists are currently working on the problem of creating efficient tools for plant disease molecular diagnostics. This chapter talks about current developments in the creation and application of molecular methods to detect several soil-borne plant diseases.

Keywords

Soil-borne plant pathogens · Diagnosis · Molecular identification · Polymerase chain reaction · Next-generation sequencing

5.1 Introduction

Healthy crops are crucial for food quality and life in sustainable farming. In reality, a problem is "detected" by objectively observing the symptoms it causes, but a problem is "diagnosed" by identifying the particular pathological condition causing it. The foundation for a healthy crop, aid in risk management, and ensure safety during agricultural production are diagnosing or quickly identifying plant pests and diseases.

A rising concern is that the biggest threat to international agriculture is soil-borne diseases (Singh et al. 2018; Kashyap et al. 2021). Agriculture today makes up around half of the land that is livable worldwide. In previous centuries, as the human population grew, the area covered by crops progressively rose. Rice, wheat, and maize were cultivated on an estimated 540 million hectares worldwide, according to McDonald and Stukenbrock (2016), and plant diseases can significantly lower crop yield. Similar to this, diseases and pests connected with maize, rice, wheat, potatoes, greengram and soybean generate yearly losses of between 17 and 30 per cent worldwide (Manzar et al. 2022a; Kashyap et al. 2022a; Manzar et al. 2021; Reznikov et al. 2018; Savary et al. 2019).

Food security and safety are provided through systematic crop disease control in agriculture, which is essential for the expanding world population (FAO 2018; Sarrocco and Vannacci 2018). Most of the harm is caused by the unintentional spread of invasive alien disease species into new locations due to international commerce and transportation (Ghelardini et al. 2017). In the Great Irish famine (caused to the late potato blight (*Phytophthora infestans*)) of Britain and Ireland (between 1845 and 1849), nearly one million people lost their lives (Cox and Large 1960). Recently, *Pyricularia graminis* f. sp. *tritici*, a blast disease that originated in South America, damaged more than 15,000 acres of wheat in Bangladesh (Callaway 2016). *Xylella fastidiosa*, a xylem-inhabiting plant pathogen, severely damaged olive trees in Italy since 2013. According to genetic research, Italian strains were

comparable to Central American isolates (Marcelletti and Scortichini 2016; Giampetruzzi et al. 2017). The planting material (white pine blister) exchange transferred the pathogen *Cronartium ribicola* from Europe to Northern America. At the same time, the subspecies Americana of the Dutch elm disease fungus *Ophiostoma novo-ulmi* arrived with rock elm logs in Europe from North America (Ghelardini et al. 2017).

Climate change influences plant-pathogen interactions, viz. the spread of diseases in agriculture can be attributed to increased temperatures, climatic extremities, and changes in yearly precipitation patterns. Most of the cultivating soil is also planted with monocultures or even just one genotype, creating a genetically homogeneous environment that makes it simple for host-specific crop diseases to spread (Schmidhuber and Tubiello 2007). Developing quick, effective, affordable tools for early pathogen identification and control is essential. Early disease identification is important since using chemicals or biological agents to cure a significant disease incidence with increased severity and incidence harms the environment and natural ecology (Padaria et al. 2016; Sharma et al. 2017). Using the resistant germplasm as the first line of defence is the most efficient strategy to combat plant diseases (Sharma et al. 2010). However, integrated disease management systems rely heavily on the availability of quick, accurate, and focused disease detection technologies without resistant strains (Tarafdar et al. 2018).

Plant pathogen detection and identification, such as commencing sampling and reaction inhibition, provide several challenges. The development of sensitive and targeted molecular techniques has transformed the identification of soil-borne pathogens in recent years. All practising plant pathologists will soon be exposed to the quick and exciting changes in diagnosis. The immunological and nucleic acid-based tests, in particular, are currently accessible for various bacteria. While conventional methods like baiting, culturing, and microscopic observations are still in use and serve as the backbone of plant pathologists, molecular techniques are readily accessible. The most important approaches for novel molecular methods to identify soil-borne diseases and their utility in agriculture are covered in this chapter. In addition to its other benefits, such as facilitating a quicker diagnosis without the need of a diagnostic laboratory, on-site diagnosis can aid in early illness assessment in domains depending on their relevance.

5.2 Major Plant Pathogens Causing Soil-Borne Diseases

Numerous soil-borne plant diseases have been identified, and high disease-suppression soils have been found. Even in the presence of pathogen inoculum and favourable conditions for the development of illness, disease growth is restricted in these soils. Even while the fundamental processes at work in these soils aren't fully understood and are known to differ according to the pathosystem, it is assumed that the suppressive effect is complex in nature, coming from a combination of general and specific suppression.

Numerous crop species are adversely affected by the diversity of soil-borne diseases, including root, vascular, and seed rot, which can be caused by fungi, bacteria, phytoplasmas, viruses, protozoa, and nematodes. Frequent severe crop destruction results in significant annual economic losses. It might be challenging to see soil-borne bacteria with the naked eye. They are tiny, relying on the biotic and abiotic components of the soil to exist, and complete their life cycle in the soil. The principal soil-borne fungi—*Phytophthora*, *Rhizoctonia*, *Fusarium*, *Pythium*, *Verticillium*, and *Armillaria*—infect roots, resulting in root rot, wilt, yellowing, stunting, and dieback, which eventually cause the death of the plants. *Armillaria* and *Rhizoctonia* induce root rot, *Verticillium* and *Fusarium* cause wilt, and *Phytophthora* causes late blight (*Armillaria* is a honey mushroom that produces brackets or flowers at the base of a tree). Compared to fungus, bacteria are a less common kind of soil-borne disease. *Erwinia*, *Rhizomonas*, *Ralstonia* and *Streptomyces* are a few soil-borne bacterial pathogens that cause the diseases soft rot, corky root, bacterial wilt and scab (Kashyap et al. 2022a). Viral infections seldom spread through the soil because they need live plant tissue, although they have been seen to move on fungus or nematodes and enter through water. Soil-dwelling creatures called plant pathogenic nematodes mostly affect roots. They cause branching and swelling by feeding on the roots' terminals.

5.3 Traditional Methods for Soil-Borne Pathogen Detection

Isolation and cultivating, reinoculation, microscopic techniques, and biochemical testing in the laboratory are conventional/traditional ways of diagnosing soil-borne pathogens. These procedures have tremendous utility since they are reasonably priced and not technically difficult. They need a high level of competence in interpretation and analysis, are time-consuming, and are usually slow. Additionally, taxonomy and fungal plant pathology knowledge and skills are required. Conidia, sclerotia, or mycelia, and symptoms that develop after infection, have historically served as the foundation for diagnosing or identifying a fungal condition. This disease diagnosis is usually cumbersome and impractical when quick results are sought (Sharma et al. 2015). They are not suitable for quick diagnosis or large-scale sample analysis, and producers must rely on specialist diagnostic facilities because they are not easily accessible.

Furthermore, correct identification needs the assistance of trained and experienced people because eye inspection is usually inadequate. Making timely disease management decisions may be more challenging due to the chance that the pathogen would remain dormant in plant tissue (Tarafdar et al. 2013; Tarafdar et al. 2013). It can be difficult to differentiate between many plant diseases due to their physical resemblance. Examples are the *Macrophomina phaseolina* and the *Phoma* species (Somai et al. 2002). A thorough understanding of taxonomy is required for determination. Identifying various populations of the same pathogen with diverse features, such as toxin production, fungicide resistance, or variations in virulence, can sometimes be getting difficult. For a high number of samples, this approach proved

inadequate. Additionally, quarantining pathogens to lower the danger of illness and the spread of the inoculum necessitates using exact, quick detection techniques.

5.4 Immunological/Serological Detection of Soil-Borne Pathogens

Immunological methods' underlying notion of antigen-antibody interaction has many drawbacks, including low test sensitivity and affinity and the possibility of contamination. Due to advancements over the past 10 years, it is now possible to detect and quantify several hazardous species using immunological approaches, including nematodes and mycoplasmas. For more than 20 years, immunological techniques have been researched. Furthermore, fungus's high inconsistency and phenotypic serological flexibility have rendered plant disease detection ineffective (Luchi et al. 2020; Meng and Doyle 2002). Applying and developing cutting-edge and efficient diagnostic procedures to prevent fungal plant disease is essential. As a result, molecular approaches that make it easier to identify and quantify pathogens are being used to diagnose soil-borne infections. The drawbacks of traditional and serological diagnostic techniques can be overcome by molecular testing.

Beginning in the 1970s, the use of antibodies in serological detection systems for the rapid and precise diagnosis of diseases accelerated with the advent of monoclonal antibody technology. Soil-borne bacteria can be discovered if bacterial antigens are used to generate antibodies. These methods were used as laborious analytical instruments. This requires using specific antibodies to find the matching antigens in test samples. Each antibody has a distinct antigen-specific binding site. Monoclonal antibodies, which may be produced indefinitely and are highly specific when utilized in immunological testing, allow for identification at the genus, species, and isolate levels (Hardham et al. 1994).

Serological diagnostic methods provide several advantages. Antibodies may take weeks to produce, but if properly kept, they are frequently stable for a long period and produce effects quickly. They have not yet been fully utilized in diagnosing plant diseases other than viruses and bacteria, although they offer a wide range of applications for the general and accurate detection of unique epitopes of numerous soil-borne microorganisms. Tests for antibodies have significantly improved. They can now distinguish between strains within a species, are nanogram sensitive, and take less time to conduct in lab and field settings. Second, diagnosis depends only on a structural element of the organism, such as the coat protein, which offers very little information about the virus.

Thirdly, serology is only useful when an antigen that can be used to create an antiserum is accessible or when the antiserum is ready. Finally, serology is worthless for identifying as-yet-unidentified soil-borne diseases. The capacity to recognize IgM or rising antibody titres determines how a serological diagnostic is organized. Serological methods are used to diagnose the majority of prevalent bacterial illnesses that are transmitted through soil. The antibody-antigen combination may be used in various ways due to its endurance. The enzyme-linked immune sorbent assay

(ELISA), which comes in various formats and offers numerous endpoint detection choices, is the most significant. The ELISA can measure a pathogen's presence and offer proof of it.

5.5 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a different method that uses the studied antibody colour change to determine the presence of soil-borne pathogens. The target epitopes (antigens) from viruses, bacteria, and fungi are accurately bound using this method by antibodies bound to an enzyme. The interaction between the substrate and the immobilized enzyme causes colour changes, which may be used to identify substances. Specific monoclonal and recombinant antibodies easily available on the market can greatly improve ELISA performance. Specific monoclonal antibodies have been used in ELISA to achieve lower detection limits in the region of 105–106 CFU/mL. For the on-site detection of plant diseases, tissue print-ELISA and lateral flow devices have been developed. Although it cannot be used to diagnose infections early on before symptoms appear because the sensitivity for bacteria is so low (105–106 CFU/mL), it may be used to confirm plant illnesses once visible signs appear. The ELISA tests can be classified as a direct, indirect, sandwich, or competitive ELISAs depending on the antigen-antibody combination.

5.5.1 Direct ELISA

A target protein (or a target antibody) placed on the surface of microplate wells is treated with an enzyme-labelled target antibody (or a specific antigen to the target antibody). The activity of the microplate well-bound enzyme is evaluated after washing.

5.5.2 Indirect ELISA

The primary antibody is treated with a target protein immobilized on the surface of microplate wells before being incubated with a secondary antibody against it. After washing, the activity of the microplate well-bound enzyme is measured. Even though indirect ELISA requires more steps than direct ELISA, the primary antibody does not need to be labelled because labelled secondary antibodies are commercially available.

5.5.3 Sandwich ELISA

A second antibody that is also specific to the target protein but has been enzyme-labelled is used to treat a target protein-specific antibody placed on the surface of

microplate wells. The activity of the microplate well-bound enzyme is evaluated after washing.

The enzyme-labelled antibody (green) and the immobilized antibody must identify various target protein epitopes (orange). Sandwich ELISA is more selective than direct ELISA because it combines antibodies to two different epitopes on the target protein. Sandwich ELISA is beneficial when extreme accuracy is needed.

5.5.4 Competitive ELISA

An antibody that is specific for the target protein and has been immobilized on the surface of microplate wells is used to treat samples that contain the protein and a known amount of the target protein. The activity of the microplate well-bound enzyme is measured after the procedure. The sample will seem lighter when there are less antibody-bound enzyme-labelled antigens present. When it is low, on the other hand, more enzyme-labelled antigen is bound to antibodies, which results in a deeper colour. When the target antigen in a sandwich ELISA test is a small molecule like dioxin, histamine, or a pesticide, two antibodies cannot attach to it simultaneously. Competitive ELISA may be used to measure low molecular weight targets.

5.5.5 Phage Display

Phage display-based antibody engineering has the potential to revolutionize the production of antibodies by making the process faster and more affordable than current monoclonal antibody techniques (Mitchell et al. 1997; Wilson and Finlay 1998; Aujame et al. 1997). To produce foreign proteins (antibodies) as fusions to phage coat proteins, cloning sites that have been introduced to filamentous phage vectors are used in this technique. Before being chosen for certain proteins with particular binding capabilities, *Escherichia coli* cells are transformed with phage libraries and cultured in culture. The technique has been used for diagnosing plant diseases and general plant biology. For example, *Ralstonia solanacearum* Race 3 and Black Currant Reversion Associated Virus have been detected using phage display to create particular antibody fragments that can be used in ELISA (Griep et al. 1998). Due to the ability to manufacture specific antibodies in large quantities without the need of expensive hybridoma technology or test animals, antibodies will soon be available at a greatly reduced cost.

5.6 Lateral Flow Devices

The lateral flow device is one of the most extensively used diagnostic tools available to farmers today (LFD). These devices are simple to use and swiftly generate results—typically in less than 10 min. The LFDs that can be purchased commercially to identify viral infections in plants are the most beneficial. As little as 3 ng mL^{-1} of

antigen may be detected by an LFD-based test for *Rhizoctonia solani*, which is equivalent to the sensitivity of conventional ELISA methods (Thornton 2008). In contrast to the plant viruses and bacterial pathogens that are typically the objectives of commercial LFD-based tests and for which specific antibodies are frequently available, this work focused on a soil-borne plant pathogenic fungus. The development of species-specific antibodies against fungi has proven to be more difficult; however as was already said, some targets have achieved success.

5.7 Biochemical Methods for Soil-Borne Pathogen Detection

Biochemical traits specific to each creature can be used to identify it. On one end of the scale, certain qualities are shared by large populations while, on the other, some are exclusive to individual populations within the species. In order to determine the taxonomic rank at which an organism is categorized, it is essential to characterize the pathogen. Embracing gel electrophoresis for soluble protein analysis are bacteria and fungi. It is crucial to standardize these procedures since gene expression is a characteristic of all of them and may be affected by environmental factors.

Similar to this, Pernezny et al. (1995) used substrate to pinpoint *Xanthomonas campestris* as the bacterial species in charge of a serious outbreak of bacterial spots in Florida lettuce crops; the pathovar presence was determined to be vitians by its fatty acid composition. In some situations, the creation of unusual metabolites by an organism can be utilized to identify it.

For instance, identifying *Aspergillus flavus* strains capable of making aflatoxin was aided by synthesizing volatile C15H24 compounds, including alpha-gurjunene, trans-caryophyllene, and cadinene. Non-toxic strains did not create these chemicals (Zeringue et al. 1993). When identifying bacterial plant pathogens using fatty acid profiles (FAME Analysis), the bacterium is often grown in pure cultures first. Wet cells are methylated and saponified to around 40 mg. By using an ether-hexane combination to extract the fatty acid methyl esters (FAME), gas chromatography is used to examine the results.

Because the fatty acid profiles of the field-collected strains most closely mirrored that of this pathovar, *Xanthomonas campestris* pv. *vitians* was discovered to be the pathogen that produced an outbreak of a bacterial spot on lettuce (Pernezny et al. 1995). The four species of the *Erwinia herbicola* group and the five species of the *Erwinia amylovora* group could be distinguished in more detailed research by Wells et al. (1994). When electrophoresizing, soluble proteins from plant diseases usually produce intricate patterns that can be used for identification. Proper staining methods may be able to disclose a particular protein dye, which, for example, may include enzyme activity, rather than utilizing a broad protein stain like Coomassie Blue. 4250 Australian isolates of *Rhizoctonia solani* were divided into 10 groups, termed zymograms, by MacNish et al. (1994), who stained for pectic enzymes.

5.8 Molecular Methods for Soil-Borne Pathogen Detection

Many experts agree that nucleic acid (NA)-based methods are among the best for finding soil-borne plant infections. More contemporary methods, including immunological methods, DNA/RNA probe technologies, and polymerase chain reaction (PCR) amplification of nucleic acid sequences, are increasingly being used to identify plant diseases (Manzar et al. 2022a). These techniques have a number of benefits over traditional diagnostic techniques, including the fact that they are more accurate, faster, and easier to use without specialized taxonomic expertise. More significantly, these techniques make it possible to identify bacteria that cannot be grown. Furthermore, molecular identification techniques aid in the discovery of new diseases with unidentified aetiologies. These instruments might be employed to accurately gauge the biomass of infections and confirm their presence (Biswas et al. 2012a, 2012b; Sharma et al. 2012b).

5.9 Nucleic Acid-Based Detection Techniques for Soil-Borne Pathogens

Most NA-based detection techniques, particularly those that employ PCR, are rapid, specialized, and sensitive. This provides a more robust diagnosis. While molecular testing verifies the diagnosis for other diseases or determines whether litigation is feasible, traditional procedures are helpful for different conditions. It is challenging to separate pathogens taxonomically because many plant pathologists cannot swiftly differentiate important disease taxa like *Pythium* or *Phytophthora* by visual inspection. To help create a genome database, various bacteria, even nonsporulating ones, can be awarded species I.D.s as sequencing expertise increases.

Diagnosticians and other applied plant pathologists are mainly situated to increase the genetic library for plant diseases due to their exposure to various conditions on diverse hosts. Sequencing the ITS or mitochondrial genes may be helpful since it provides a DNA fingerprint for many plant illnesses. Many of these diseases must be cultivated before being detected. The study of this area may easily recognize these sequences. Massive sequencing technology advancements have profoundly influenced genomic research and considerably increased the throughput of cost-effective sequences. The pyrosequencing method of DNA sequencing is built on the sequencing-by-synthesis methodology. The management of fungal plant diseases currently does not make extensive use of pyrosequencing technologies.

5.10 Polymerase Chain Reaction (PCR)

For developing monoclonal antibodies and using the polymerase chain reaction to amplify nucleic acid sequences, J.F. Kohler, C. Milstein, and K. Mullis were awarded two Nobel Prizes in 1984 and 1993 (PCR). A thermostable DNA polymerase catalyses an exponential amplification of a target DNA strand in the polymerase

chain reaction (PCR), the mainstay of NA-based disease detection. This valuable and inexpensive molecular method can duplicate or amplify tiny fragments of DNA or RNA. By connecting two synthetic oligonucleotides, or "primers", to the target genomic sequence and extending them using a Taq polymerase, this in vitro amplification technique amplifies a single copy of the nucleic acid target (a thermostable DNA polymerase). Because of the DNA hybridization and replication fidelity, PCR was initially used to detect illnesses caused by bacteria and viruses. These days, both plant illnesses and diseases transmitted through the soil are frequently identified using it. Due to its exceptional sensitivity, advanced PCR methods, such as reverse-transcription PCR (RT-PCR), have also been used in addition to traditional PCR technology for the identification of plant pathogens. The many PCR types used in pathogen detection are described in the section below.

5.11 Random Amplified Polymorphic DNA (RAPD)

The Random Amplified Polymorphic DNA (RAPD) technique is a simple, rapid, and inexpensive way to amplify a tiny amount of total genomic DNA at low annealing temperatures. It uses short synthesized oligonucleotides of random sequences as primers. A somewhat unique profile pattern is visible when the ensuing PCR product is resolved. As a result, RAPD markers have established themselves as useful tools for studying the genetics of fungal populations (Nasir and Hoppe 1991). This marker makes it possible to detect even the smallest DNA changes in the organism. For molecular taxonomy, genomic mapping, and evolutionary studies, several fungal species have been identified using RAPD (Nasir and Hoppe 1991). By examining DNA products created by RAPD, it has been possible to learn about the variation and segregation of genetic traits among strains.

5.12 Restriction Fragment Length Polymorphism (RFLP)

The phylogenetic separation, description, and categorization of soil-borne illnesses is made possible by nuclear ribosomal DNA (rDNA) amplified using restriction fragment length polymorphism (RFLP) (RFLP). Restriction fragment length polymorphisms in DNA encoding specific genes can be used to identify the species of a pathogen. This method of identifying a species depends on having a good database on the variability in fragment length polymorphisms that may be found among isolates of individual species because conspecific isolates may differ in the presence or absence of specific restriction sites, changing the RFLP banding.

As an illustration, Camele et al. (2005) employed thorough RFLP of PCR-amplified rDNA to identify and separate 10 *Phytophthora* species infecting different crops, enabling selective identification of these *Phytophthora* spp. The restriction patterns of 27 other *Phytophthora* species were identified and used to amplify and further digest the amplicons generated by PCR using *Phytophthora*-specific primers (Drenth et al. 2006, 2006). Following analysis of the ITS region

using PCR-RFLP, several anastomosis groups were discovered in *Rhizoctonia solani* isolates (Pannecoucque and Hofte 2009). The ability to discriminate between pathogenic and non-pathogenic *Pythium myriotolum* strains was also made feasible (Gómez-Alpízar et al. 2011). Sharma et al. have identified the genetic diversity in populations of *M. phaseolina*, a PCR-amplified rDNA-targeting microbe isolated from chickpea (2012a).

5.13 Amplified Fragment Length Polymorphism (AFLP)

A PCR-based tool and variation of the RFLP, the amplified fragment length polymorphism (AFLP) is used in genetic research, DNA fingerprinting, and the practice of genetic engineering. It has been used to distinguish between different species, although it is most usually employed to examine genotypic diversity in a population (Gargouri et al. 2006). Infections connected to recent disease outbreaks, such as sorghum ergot, can be traced back to their geographic source using the latter trait. Despite being an effective diagnostic tool, AFLP analysis takes a lot of time, requires complex technical skills, and is not suited for everyday use in diagnostic clinics.

5.13.1 Simple Sequence Repeats (SSR)

Simple sequence repeats (SSRs), often referred to as microsatellites or short tandem repeats (STRs), are repeating patterns made up of one to six nucleotides that are found in every eukaryotic genome. They are known for producing the best and most precise markers, which are frequently applied in soil-borne diseases to identify genetic changes between even among closely related species (Prospero et al. 2004). The distribution of these nucleotide units across the genome is essentially random, and their recurrence patterns may differ from person to person. To produce PCR products of various lengths, one can employ primers that surround such varied locations.

Microsatellites are a common genetic marker used for DNA fingerprinting due to their extraordinary versatility. The abundance of thousands of potentially polymorphic markers and a high degree of polymorphism in SSRs are advantages. SSR markers are a reliable solution for a broad range of applications, such as genome analysis and genetic mapping (Szabo and Kolmer 2007). Microsatellite markers exclusive to the *Phytophthora ramorum* pathogen were used in the additional study to discriminate between the A1 and A2 mating types of isolates from this disease that originated in two distinct countries.

5.13.2 Multiplex PCR

Using a single reaction mixture and many primer pairs, the multiplex PCR test enables the simultaneous amplification of numerous pathogens (Sint et al. 2012).

The generated amplicons can then be separated and shown using electrophoresis. The multiplex test requires the creation of primers, and specific sets of primers should have equivalent annealing temperatures for effective amplification. It makes it possible to accurately and simultaneously detect several DNA or RNA targets using a single procedure. It is advantageous in plant pathology because sensitive detection is necessary to produce pathogen-free plant material, and different soil-borne pathogens frequently infect a single host. Wheat (Sun et al. 2018), strawberries (Li et al. 2011), and turfgrass are a few examples of hosts where several infections can be found at the same time in a single multiplex PCR test (Asano et al. 2010).

5.13.3 Real-time PCR

Real-time PCR, which is based on the nucleic acids of bacteria, fungi, and viruses, is used to rapidly identify plant illnesses. The important component in managing plant diseases is detection and pathogen quantification (Le Floch et al. 2007; Lees et al. 2002). Real-time PCR has significantly improved pathogen identification and quantification, while quantification based on culture techniques is frequently considered inaccurate and unreliable (Tarafdar et al. 2018). Real-time PCR differs from end-point PCR in that each PCR cycle includes a measurement of the amplified PCR product. Since the exponential phase of the reaction is being monitored as it progresses, real-time PCR allows for accurate template quantification. Real-time PCR is gaining popularity for identifying and quantifying a variety of pathogenic fungus, oomycetes, bacteria, nematodes, viruses, and biocontrol agents that affect plants. A specific increase in fluorescence during PCR amplification can be used to identify pathogenic fungi.

5.13.4 Colony PCR

This efficient method for crude mycelium-based amplification utilizes the ITS1–5.8S-ITS2 section of the fungal ribosomal DNA cluster. PCR generally has a high success rate. This method ought to be widely applied to streamline molecular taxonomic studies and enable more in-depth, sequence-based analyses of fungal isolates. The data were directly obtained from fungal hyphae without any prior DNA extraction or other processing. It is possible to successfully amplify DNA from the fungus *Cladosporium*, *Geomyces*, *Fusarium*, and *Mortierella*. Yeasts discovered in the soil may always have their DNA enhanced. Mutualistic *Basidiomycota* and *Ascomycota* were also successfully amplified without the need for DNA extraction from cleaned mycorrhized root tips, and *Tuber melanosporum* fruiting bodies could be swiftly recognized using a direct PCR using species-specific primers (Walch et al. 2016; Bonito 2009).

5.13.5 Nested PCR

Nested PCR is an endpoint PCR variation that uses two sets of primer pairs for two rounds of PCR amplification to boost specificity and sensitivity. Nesting makes it easier to employ non-specific PCR primers for amplifying different pathogens in the first round of PCR, followed by the use of pathogen-specific primers in the second round. The main goals of the PCR modification were to improve sensitivity and specificity. Two primer sets are used to carry out two successive PCR reactions, treating the results of the first round of amplification with the same treatment in the second round (Ni et al. 2011; Grote et al. 2002; Kamolvarin et al. 1993).

5.13.6 Bio PCR

The bio-PCR test amplifies the endpoint PCR technique, which involves a pre-assay incubation step in a sick sample to increase the biomass of the causal agent. This method focuses solely on the target pathogens by cultivating the target pathogen in a growing medium that prevents the growth of non-target microorganisms to maximize detection.

5.14 DNA or RNA Probe-Based Assays

5.14.1 In Situ Hybridization

Using the in situ hybridization (ISH) technique, the mRNAs present in the fixed sample may be identified. The main goal of this test is to design an antisense small-scale RNA probe that will bind the target mRNA (interesting sequence). But it's also feasible to use cDNA probes and artificial oligonucleotide probes. Because they are detectable and straightforward to measure for, the radioactive isotopes 35S, 125I, and 32P are widely employed to label probes. Tyramide, bromodeoxyuridine, biotin, digoxigenin, alkaline phosphatase, and digoxigenin can all be used to label nonisotopic probes. Photographic, X-ray film autoradiography, liquid emulsion, and microscopic techniques are a few examples of signal detecting techniques.

5.14.2 Fluorescent In Situ Hybridization

Fluorescent in situ hybridization (FISH) is a cutting-edge approach for the diagnosis of plant diseases that are still relatively new. The specificity of DNA sequences is combined with the sensitivity of fluorochrome-based detection methods (Hijri 2009; Cui et al. 2016). Using DNA or RNA probes that are fluorescently coloured either directly or indirectly, FISH assays may locate specific DNA or RNA sequences in cells or tissues (Shakoori 2017). Using wide-field epifluorescence or confocal laser

scanning microscopy, stained cells from the standard FISH methods are seen when fluorescently mono-labelled oligonucleotide probes hybridize the ribosomal RNA (rRNA) of microbial cells (Lukumbuzya et al. 2019). The rRNA sequences of plants that have been infected with a pathogen are specific to that pathogen. FISH can recognize this specific information provided by RNA (Fang and Ramasamy 2015). Southern tomato blight is brought on by the disease *Sclerotium rolfsii*, which can be found in soil. The FISH technique that used an oligonucleotide probe dyed with Cy3 and Cy5 successfully identify soil smears in DNA isolation with 0.06 pg L^{-1} of *S. rolfsii* (Milner et al. 2019). FISH's most vital points are repeatability, sensitivity, specificity, precision, and speed (Bozorg-Ghalati et al. 2019). In mixed-species specimens, it could also pinpoint the primary pathogens and offer details on resolution and morphology (Frickmann et al. 2017). A common pitfall that reduces test specificity is false-positive results using autofluorescence materials.

5.15 Isothermal Amplification Techniques

5.15.1 Loop-Mediated Isothermal Amplification (LAMP)

Due to its outstanding efficacy, specificity, ease of use, and speed, LAMP requires four primers, two long outside and two short inside, each recognizing six different sequences in the target DNA. DNA synthesis will begin when the target sequence hybridizes with the first inner primer, which comprises a sense and antisense DNA sequences. The single-stranded DNA produced by the outer primer serves as a template for the creation of a DNA molecule with a loop structure by the second inner and outer primers. The term "strand-displacement DNA synthesis" refers to this procedure. The constant cycle reaction causes products with repeated target DNA sequences of varying lengths to accumulate.

The reaction tube is incubated at 63–65 °C in a standard water bath or heat block in a laboratory setting to maintain a constant temperature. Unaided eyes can perceive the amplified product as a white precipitate or a yellow-green-coloured solution after adding SYBR green to the reaction tube. The primary benefit of LAMP is that it may be completed rapidly and at a constant temperature. Since it uses an expedient isothermal technique, it is ideal for plant pathogen identification at the point of care in the field.

It also has a high amplification efficiency and sensitivity since it can generate many PCR products from a small quantity of DNA input. Due to the assay requiring only a few pieces of essential equipment, this process is also affordable. The sensitivity of hybridization assays, such as LAMP-ELISA hybridization and LAMP paired with colorimetric gold nanoparticle hybridization probes, may be improved by using amplicons containing many inverted repeats produced by LAMP, according to specific reports. The electrochemical sensor, in conjunction with LAMP offered a reliable platform for pathogen detection due to its outstanding sensitivity, which allowed it to recognize as little as ten copies of pathogen genomic DNA. LAMP-biosensor technology has a significant potential for in-field testing,

detection, and identification of plant diseases (Tsugunori et al. 2000; Fukuta et al. 2003; Ghosh et al. 2016; Ghosh et al. 2017).

5.15.2 Rolling Circle Amplification

Rolling circle amplification is a widely used isothermal enzymatic assay that utilizes DNA or RNA to diagnose plant diseases. In addition to RCA, several techniques, like direct sequencing and RFLP, have effectively discovered and classified plant diseases with much less time and price than conventional methods. The main components required for this experiment are deoxynucleotide triphosphates, a circular template, a short DNA/RNA primer, and a homologous buffer. For 40 *Fusarium* strains, naked eye viewing of the RCA result has been made possible by adding fluorescent dye to the reactions (Davari et al. 2012). Ligating padlock probes with RCA has also been shown to detect fungal infections (Najafzadeh et al. 2011). The RCA test offers the advantages of simplicity, efficacy, and lack of temperature cycling apparatus (Dong et al. 2013; Goo and Kim 2016). Using this method, it is also possible to analyse gene expression, single nucleotide polymorphism, mRNA splicing, and post-translational modifications of protein molecules (Gao et al. 2019).

5.16 DNA-Based Point-of-Care Diagnostic Methods

Diagnostic tests that can be performed at the point of care (POC) and without costly equipment are desperately needed. Despite having several advantages over other technologies, PCR-based methods are much less effective for POC applications because they require energy to carry out the temperature modifications necessary for DNA amplification. The best way to overcome this constraint uses isothermal DNA amplification. For instance, POC detection of pathogen DNA utilizing isothermal amplification combined with lateral flow strips and portable fluorometers has been accomplished.

- POC—DNA Extraction methods: To successfully extract DNA from plant tissues, it is necessary to be able to properly remove a variety of contaminants that may otherwise interfere with the DNA amplification process. A rapid and efficient DNA extraction method using a lateral flow device (LFD) has been devised for POC testing and plant pathogen identification.
- In an extraction buffer, the sample is agitated with metal ball bearings before the lysate is transferred to the release pad of an LFD nitrocellulose membrane. The membrane is then added to the DNA amplification process using PCR or another isothermal amplification technique after being partly removed. It is possible to do the extraction outside since the isolated DNA is very stable on the membrane at ambient temperature.
- Another method uses a simple dipstick composed of cellulose, which can analyse plant samples in as little as 30 s. Plant tissues are macerated by giving them a

vigorous 8–10 s shake in a tube with extraction buffer and one or two ball bearings. Before entering the tube containing the amplification mix, the sample is first put in a cellulose dipstick tube and three times rinsed with wash buffer in a separate tube. The technique works on various domesticated species, including mature tree leaves and notoriously tricky tissues such as rice, tomato, and sorghum (mandarin, lime, and lemon). It is compatible with a variety of amplification methods, such as PCR, LAMP, and RPA, and it may be used to detect pathogen DNA and RNA in tissues that have been infected.

5.17 Recent Advances in Soil-Borne Pathogen Detection

5.17.1 Ancillary Ways of Pathogen Detection

Thermography, fluorescence imaging, hyperspectral imaging, and gas chromatography are a few techniques for indirectly identifying infections.

Thermography is a promising method for evaluating the heterogeneity in the infection of soil-borne diseases and can record changes in the surface temperature of plant leaves and canopies. Thermography uses thermographic cameras to record and analyse colour variations in emitted infrared light. Plant diseases affect how much water a plant losses when its stomata open and close (Hillnhütter et al. 2011). Thermographic imaging shows the disease that results may be observed, and without the effect of outside temperatures, the amount of water lost can be determined (Oerke et al. 2006).

Another cutting-edge technique is hyperspectral imaging, which may be used to indirectly detect plant illnesses and gather crucial information on the health of plants over a wide spectrum of wavelengths between 350 and 2500 nm. For the diagnosis of agricultural diseases and plant phenotyping, it is increasingly frequently utilized in large-scale agriculture. This method allows for quick processing of imaging data and is exceptionally accurate. Because they monitor variations in reflectance brought on by the biophysical and metabolic impacts of infection, hyperspectral methods are used to detect plant infections. Hyperspectral imaging methods have been used to identify and report infections caused by *Magnaporthe grisea* in rice, *Phytophthora infestans* in tomatoes, and *Venturia inaequalis* in apple trees (Delalieux et al. 2007; Zhang et al. 2003).

5.17.2 Gas Chromatography

Identifying the volatile chemical signature of diseased plants is another non-optical indirect way of plant disease identification. Plant pathogen infections may cause the emission of certain volatile organic compounds (VOCs) that are highly diagnostic of the sort of stress the plants are experiencing. When strawberries are infected with *Phytophthora cactorum*, a fungus that causes crown rot, *p*-ethyl guaiacol and *p*-ethyl phenol are released as identifiable VOCs from the damaged section of the plant/fruit.

The volatile signature of plants may be examined using gas chromatography (GC) technology to check for a particular VOC that may indicate the presence of a specific disease. Gas chromatography and mass spectrometry (GC-MS) are widely employed to detect unidentified molecules in volatile samples and improve compound separation and analysis effectiveness. Due to its high specificity, GC/GC-MS can offer more accurate details on plant disease than the optical imaging-based detection techniques listed above. The quantitative information collected from the VOC sample shows that illnesses can be identified at various stages (Kashyap et al. 2022a).

5.18 On-Site Direct Diagnosis of Plant Diseases

There are now several on-site direct diagnostic methods available. They are straightforward to understand and useful for farmers in making prompt decisions and early adoption of this technology for precise disease management strategies that might lessen the effect of plant illnesses. On-site testing can provide immediate response without shipping the sample to an off-site laboratory if it is done "field-side" in the farmer's presence. Utilizing a fluorogenic probe-based test, for instance, which entails magnetic bead-based nucleic acid extraction followed by qPCR using portable real-time PCR, *Spongospora subterranean*, a soil-borne disease of potatoes, may be quickly and easily diagnosed on-site. Compared to the laboratory-based method, the portable real-time PCR methodology can identify the pathogen with as little as 100 copies of *Spongospora subterranea* DNA, even when the pathogen colonization in the host is very low. The revolutionary portable real-time PCR may be used in place of laboratory-based methods to detect infections.

X-ray crystallography is now one of the most sophisticated techniques for diagnosing certain diseases using a particular protein released by the pathogen or host during contact. Using X-ray crystallography equipment at Diamond Light Source, researchers at the Iwate Biotechnology Research Centre (Japan) found the deadly rice blast disease *Magnaporthe oryzae*. The gene-for-gene paradigm was used for the first time to identify a pathogen at the molecular level using a crystallographic-based technique.

5.18.1 Immunofluorescence (IF)

A fluorescence microscopy-based optical method is applied to detect pathogen infections in root tissues. Plant samples are cut into tiny tissue slices and adhered to microscope slides for this operation. The specific antibody is detected by conjugating a fluorescent dye to observe the distribution of the target molecule across the sample. They are using IF, and the onion crop infection caused by *Botrytis cinerea* was found. Crown rot, a novel disease in Europe, may be found using IF and FISH together (Wullings et al. 1998). Similar to FISH, a flaw in other fluorescence-based methods such as photobleaching results in erroneously negative

consequences. The reduction in sensitivity brought on by photobleaching may be controlled, though, by reducing the amount of light exposure time and intensity, increasing the concentration of fluorophores, and choosing fluorophores that are more resistant to photobleaching.

5.18.2 Flow Cytometry (FCM)

It is a widely utilized laser-based optical technique for cell sorting, biomarker detection, and protein modification. FCM is a unique tool for detecting plant illnesses even though it has been used to count bacteria, distinguish between live and non-viable bacteria, describe bacterial DNA, and examine fungal spores. It has also been used to research antibiotic susceptibility and cell cycle dynamics.

5.18.3 Next-Generation Sequencing

Next-generation sequencing (NGS), high-throughput sequencing (HTS), and pyrosequencing are cutting-edge diagnostic techniques that revolutionize the detection of pathogens in various plant samples. As opposed to conventional molecular technologies, which require prior knowledge of the pathogens' sequence information, the NGS approach is unlimited, making it possible to identify any known and undiscovered pathogens in a single experiment. At its genomic core, phytopathogens are a collection of soil-dwelling bacteria, and the development of NGS technology has spawned novel methods for the detection and taxonomic identification of phytopathogens. The organism need not be cultivated or have its past sequencing data to apply this procedure, which takes some time but is essential for finding novel bacteria, viruses, and viroids (only around 10% of bacteria are culturable). NGS can quickly identify both known and unknown plant diseases. The primary steps in DNA-based NGS include DNA isolation and fragmentation, library preparation, massively parallel sequencing, bioinformatics analysis, variant/mutation annotation, and interpretation. Massive parallel signature sequencing, pyrosequencing, colony sequencing, and sequencing by oligonucleotide ligation detection (SOLID) are some of the most frequently employed advanced sequencing methods in HTS (Rajesh and Jaya 2017). Using RNA-sequencing, it may be possible to comprehend and study the dynamic nature of the transcriptome (RNA-Seq). The most popular NGS platform for RNA-Seq is the Illumina HiSeq platform, which has taken the NGS market by storm. The most recent release for the platform was a desktop sequencer named MiSeq (Kukurba and Montgomery 2015; Hariharan and Prasannath 2021).

When identifying early-stage infections in plants brought on by various fungal/oomycete diseases, symptoms in the host plant are typically necessary. Several of the abovementioned molecular and serological methods are often utilized to find these infections. But since it may target several different pathogen loci in a plant metagenome that is affected, next-generation sequencing (NGS) has the most potential as a diagnostic tool (Sharma et al. 2016). Finding significant eukaryotic plant

diseases with NGS has several possibilities. It may raise the fraction of NGS readings for targets with low abundance by concentrating specific nucleic acids in heterogeneous samples using targeted genome capture (TGC) oligonucleotide probes. Metagenomes and the Electronic Probe Diagnostic Nucleic Acid Analysis (EDNA) have the potential to simplify the detection of oomycete and fungal plant diseases significantly. EDNA is more reliable than electronic probes, which simply rely on matches between queries and metagenome data in diagnosing oomycete and fungal plant diseases.

By amplifying certain DNA regions, the PCR method may identify diseases like bacteria, viruses, and fungus. The drawback of the approach is that the search is quite selective since one base their study on which pathogen is most likely to be present based on certain symptoms. NGS eliminates the need for a prior decision because it can directly identify all possible pathogens. This rapidity is a significant asset in a sector where time is money. The same principle applies to cultivation: the longer something is developed, the longer it takes to battle disease. All parties in the supply chain benefit from rapid diagnostics since they may help producers, importers, and exporters save much money. The disadvantage of this strategy is the time and effort required to generate and assess a large number of sequences.

5.18.4 Disease Diagnostics Kits

Biotechnology has made it feasible to develop diagnostic tools which assist farmers worldwide in managing various diseases that affect their crops. Thanks to improved diagnostic techniques that take up less processing time, infections may be identified with greater precision. The fast identification of DNA or proteins particular to each disease, ailment, or condition is how these diagnostics function. A qualified person must use the tools and procedures. Diagnostic kits offer a large selection of ELISA kits for plant pathogen detections with good test performance characteristics for the precise, quick, simple, and high-throughput identification of the organisms that cause plant disease. Compared to conventional diagnostic procedures and PCR-based approaches, immunological techniques based on ELISA kits provide several benefits. A range of ELISA-based rapid test strips with obvious colour change indicators is now readily available due to the usage of lateral flow devices (LFD), which are designed for on-site, accurate, and quick diagnosis of plant diseases by untrained workers.

5.19 ELISA (Enzyme-Linked Immunosorbent Assay) Kits

The ability of an antibody to recognize a particular protein fragment or antigen linked to a plant pathogen is the basis for ELISA kits. The kits are simple to use and take around 5 min to measure sickness in the field. Additionally, they don't require specialized knowledge or pricey laboratory equipment. Several ELISA test kits are available; infections are already caused by pathogens such *Erwinia amylovora*,

Ralstonia solanacearum, *Phytophthora* sp., etc., in grains, root crops, ornamentals, fruits, and vegetables.

5.19.1 Direct Tissue Blotting

Additionally, this approach searches for plant pathogens using specific antibodies. Before introducing antibodies, samples of the diseased tissue are pressed onto specialized paper to be tested for protein content. The antibody-pathogen combination is then exposed to a dye-inducing reagent for reaction. The colour reaction shows a positive result and the presence of the pathogen in the affected tissue.

5.19.2 DNA/RNA Probes

An additional set of tools that may be used to identify plant diseases are nucleic acid (DNA/RNA) probes. These probes are nucleic acid fragments arranged like the DNA or RNA of the pathogen. Since the sequences complement one another, the probes may be utilized to identify specific diseases (Goodwin et al. 1989).

5.19.3 Squash Blot Method

The squash blot method uses a specific type of paper called a membrane to "squash" plant tissue from a plant that is suspected of having a disease. A probe that can interact with the DNA or RNA of the plant pathogen alleged to be present in the tissue is then applied to this membrane. The binding will occur when there exist complementary sequences. A colour reaction demonstrates the existence of the disease after adding several more chemicals to the membrane, which indicates that the probe and the pathogen DNA/RNA have formed a bond. Lack of a colour reaction means a bad result or the absence of sickness.

5.19.4 Use of Pocket Diagnostic Rapid Test Strips for Plant Diseases

Different lateral flow rapid test strips identify various plant pathogens. After breaking it up into small bits, place the sample in the container with the buffer and ball bearings. Shake the sample in the liquid for about a minute to break it up. While drawing liquid into the pipette, watch out for sample debris and air bubbles. To acquire reliable findings in less than 10 min, add 2 drops to the sample well of the testing device while maintaining levelness.

5.19.5 PCRD-Nucleic Acid Detection

The traditional method for confirming the presence of nucleic acid following DNA amplification in PCR is DNA agarose gel electrophoresis. PCRD offers a rapid and simple alternative to gel electrophoresis that may be finished in minutes without requiring expensive equipment, exposure to intercalating dye, or UV radiation. PCRD is a nucleic acid lateral flow immunoassay (NALFIA), which may be used in conjunction with PCR, loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), or helicase-dependent amplification (HDA). The PCRD format may be used by large throughput laboratories and small field-based laboratories.

5.19.6 Diagnostic Kits' Advantages

Quick tests that may be performed in the field in a matter of minutes allow for making judgements on the spot, which is favourable for yield since it enables the implementation of management measures earlier than if a sample were sent to the lab. A rapid test can lower the cost per sample since fewer samples must be sent to the lab, reducing the cost per sample.

5.20 Conclusions

It is now feasible to quickly and precisely identify the major genera and species of disease-causing organisms by combining contemporary, sophisticated immunological, and nucleic acid-based methods. Due to their high sensitivity and accuracy, monoclonal antibodies and PCR-based techniques can potentially displace current technologies. Thanks to NA-based methodologies, often regarded as fast pathogen detection tests, an increasing range of strategies are now accessible for addressing disease challenges that are of relevance in applied plant pathology programmes. Molecular processes may be put to use right now to advance our lab's technical capabilities and get ready for any threats. Given that these techniques are a bit challenging and time-consuming for data analysis, they must be carried out by qualified specialists.

Additionally, since the majority of these approaches do not give real-time detection, early warning systems and in-field testing are less suitable for them. Any pathogen detection methodology's limitations must be understood for optimal implementation, and NA-based procedures are no exception. Utilizing the right parameters is crucial when using NA-based tools to assure accuracy. Understanding the reliability of customary laboratory techniques and the need to accumulate several lines of evidence is also necessary for critically using such technologies. Modern, cutting-edge techniques have reproducible sensitivity and are frequently noticeably quicker than traditional techniques. Prompt assessment of fungal resistance levels may also help in the creation of successful resistance management techniques.

However, there is still a glaring knowledge deficit in this field of research since no single technique can satisfy the growing need for speedier, more efficient, reproducible, and sensitive results.

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Genetic Enhancement of Biocontrol Agent as Effective Management of Soilborne Disease

6

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Abstract

Reduction in the use of agrochemicals is regarded as a crucial task for sustainable agriculture. For decades, many microbial biocontrol agents have been proved to be a fruitful research field in development of agriculture. To meet the demand for nutritious food with more challenging resources and less use of pesticides, biocontrol agents became helpful to a large extent. The use of biocontrol agents is economic, environment-amicable and preventive for soilborne pathogens. However, practical application and commercialization of biocontrol agents are yet confined owing to less consistency in the field level performance. By employing biotechnological tools like, genomics and genetic engineering, performance of BCA could be enhanced to provide cost-effectiveness and efficacy demanded by farmers. The current chapter has presented different biotechnological approaches for genetic enhancement of BCAs to manage soilborne diseases effectively.

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Keywords

Biocontrol agent · Soilborne disease · Protoplast fusion · Mutagenesis · Transformation · Genome shuffling · Genetically modified organisms

6.1 Introduction

Upcoming future needs up to 70% of more food to feed approximately 9 billion population till 2050 for which food protection is an utmost requirement (www.fao.org). Despite the significant achievement in plant breeding and disease management practices, losses due to pathogen and pests remain one third of the crop produce. Pathogens lessen the production and quality of food, feed and fibre, either before or after harvest. Most post-harvest pathogen generates toxins that can cause illness to consumers. Moreover, plant disease could destroy natural ecosystems, aggravating ecological concerns resulting from habitat shrinkage and improper land management. Various strategies may be utilized to prevent, reduce or manage plant diseases. Besides good agronomic practices, farmers frequently depend on agrochemicals. Many studies have shown agrochemical usage to protect crops and fulfil population needs but with severe pollution and land degradation as a side effect. Additionally, the raising health awareness among the people related with the development of resistance to pathogens due to overuse of the chemicals also leads to the limited usage of chemicals in crop protection. At present, there are strict regulations on the use of agrochemicals, as well as social influence to obviate the most hazardous chemicals from the market. In this context, the application of biocontrol agents (BCAs) proves to be a potent microbiological approach to maintain integral plant disease management (IDM). Biocontrol agents provide long-term positive impacts on the field in an environment-friendly manner. They control disease-causing pathogens without affecting flora and fauna and increase the soil fertility as well.

Microbial biocontrol agents hamper pathogen growth and development by following one or complex array of many direct and indirect mechanisms. Direct modes are competition, antibiosis and parasitism. The competition for space and nutrients occurs between two hosts while antibiosis involves the secretion of metabolites (enzymes like chitinases, glucanases and compounds like terpenoids and siderophores) which inhibit growth of pathogenic microorganisms (Cao et al. 2018). Parasitism is the act of biocontrol agents (producing pectinase and cutinase) to infect pathogen, thereby inhibiting its activity (Elad and Kapat 1999).For instance, *Sarocladium oryzae* produces an antifungal agent cerulenin, which helps to inhibit metabolic pathways of biosynthesis of fatty acid of yeast and fungi (Omura 1976). In indirect mode, BCA can also show its potential via interacting plants and inducing resistance or priming plants without any direct contact with the targeted pathogen. Several microbial species and strains have been evaluated for their expertise in controlling soilborne pathogens. Moreover, biocontrol companies register the BCA in the form of living microorganisms and antimicrobial metabolites with or without living cells.

6.2 Biotechnological Strategies to Unravel/Decipher the Genetic Basis of Specific Mechanism of Action

Enhancement of the genetic power that lies within biocontrol agents to manage plant diseases necessitates, at very first step, identification of the key players behind it. To investigate about the molecular strategies deployed by biocontrol agents for modulating plant disease response, it is important to identify and functionally characterize the genes and pathways involved. Though much case studies are not reported to expose the molecular players in BCA for managing harmful soil microorganisms, a range of biotechnology and bioinformatics tools are available out there to shed light on these aspects.

6.2.1 Homology-Based Search

The genes present over different organisms with high sequence similarity are believed to belong to the same family and having similar or closely related functions. They are named as homologs. Homologs with a particular function or trait of interest are widely used to mine gene(s) coding for the same protein(s) not reported earlier in the organism under the study (Cui et al. 2007). BLAST is a common and robust bioinformatic tool for homology searching based on sequence similarity. Predicted exon/CDS/ORF are searched against non-redundant protein database, e.g. NCBI and SwissProt. Also, known gene from the available database of closely related organism is selected to be used as query against the genome or CDS sequence library of the subject organism to identify gene(s) with same putative function along with their predicted cellular location, other structural features and interaction with other proteins. There are many tools available online, each with own features and advantages (Chao and Zhang 2009), for this purpose. All these tools can be used to unveil the model pathway recruited by biocontrol agents against harmful soil microorganism. The knowledge can be utilized in the future to make them genetically more empowered towards the same. For example, as genome sequencing and analysis of *Bacillus subtilis* (gram-positive beneficial soil microbe) has been done thoroughly (Kunst et al. 1997; Leal et al. 2021), the information can be leveraged for digging out genomic information of other gram-positive beneficial soil microbes, specifically of the same genera, based on homology search.

6.2.2 Forward Genetics Approach

Forward genetics approach is one of the approaches of determining genetic basis of the phenotype or trait of interest. It progresses through creating untargeted mutation (s) followed by searching for individual with alteration in target trait. This approach is mainly suitable for easily identifiable morphological trait (Raingam et al. 2018). If the mutations get to disrupt the gene controlling the target trait, it gets reflected in the phenotype. Thus, it paves the way to the discovery of the underlying gene.

Identification of plant colonization gene in *Pseudomonas fluorescens* have been carried out using forward genetics screening which can be deployed to track the genes in biocontrol agents involved in managing soilborne diseases (Cole et al. 2017; Dekkers et al. 1998). The approaches used for incorporating mutations are: a. point mutation using chemical mutagens and b. insertional mutations using T-DNA or transposons (for loss-of-function) or through activation tagging (for gain of function).

While deploying point mutations is much easier than insertional mutation, its main setback lies into recovering the information about its location. Map-based cloning (MBC), a cumbersome biotechnology technique, was exploited for this purpose before the advent of NGS technology. In the era of NGS technology, it has become more automated and still involves whole genome sequencing and analysis (Moresco et al. 2013).

Insertional mutation is created by inserting a stretch of foreign DNA into the genome to modulate the expression of the internal gene which is manifested as abnormality in phenotype. Insertional mutagenesis approaches use entrapment tagging technology to discover the gene disrupted in the event. It uses different types of traps such as gene trap, promoter trap or enhancer trap to actualize the effect (Ram et al. 2019). Though applying insertional mutagenesis is of a good amount of scientific effort, ease of recovering information about flanking site is the important advantage over point mutation.

6.2.3 Reverse Genetics Approach

Reverse genetics approach is employed to unravel the function of a gene by analysing the phenotypic effects of specific gene. The use of reverse genetics approach in soil microbes has already been shown by Melnyk et al. (2019) and Beskrovnaya et al. (2020) to address molecular mechanism behind different biological process, e.g. pathogenesis and induced systemic susceptibility, respectively, in *Pseudomonas* spp. As opposed to forward genetics, it progresses through engineering a particular genetic sequence to verify its phenotypic effect, thereby functionally characterizing it. The approaches under reverse genetics are less time-consuming and more targeted than those of forward genetics. This popular strategy uses different tools such as, to name a few, targeted insertional mutagenesis, RNAi, TILLING and lately gene editing or genome editing.

RNAi or RNA interference is the technology of silencing gene(s) by introducing dsRNA. The exogenously introduced antisense strand, accompanied with RISC protein complex, recognize complementary sense strand of endogenous RNA. Upon sensing the dsRNA, host cell starts the procedure of degrading the RNA and thereby blocks the production of the corresponding protein. TILLING (Targeting Induced Local Lesions IN Genome) is another reverse genetics technique which combines induced mutation with PCR detection of mutation in target gene. Major advantage of TILLING is it waives off the requirement of transformation.

Genome editing involves making alteration in a precise position in the genome. In spite of the presence of ZFN (zinc finger nucleases) and TALENs (transcription activator-like effector nucleases) to mediate genome editing, genome editing approach got the real popularity with the advent of more advanced CRISPR (clustered regularly interspaced short palindromic repeats) technique. The biggest advantage of this technique is only a 20 nt stretch of sequences, named as sgRNA and designed from the target gene, is enough to guide the Cas9 protein to make the specific cleavage disrupting the target gene.

Though RNAi ruled the field for the past decades, CRISPR/Cas, the most robust gene editing or genome editing technique, has become the strategy of choice worldwide for executing reverse genetics approach since it stepped into the field. With its power of mediating precise and customizable location of action within a gene or regulatory region, scientists can go beyond a gene and up to nucleotide level to assign its role.

6.2.4 Transcriptomics Study

Transcriptome represents the mRNA pool present in a tissue under a given condition. Any external or internal stimuli in an organism prompt suitable molecular changes in order to react to the stimuli which, in turn, get reflected in mRNA pool. Differential display PCR, microarray technique and RNAseq are the approaches to study the transcriptome pool quantitatively. DD-PCR and microarray is based on nucleic acid hybridization technique (Mong et al. 2002), while RNAseq is the advanced technique which couples quantitative measurement with the sequencing of mRNA. These techniques compare the transcriptomes to determine the changes in the transcript profile in a specific tissue of the target organism under a specific situation.

Interrogating the expression pattern of genes by inspecting the transcriptome of the target organism under a particular natural or imposed condition is another way to speculate the function of a gene and its involvement in specific mechanism of action. The transcriptome pool of biocontrol agent effectively interacting with harmful soil microorganism can give sufficient clues about the genes engaged ameliorating the soilborne diseases. This has been demonstrated by Perazzolli et al. (2016) when their study about transcriptome analysis of soil microbial community revealed upregulation of defence response in the presence of biocontrol agent *Trichoderma atroviride*.

6.2.5 Whole Genome Sequencing

WGS of first microbe was carried out in 1995 as *Haemophilus influenzae* with 1.83 Mb genome size was sequenced using shotgun sequencing approach. Since then, sequencing technologies have improved enormously through almost 3 decades making genomics study more straightforward.

Sequencing of whole genome gives a holistic view about the genetic makeup and helps the scientists to comprehend the special features lying under the genome sequences. This strategy exposes the alteration of sequences, insertion and deletion events, SNPs along with their position at the same time in the of the organism in question and thereby easily shed light on the functional aspect of the gene or other regulatory region (Raingam et al. 2018).

6.2.6 Cloning and Characterization

The master approach to characterize one or more genes at a time is cloning and transformation of the same into a suitable host organism. It involves cloning of a putative gene into a suitable vector and getting it expressed into a compatible host to monitor and validate the function of the cloned gene(s). Genes identified by other techniques are often subjected to cloning for final authentication about its role in the host system. Characterization of different genes involved in plan growth promotion in a range of beneficial soil microbes through cloning has been reviewed by Hayat et al. (2010). In a BCA system, the gene along with its regulators and related interactors, once identified, can be fine-tuned to make the host system more efficient in suppressing the corresponding microbial population present in the soil.

6.3 Genetic Enhancements of Biocontrol Attributes/Mechanism/Activity

So far, many microorganisms have been isolated and investigated for their biocontrol potential. However, a few limitations restrict their performance, e.g. poor survivability of inoculant in the field, less production of desirable metabolites, etc. Modern agriculture demands a biological control agent against a wide array of pathogens having enhanced antagonistic potential, improved antifungal/antibacterial activities, increased host colonization ability and tolerance to stress conditions. Biotechnology offers various means to enhance the genetic potential of a biocontrol agent for effective disease management. Genetic enhancement could be achieved by protoplast fusion, mutagenesis (directed and random), transformation, genome shuffling, etc.

Genetic modification via transformation involves vector-mediated transfer of specific genes in a wild strain of BCA to improve its effectiveness of biocontrol activity. The desired DNA insert containing vector is designed and introduced into a suitable host to multiply and produce proteins. *Trichoderma* spp. produce cell wall-degrading enzymes which are greatly attributable to its biocontrol activity. To increase the chitinase activity during biocontrol, *ChiV* gene was transformed into *Trichoderma harzianum* strain and results showed a higher level of pathogen suppression (Yang et al. 2011). Another technique, mutagenesis creates genetic variants of BCA by random mutation, directed evolution, transposon mutagenesis and site-directed mutation method. In random mutagenesis (classic method),

mutation is induced in the genome of target microbe by exposing mutagens (physical and chemical) followed by screening for desired phenotype. Random mutagenesis has offered many successes; however, it is generally impeded for the reason that, in addition to desired mutation, many unanticipated mutations that could possess an adverse impact on performance are inserted. Abbasi and co-workers (2016) created random mutagenesis by using gamma radiation in *Trichoderma harzianum* to enhance antagonistic ability. UV irradiation elevated phytase activity *Thermomyces lanuginosus* in comparison to wild strain. Biocontrol agent can also be improved by deletion or suppression of unwanted characteristics using transposon mutagenesis. Site-directed mutagenesis creates targeted, specific changes into DNA sequences. Protoplast fusion is an outstanding technique of genetic recombination by which beneficial attributes of different promising strains can be merged in a single hybrid. It overcomes the crossing barrier, thereby permitting the formation of interspecific as well as intergeneric hybrids. In *Trichoderma reesei*, two intra-strain protoplast fusants exhibited improved carboxymethyl cellulase activity than parental strain PTr2 (Prabavathy et al. 2006). Investigations have been done for improving the efficacy of BCA by combining protoplast fusion with other techniques such as mutagenesis, etc. Hatvani et al. (2006) tried to improve multiple-fungicide resistance in UV-induced mutants of *Trichoderma* strains. Genome shuffling, the latest non-recombinant technique, allows combinatorial recombination among phenotypically selected genotypes by recursive recombination/protoplast fusion, without the knowledge of genome sequence of target strains (Zhang et al. 2002). It is a quick and efficient approach for producing potential BCA strain. The genetic potential of *Streptomyces melanosporofaciens* EF-76 were improved by protoplast fusion or genome shuffling. The improved fusants produced broad range of secondary metabolites to control diseases in potato (Clermont et al. 2011). Côrtes and co-workers (2021) have improved a fungal BCA, *Sarocladium oryzae*, by the construction of a mutagenic library followed by genome shuffling and a high-throughput screening technique.

6.4 Improved Biocontrol Properties

The application of synthetic microorganisms yields inconsistent results due to the competition in between the introduced and indigenous microbial community. The use of synthetic microbial formulations leads to displacement of indigenous microbiota for disease management. However, application of single or a consortium of microbiota with efficient utilization of suppressive activity, optimizes the interplays among introduced synthetic and indigenous microbial metabolic activities (Olorunleke et al. 2015; Fujiwara et al. 2016). Such interplay leads to identification of microbial clicks which help to manage soilborne pathogens via different mechanisms including antibiosis, competition, parasitism and induced resistance. Microbial clicks are an interplays between introduced synthetic microbiota (ISM) and indigenous microbiota (IDM). IDM is the interaction between pathogenic microbiota (PM) and non-pathogenic microbiota (NPM). The NPM includes

endosphere (NEdM), rhizoplane (NRpM), rhizosphere (NRzM) and bulk soil (NBkM) microbiota. It has reported that positive outcome of ISM depends upon compatibility, loading capacity and priority effects (Verbruggen et al. 2013). Pirttilä et al. (2021) have reviewed in details in their study how to develop new potent microbial strains to develop an improved biocontrol agent. Niu et al. (2020) have reviewed the role of microbial interplays within multiple strain biological control agents (MSBCA) impact on soilborne plant disease and their potential to be significantly important BCAs in their review. Tables 6.1, 6.2 and 6.3 have showed different mechanisms of biocontrol agent.

6.4.1 Competition, Colonization and Growth Promotion

Pathogen and introduced biocontrol agent compete for the availability of space and nutrients for their colonization with plant. It has been indicated that non-pathogenic plant-associated microorganism generally protects the plant by quick colonization and accordingly debilitating the limited accessible substrates with the goal that none are accessible for pathogen to develop (Zamioudis and Pieterse 2012). Colonization of microorganisms in roots is accomplished by development, chemotaxis and motility. Adesina et al. (2009) observed that *Pseudomonas jessenii* RU47 protect lettuce from bottom rot disease caused by *R. solani* by effective and reliable concealment. Kakraliya et al. (2020) reported that *Trichoderma harzianum* acts as BCA to prevent collar rot disease of elephant foot yam by 80–85%. Fujiwara and co-workers (2016) observed that consortium of *Sphingopyxis* sp. TBD181, *Bosea* sp. TBD101, *Kaistia* sp. TBD58, *Brevibacillus* sp. TBD179, *Sphingopyxis* sp. TBD84, *Cupriavidus* sp. TBD162 and *Ancylobacter* sp. TBD132 inhibits the development of *Fusarium oxysporum* f. sp. *conglutinans* (FOC), a fungal phytopathogen of crops. Soilborne wilt diseases is another serious problem caused by *Verticillium dahliae* in strawberry and tomato (Berg et al. 2005) and *Alternaria solani* in tomato (Babu et al. 2015). Niu et al. (2020) observed that efficacy of BCAs depends upon application methods, strain specificity and timing of application. Rahman et al. (2021) studied the efficacies of BCAs, *Bacillus subtilis* (Serenade) and *Gliocladium catenulatum* (Prestop), to a resistant rootstock in suppressing tomato wilt disease. They found that *Bacillus subtilis* (Serenade) could be used as a BCA as it showed better disease suppression and improved yield. Barka et al. (2000) reported in their study that grapevine plantlets, inoculated with beneficial microbes, grew faster, became sturdier and develop better root system. Kavino et al. (2007, 2010) observed that inoculation of endophytic *Pseudomonas* and *Bacillus* species in banana plantlets show improved physiological attributes and vegetative growth and strong resistance against bunchy top diseases. In tomato, *Fusarium oxysporum* f. sp. *radicis-lycopersici* causes root and crown disease. Baysal et al. (2008) reported that *B. subtilis* strain EU07 reduced disease incidence by 75% and could be exploited as a potential BCAs for tomato root and crown disease. Prasad et al. (2020) found that *Fusarium oxysporum* f. sp. *ricini*, *Macrophomina phaseolina* and *Aspergillus niger* were reported as seed- and soilborne pathogens of groundnut and safflower

Table 6.1 Biocontrol mechanism of bacterial and fungal BCAs against different pathogen

Diseases/pathogen	Biocontrol agents	Mechanisms involved (hypothesized or demonstrated)	Benefited host	References
Sclerotium rolfsii, *S. sclerotiorum*, *R. solani*, *F. solani* and *penicillium* sp.	*B. Amyloliquefaciens* PGPBacCA1	Antibiosis	Common bean seeds	(Torres et al. 2017)
Bacterial wilt/brown rot (*Ralstonia solanacearum*)	*Bacillus subtilis* and *Paenibacillus macerans*	Induced systemic resistance	Potato	(Aliye et al. 2008)
Scab (*streptomyces* spp., mainly *Streptomyces scabiei*)	*Streptomyces bacteriophage*; non-pathogenic *streptomyces*	Cell lysis; antibiosis, competition	Potato	(McKenna et al. 2001; Hiltunen et al. 2009)
Fusarium dry rot (*fusarium roseum and fusarium oxysporum*)	*Bacillus* spp.	Antagonism	Potato	(Kotan et al. 2011)
Late blight/mildew (*Phytophthora infestans*)	*Pseudomonas koreensis* or its biosurfactant	Antagonism	Potato	(Hultberg et al. 2010)
Rhizoctonia black scurf and stem canker (*rhizoctonia solani*)	*Trichoderma harzianum*	Competition, induced systemic resistance	Potato	(Gallou et al. 2009)
Heterobasidion annosum	*Phlebiopsis gigantea*	Competition for resources	–	(Pertot et al. 2015)
Pythium spp., *R. solani*	*Pseudomonas* spp.	Production of antibiotics, siderophores, volatiles	–	(Pertot et al. 2015; Howell and Stipanovic 1980)
Species of *Alternaria*, *botrytis*, *fusarium*, *Gaeumannomyces*, *Ophiostoma*, *Phoma*, *Pseudocercosporella*, *pythium*, *sclerotinia* and *sclerotium*	*Pythium oligandrum*	Hyperparasitism	–	(Pertot et al. 2015)
Species of *fusarium*, *rhizoctonia*, *phytophthora*, *pythium*, *Phytomatotricum*, *Aphanomyces*, *Monosprascus*, *armillaria*, *sclerotinia*, *verticillium*, *Geotrichum*	*Streptomyces* spp.	Mycoparasitism	–	(Pertot et al. 2015)

(continued)

Table 6.1 (continued)

Diseases/pathogen	Biocontrol agents	Mechanisms involved (hypothesized or demonstrated)	Benefited host	References
Species of *rhizoctonia*, *fusarium*, *Alternaria* and *Colletotrichum* as well as oomycetes, such as *pythium* and *phytophthora*	*Trichoderma* spp. (*T. Atroviride*, *T. asperellum*, *T. harzianum*, *T. viride*, *T. gamsii and T. polysporum*)	Competition, resistance and hyperparasitism	–	(Pertot et al. 2015)
Pythium spp., *fusarium* spp., *rhizoctonia solani, aspergillus flavus*	*Bacillus* spp. (*B. subtilis*, *B. amyloliquefaciens*, *B. firmus* and *B. pumilus*)	Competition, direct antibiosis, induced resistance	–	(Pertot et al. 2015; Shafi et al. 2017)
Sclerotinia sclerotiorum and *S. trifoliorum*	*Coniothyrium minitans*	Lysis by chitinase and β-1,3 glucanase	–	(Pertot et al. 2015)
Verticillium dahliae, *R. solani* and nematodes	*Purpureocillium lilacinum* QLP 12 (previously *Paecilomyces lilacinus*)	Parasitism	–	(Lan et al. 2017)
Clubroot disease *Plasmodiophora brassicae*	*Lysobacter capsici* ZST1–2, *L. antibioticus* 13-B-1, *L. antibioticus* 6-B-1 and *L. antibioticus* 6-T4	Antibiosis	Chinese cabbage	(Fu et al. 2018)
Plasmodiophora brassicae	*B. subtilis* NCD-2	Antibiosis	Chinese cabbage	(Guo et al. 2019)
R. Solanacearum	*B. cereus*	Niche exclusion	Eggplant	(Achari and Ramesh 2018)
Xanthomonas campestris, *S. sclerotiorum*	*P. viridiflava*	Antibiosis and ISR	Canola	(Romero et al. 2019)
F. solani	*B. Amyloliquefaciens* (GB03) and *microbacterium imperial* (MAIIF2a)	Antibiosis	Cassava	(Freitas et al. 2019)
P. Syringae pv. *Actinidiae*	*Pseudomonas* sp.	Antibiosis	Kiwi fruit plant	(Wicaksono et al. 2018)
Fusarium graminearum	M6 *Enterobacter* sp.	Hyperparasitism	Finger millet	(Mousa et al. 2016)
	B. Velezensis	Antibiosis	Tomato	(Gao et al. 2017)

A. solani, *Botrytis cinerea*, *Valsa Mali*, *Monilinia fructicola*, *F. oxysporum* f. sp. *capsici* and *C. lindemuthianum*				
Candidatus Liberibacter asiaticus	*B. subtilis* L1–21	ISR	Citrus	(Munir et al. 2020)
Pyricularia grisea	*P. aeruginosa* and *P. pseudoalcaligenes*	ISR	Paddy	(Jha 2019)
F. Oxysporum, *Curvularia* sp., *Alternaria* sp. and *sclerotinia homoeocarpa*	*B. Amyloliquefaciens*	Antibiosis	Brown millet	(Verma and White 2018)
R. solani	*S. Maltophilia* H8, *P. aeruginosa* H40 and *B. subtilis* H18	ISR	Cotton	(Selim et al. 2017)
Macrophomina phaseolina	*K. pneumoniae* HR1	Antibiosis, ISR	*V. mungo*	(Dey et al. 2019)
P. Capsici	*P. Putida* BP25	Antibiosis	Black pepper	(Agisha et al. 2019)
S. Cepivorum	*B. siamensis*	Antibiosis, ISR	Ginger plant	(Wang et al. 2020)
F. Fujikuroi	*B. oryzicola* YC7007	ISR	Rice	(Hossain et al. 2016)

Table 6.2 Some antibiotics produced by BCAs and their role in controlling plant diseases by antibiosis

Sources	Antibiotics	Plant disease	References
Agrobacterium radiobacter	Agrocin 84	Crown gall	(Kerr 1980)
Bacillus amyloliquefaciens FZB42	Bacillomycin, fengycin	Wilt	(Koumoutsi et al. 2004)
Bacillus cereus UW85	Zwittermicin A	Damping-off	(Smith et al. 1993)
Bacillus subtilis AU195	Bacillomycin D, aflatoxin	Contamination	(Moyne et al. 2001)
B. subtilis QST713	Iturin A	Damping-off	(Kloepper et al. 2004)
Bacillus subtilis BBG100	Mycosubtilin	Damping-off	Leclère et al. (2005)
Pantoea agglomerans C9–1	Herbicolin	Fire blight	(Sandra et al. 2001)
Pseudomonas fluorescens F113	2,4-Diacetylphloroglucinol	Damping-off	(Shanahan et al. 1992)
P. *fluorescens* 2–79 and 30–84	Phenazines	Take-all	(Thomashow et al. 1990)
P. fluorescens Pf-5	Pyoluteorin, pyrrolnitrin	Damping-off	(Howell and Stipanovic 1980)
Lysobacter sp. strain SB-K88	Xanthobaccin A	Damping-off	(Islam et al. 2005)
Trichoderma virens	Gliotoxin	Root rot	(Wilhite et al. 2001)
Burkholderia cepacia	Pyrrolnitrin, pseudane	Damping-off and rice blast	(Homma et al. 1989)

crops. They developed a novel chitosan-PEG (polyethylene glycol) (Cts-PEG) mixture comprising *Trichoderma harzianum* (Th4d) (Cts-PEG-Th) spores and observed that Cts-PEG-Th significantly reduce the disease caused by these fungal pathogens in groundnut and safflower crops. Chandra et al. (2020) reported *Pseudomonas aeruginosa* (isolate-2) from *Valeriana wallichi*, as potential PGPR and BCA against fungal pathogen: *Alternaria alternata*, *Aspergillus flavus* and *F. oxysporum*. Das et al. (2010) observed that IAA producing biocontrol strain *Bacillus subtilis* SRB28 show significant plant growth promotion of sorghum by root colonization and significantly improve root architecture. Tariq et al. (2020) reviewed and reported the utilization of bacterial taxa to increase crop production that has been evaluated previously for *Bacillus*, *Herbaspirillum*, *Actinobacteria*, *Lactobacillus*, *Paenibacillus*, *Pseudomonas*, *Serratia*, *Burkholderia*, *Acetobacter*, *Azospirillum* and *Rhodococcus*. In this way, numerous BCAs are also involved in improving plant biomass, augmenting surface area, improving root architecture, nutrient cycling, nitrogen fixation, phosphate solubilization, siderophore and IAA and HCN production, showing antagonism towards fungal pathogens (Tariq et al. 2020; Pii et al. 2015).

Table 6.3 List of bioactive compounds produced from BCAs for pathogen management

BCA	Compound	Benefitted plant host	Pathogen	References
B. Velezensis	Surfactin, iturin and fengycin		*Ralstonia solanacearum* and *fusarium oxysporum*	(Cao et al. 2018)
B. Amyloliquefaciens		*O. sativa*	*Curvularia lunata*, *F. semitectum* and *Helminthosporiumoryzae*	(Saechow et al. (2018)
B. Velezensis	Bacillomycin D and fengycin	Chilli pepper	*Botrytis cinerea*	(Jiang et al. 2018
Metschnikowia fructicola		Strawberry fruit	*Botrytis cinerea*	(Zhimo et al. 2021)
Aspergillus flavipes	3-Hydroxy-2′,4,4′,6′-tetramethoxychalocone	*N. benthamiana*	*Phytophthora* spp.	(El-Sayed and Shad Ali 2019)
Candida oleophila I-182	Rlm1 protein	Tomato fruit	*Botrytis cinerea*	(Sui et al. 2020)
Pseudomonas chlororaphis	Pyrrolnitrin and phenazines	Barley, wheat and oats	*Fusarium graminearum*	(Huang et al. 2018)
Streptomyces sp.		Wheat	*Rhizoctonia solani*	(Araujo et al. 2019)
Acremonium strictum	Verlamelin		*Erysiphe graminis* f. sp. *hordei*, *Puccinia recondita*	(Kim et al. 2002)
Trichoderma citrinoviride		Panax ginseng	*Rhizoctonia solani*, *Botrytis cinerea*, *Alternaria panax*, *Cylindrocarpon destructans*, *Phytophthora cactorum*, *pythium* spp. and *Botrytis cinerea*	(Park et al. 2018)
Rhizopycnis vagum		*Zingiber officinale* Rosc	*Rhizoctonia solani*, *Corynespora cassiicola*, *Colletotrichum acutatum*, *Phytophthora infestans*, *fusarium oxysporum*, *sclerotium rolfsii*	(Anisha et al. 2018)
Trichoderma asperellum		Lettuce leaves	*Corynespora cassiicola*, *Curvularia aeria* (leaf spot fungi)	(Baiyee et al. 2019)
Penicillium spp.		*Musa* spp.	*Fusarium oxysporum*	

(continued)

Table 6.3 (continued)

BCA	Compound	Benefitted plant host	Pathogen	References
				(Ting et al. 2010)
Pseudozyma sp.	Pep1, Cmu1, Cwh41 and Hum3	*Ustilago maydis* and *Hordeum vulgare*	*Blumeria graminis*	(Hemetsberger et al. 2015)
Pseudozyma tsukubaensis	Mycocins		*Ustilaginomycetes*	(Golubev et al. 2006)
T. virens	Sm1 and 18 mer peptaibols	Cucumber	*P. Syringae*	(Djonovic et al. 2006)
Muscodor yucatanensis		*Monarda citriodora* (lemon mint)	*Sclerotinia* sp. *Colletotrichum capsici*, *aspergillus flavus*, *aspergillus fumigatus*	(Katoch and Pull 2017)

Genetic engineering of BCAs with specific genes upgrades the colonizing ability, uniformity in performance and spectrum of activity. Raaijmakers et al. (1995) reported that expression of ferric siderophore receptor *pupA* gene of *Pseudomonas putida* WCS358 in strain WCS374 increased the competitiveness of WCS374 against WCS358 when both strains were co-inoculated. Dekkers et al. (2000) reported that increasing the copy number of the *Pseudomonas fluorescens* WCS365 site-specific recombinase gene *sss* in F113 and WCS307 strains increased the competitive colonization ability of the recombinant strains against *Fusarium oxysporum* f.sp. *radicis-lycopersici*, on tomato root tips. Mark and co-workers (2006) reported a modified IAA overproducing strain *P. fluorescens* CHA0 which causes DNA rearrangements and prevent BCAs from "locked in" under unfavourable state of competitive colonization show increased root yield in natural soil.

6.4.2 Antibiosis

Antibiotics execute pathogens legitimately. BCAs produce antibiotic in the specific micro niche of roots for successful control of phytopathogenic diseases (Lugtenberg and Kamilova 2009). Liu et al. (2007) reported that mutagenesis play role in antibiotics production by the BCAs to control plant diseases. Various microbe including bacteria (2900), fungi (4900) and actinomycetes (8700) found to generate enormous quantities of antibiotics (Bérdy 2005). Ongena and Jacques (2008) studied *Bacillus*, lipopeptides, iturin, surfactin and fengycin as biocontrol metabolites. Raaijmakers and Mazzola (2012) reported antibiotic metabolites, DAPG, pyrrolnitrin and phenazine from *Pseudomonas*. In another study, *Pseudomonas* spp. produce 2,4-diacetylphloroglucinol, cyanide, siderophores, pyoluteorin, phenazines and pyrrolnitrin as antimicrobial compounds (Compant et al. 2010). Numerous antifungal antibiotics, such as heptelidic acid, gliovirin, gliotoxin, viridin, viridiol and valinotrocin, have been produced by the biocontrol fungus, *Gliocladium virens*. Singh et al. (2005) demonstrated that antibiotic gliotoxin effectively control *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Rhizoctonia bataticola*, *Pythium debaryanum* and *Pythium aphanidermatum.* Vinale et al. (2009) reported that *T. harzianum* strains T22 and T39 produce different antibiotics such as T22 azaphilone, T39 butenolide, 1-hydroxy-3-methylanthraquinone, harzianolide, 1,8-dihydroxy-3-methyl-anthraquinone and harzianopyridone. They observed that T22 azaphilone and harzianopyridone inhibited the growth of the pathogens *Leptosphaeria maculans*, *Phytophthora cinnamomi*, *Botrytis cinerea*, *Rhizoctonia solani* and *Pythium ultimum*. Lahlali et al. (2013) observed the suppression of clubroot disease of canola by the biofungicide Serenade occurred via antibiosis and induced systemic resistance. Alternatively, the incidence of stem and root rot diseases of cucumber were decreased by the application of Prestop at seeding stage (Rose et al. 2004). Berg and associates (2005) showed the usage of different plant extracts, bio-fumigation and BCAs: *Trichoderma*, *Serratia* and *Pseudomonas* can effectively reduce wilt symptom and growth of fungal microsclerotia. D'aes

et al. (2011) found that *Pseudomonas* CMR12a produces phenazine, orfamides and sessilins and results in suppression of *Rhizoctonia solani* which causes root diseases in Chinese cabbage and beans.

Actinobacteria are known as largest producer of antibiotics and natural bioactive metabolites (70%) and thus are potential BCAs (Chen et al. 2018). *Streptomycetes* due to their diverse metabolic activities are attaining interest in agriculture as BCAs, plant growth promoters, possess intense antagonistic effects against phytopathogens and control soilborne diseases (Viaene et al. 2016; Dias et al. 2017). Yun et al. (2018) reported 80% of antimicrobial metabolites reported from actinobacteria were obtained from *Streptomyces* sp. only. Zheng et al. (2019) isolated *Streptomyces diastaticus* FJAT-31547 from tomato rhizospheric soil which antagonist *Fusarium oxysporum* and *Ralstonia solanacearum* growth. They reported that n-hexadecanoic acid as principal component responsible for this antimicrobial effect. The application of crude extract show that disease incidence was reduced by 80.59% and 76.92% for *Fusarium* and bacterial wilt, respectively, with promising growth-promoting effect on tomato plant in pot trail. González et al. (2020) have reported that *Epicoccum purpurascens* extract inhibited the growth of *F. oxysporum* f. sp. *melonis*, *Neocosmospora falciformis*, *F. solani* f. sp. *cucurbitae* and *N. keratoplastica* via antibiosis and thus decreases the incidence of soilborne fungal diseases like carbonaceous rot, collapse. Begum et al. (2008) found that *Pseudomonas aeruginosa* show the property of antibiosis and hence malformed mycelia of *Colletotrichum truncatum* which causes anthracnose disease in soybean. *Plasmodiophora brassicae* is another fungal pathogen reported to cause clubroot disease in cruciferous plants by causing root infection and impair plant growth and developments. Ahmed et al. (2020) reviewed how endophyte-mediated BCAs achieved the exclusion of pathogen via niche and nutrient competition, by producing anti-microbial compounds and inducing host defence responses.

Mark et al. (2006) genetically engineered the *P. fluorescens* F113 and CHA0 and *P. putida* WCS358 and observed increased production of 4-diacetylphloroglucinol (PHL) and pyoluteorin (PLT). *P. fluorescens* CHA0 show overproduction of PHL and PLT, inhibiting the growth of *Sinorhizobium meliloti* and nodulation in alfalfa. Glandorf (2019) and Shelake et al. (2019) studied that utilization of CRISPR/Cas system for genome editing of microorganisms can be exploited to fight against phytopathogen-induced plant diseases. Muñoz et al. (2019) recently proposed that creation of non-pathogenic strains of fungal pathogens by CRISPR/Cas could be a promising approach for biocontrol.

Genome shuffling (GS) is a nonrecombinant DNA technology for rapid phenotypic improvement. It allows recursive combinatorial recombination among parental genotypes for any desired traits, such as production of bioactive metabolites against environmental stresses or microbial pathogens (Magosha et al. 2018). Different studies have been carried out to control soilborne plant diseases via antibiosis mechanism by exploiting GS technology to genetically improve bacterial BCAs such as *Bacillus subtilis*, *Streptomyces melanosporofaciens* and *Streptomyces bikiniensis* with enhanced antagonism against fungal phytopathogens such as *Fusarium oxysporum* f. sp. *melonis*, *Phytophthora infestans* and *Fusarium oxysporum*

f. sp. *cucumerinum*, respectively (Chen and Chen 2009; Clermont et al. 2011; Zhao et al. 2014). *Burkholderia glumae* are known to cause panicle blight disease in rice plants. *Streptomyces* A20 produces streptothricins D, E and F with antimicrobial activity proved to be potential biocontrol agents against panicle blight disease and efficiently protect rice plants from *Burkholderia glumae* infection. It also forms strong colonization with root hairs of rice plants and show plant growth promotion (Suárez-Moreno et al. 2019). *Sarocladium oryzae* BRM 6461, a model fungal BCA, are well known to produce antifungal agent cerulenin. De Carvalho de Carvalho BarrosCôrtes et al. (2020) reported for the first time the pipeline to genetically improve fungal BCAs by GS technique. They have constructed a parent library using mutagenic agents, further genome shuffling and high-throughput screening. Superior mutant *S. oryzae* GS4–03 was selected after GS, showing antagonism, enhanced cerulenin production, UV-B irradiation tolerance and heat tolerance. It prevented the mycelial growth of *Rhizoctonia solani* and *Sclerotinia sclerotiorum* and suppressed white mould and root rot disease.

Voisard et al. (1989) created a transgenic strain *Pseudomonas fluorescens* P3 with HCN biosynthesis operon *hcnABC* of *Pseudomonas protegens* CHA0 for improved control of tobacco black root rot disease. Fenton et al. (1992) reported that transgenic *Pseudomonas* sp. strain M114 (pCU203) containing recombinant plasmid pCU203, with 2,4-diacetylphloroglucinol (DAPG) biosynthetic gene of *Pseudomonas* sp. F113 results in enhanced control of sugar beet damping-off disease. Leclère et al. (2005) observed that substituting the native promotor of the *mycosubtilin* operon in *Bacillus subtilis* strain ATCC 6633 with a constitutive promotor produced the recombinant strain BBG100 with enhanced mycosubtilin production and results in improved control of *Pythium aphanidermatum* infection in tomato. Ligon et al. (2000) showed overexpression of pyrrolnitrin biosynthesis genes (*prnABCD*) in *P. fluorescens* BL915 either by adding additional plasmid-borne copies of *gacA* or through replacing the native promoter of *gacA* with stronger *Ptac* promoter and results in enhanced control of *R. solani* infection in cucumber.

6.4.3 Lysis (Mycoparasitism)

Parasitism is the close relationship between two different organisms in which the parasite receive benefit in any form such as nutrients, food, shelter or protection from its enemy from the other organisms, i.e. host on which it is depending. Hyperparasitism is the example of interaction between plant and plant pathogens. The hyperparasitic interactions between plant and fungi are termed as "mycoparasitism". The first level of mycoparasitism is the chemotaxis of BCAs towards the target pathogen. The second level is recognition of specific interaction between lectin of pathogen or carbohydrate receptors on the surface of BCAs. The third level is adherence by cell wall followed by degradation via chitinases and β-1,3-glucanase enzymes (Di Pietro 1993). The final level is penetration, where the BCAs could generate structures like appressoria for penetrating the cell wall of pathogens (Chet 1987). *Bdellovibrio bacteriovorus* is a pathogenic bacterium which depends on its host and utilizes the

host cytoplasm for nutrients (McNeely et al. 2017). When the parasite does not kill the host, such kind of interaction is known as biotrophic mycoparasitism, here the parasite form haustoria like structure to invade the host cell for obtaining food and nutrient from its host fungus (Kohl et al. 2019). However, when the pathogens or the parasites kills its host cell upon invading, the host is termed as necrotrophic hyperparasites. They produce cell wall-degrading enzymes (CWDEs) such as chitinases, β-1,3-glucanases, proteases and cellulases along with different metabolites which cause the cell wall disruption and leads to cytoplasmic disorientation. These CWDEs also play important role in nutrient recycling process (Kohl et al. 2019). In a study, Sharma and Bhat (2011) observed that *Trichoderma* secretes chitinase enzyme in its micro niche, i.e. in the decomposing bark which is being hyperparasitized by *Rhizoctonia solani*. These hyperparasitic phytopathogens produce endospores such as chlamydospores and sclerotia against BCAs. But no cases of development of resistant against BCAs have been observed by any phytopathogens (Kohl et al. 2019).

Trichoderma is essentially mycotrophic and shows antagonism by mycoparasitism, competition for space and nutrients and induced systemic resistance (Atanasova et al. 2013; Guédez et al. 2009). Black sigatoka disease of banana is known to be caused by *fijiensis*. Cavero et al. (2015) reported that *Trichoderma atroviride* was found to be equivalent to fungicide azoxystrobin to control black sigatoka disease of banana in field condition via mycoparasitism. *T. harzianum* 1051 show mycoparasitism against *Crinipellis perniciosa* by producing different CWDEs chitinase, protease and N-acetylglucosaminidase (De Marco et al. 2000; De Marco and Felix 2002; De Marco et al. 2004) whereas β-1,3-glucanase displayed no phytopathogenic effect (Marco and Felix 2007). Hernandez-Leon et al. (2015) reported that *Pseudomonas* spp. produces cellulase, chitinase, proteases and β-glucanase to lyse fungal cells. Yellowing disease is common pepper (*Piper nigrum* L.) disease caused by phytopathogens *Meloidogyne incognita* and *Fusarium oxysporum. Trichoderma* spp. isolate (JI) shows mycoparasitism against *Fusarium* sp. and *Trichoderma* spp. JA and JL cause lysis in *Meloidogyne* sp. and were observed as biocontrol of yellowing disease of pepper (Mayang and Suryanti 2012). González et al. (2020) observed that *T. lentiforme* suppresses soilborne fungal diseases like carbonaceous rot caused by *F. oxysporum* f. sp. *melonis*, *F. solani* f. sp. *cucurbitae*, *Neocosmospora falciformis* and *N. keratoplastica* hyperparasitism. *T. harzianum* Tc-Jjr-02 show growth inhibition of *Colletotrichum capsici* and *Colletotrichum gloeosporioides* by damaging hyphae cell walls by mycoparasitism and prevent chilli anthracnose disease (Miftahurrohmat et al. 2021). Begum et al. (2008) reported that *T. virens* and *T. harzianum* inhibited the growth of *C. truncatum* by mycoparasitism and antibiosis resulting in coiling and penetration into the hyphae and protect soybean anthracnose disease. Expression of *chiA* gene of *Serratia marcescens* in *Pseudomonas putida* inhibits the infection of *Sclerotium rolfsii* and protect beans (Chet et al. 1993). Similarly, recombinant *Stenotrophomonas maltophilia* W81M3 or W81M4 with overproduction of an extracellular serine protease improved control of sugar beet pythium damping-off disease (Dunne et al. 2000).

6.4.4 Induced Systemic Resistance (ISR)

When the phytopathogens infect the host plant, naturally occurring BCAs helps to provide protection to the host plant. These BCAs triggers the expression of resistance gene in host by releasing biochemical stimuli which stimulates the host defence mechanism (Nega 2014). Pathogen-associated molecular pattern (PAMP) and microbe-associated molecular pattern (MAMP) are examples of the stimulus produced by plants and microorganisms, respectively, for induction of resistance (Kohl et al. 2019). Conrath et al. (2015) reported the application of non-pathogenic bacteria for acquiring induced systemic resistance (ISR) in host plant against phytopathogens. Nega (2014) observed that ethylene and jasmonic acid pathways play crucial role to operate ISR. Few examples of ISR include production of peroxidase, chitinase and β-1,3-glucanase by *Bacillus mycoides* in sugar beet (Bargabus et al. 2003), production of 2,3-butanediol in *Arabidopsis* by *B. subtilis* GB03 and IN937 (Ryu et al. 2004), production of lipopolysaccharide in *Arabidopsis* by *Pseudomonas putida* (Meziane et al. 2005) and secretion of siderophore in cucumber by *Serratia marcescens* 90–166 (Press et al. 2001). Mauch-Mani and co-workers (2017) found that to enhance the defence mechanism "priming of plant with stimuli" play an important role for future, long-lasting system and faster defence response.

Soilborne pathogens such as *Fusarium* and *Rhizoctonia* and pathogenic microbial consortia such as *Phytophthora capsici*, root knot nematodes and *Verticillium dahliae* are responsible for causing wilting in chilli (Sanogo and Carpenter 2006; Sanogo et al. 2013). Carrion et al. (2019) found that *R. solani* favours the growth of beneficial microbiota, which produces metabolites and expresses genes to provide protection to host plant.

Liu et al. *(2021) reported that application of novel biocontrol agent prepared by* attapulgite coating and *Bacillus amyloliquefaciens* FZB42 known as attapulgite-coated biocontrol agent (APBA) significantly reduced *Fusarium* root rot in a medicinal herb *Angelica sinensis* and improve soil chemical properties and alter microbial community composition in the rhizosphere. A study of Li et al. (2021) first time reported the use of *Ochrobactrum intermedium* (I-5), isolated from alfalfa rhizospheric soil, as a BCA against *Fusarium tricinctum* to inhibit alfalfa root rot disease. This strain also promoted invertase, ureases, cellulase and neutral phosphatase activity in alfalfa rhizosphere and significantly reduces the damage to the rhizosphere soil quality caused by alfalfa root rot and also inhibited the germination and growth of *F. tricinctum* up to 78.93%. White rot disease *of Allium* genus (garlic, onion) are well known to be caused by *Sclerotium cepivorum*. Ocegueda-Reyes et al. (2020) reported that *B. amyloliquefaciens* and *B. subtilis* as potential BCAs as it produces ACC deaminase, IAA, siderophores and cell free extracts show antifungal activity against *S. cepivorum.* All these attributes make these rhizobacteria a potential alternative to control of *S. cepivorum* in onion. Zaccardelli et al. (2020) found that *Bacillus amyloliquefaciens* and *B. subtilis* strains isolated from composted aromatic plant waste are potential siderophore producer and P-solubilizer and possess five antimicrobial lipopeptide genes in their genome that are proved to be

potential biocontrol agent for soilborne rocket damping-off diseases caused by *Sclerotinia minor* and *Rhizoctonia solani*. N. D. et al. (2021) have deeply reviewed that how potential bacterial and fungal BCAs and their metabolic products such as antioxidants, phenolics, secondary metabolites, crude extracts and different PGP attributes can be utilized for management of *Fusarium verticillioides* and its fumonisin in cereals.

Arbuscular mycorrhizal fungi (AMF) are known BCAs for phytopathogens such as *A. solani*, *Aphanomyces euteiches*, *Cercospora arachidicola*, *Cercosporidium personatum*, *Erysiphe graminis*, *F. solani*, *F. verticillioides*, *Gaeumannomyces graminis*, *M. phaseolina*, *P. cactorum*, *P. aphanidermatum*, *R. solani*, *S. cepivorum* and *V. dahliae* (Spagnoletti et al. 2018; Zhang et al. 2018; Mohamed et al. 2019). *Glomus clarum* and *G. deserticola* are well-known BCA for maize ear rot (Olowe et al. 2018). El-Sharkawy et al. (2018) observed that application of AMF and *Trichoderma* spp. show significant biocontrol of wheat stem rust with induction of defensive enzymes and total phenol content. The mechanisms exerted by AMF comprise direct competition for nutrients, space and colonization sites with the soilborne pathogenic fungi and also heal the damage caused by phytopathogens during disease (Vierheilig et al. 2008; Vos et al. 2014; Spagnoletti et al. 2018; Mohamed et al. 2019). Abdel-Fattah et al. (2011) reported that AMF application leads to ultrastructural and biochemical changes such as thickening of cell wall, cytoplasmic granulation, increased numbers of cell organelles, nuclear hypertrophy, accumulation of fungitoxin and activation of defence enzymes in bean plants against *Rhizoctonia* root rot infection which.

Genetically improved *Pseudomonas protegens* strain P3 with *pchCBA* gene obtained from *Pseudomonas protegens* strain CHA0 activates the salicylic acid pathway and thus ISR against tobacco necrosis virus (Maurhofer et al. 1998). Barahona et al. (2011) develop a mutant of *Pseudomonas fluorescens* F113 for the genes *sadB*, *wspR* and *kinB*, which show significantly enhanced antagonism against *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato and *Phytophthora cactorum* in strawberry due to hypermotility and better root colonization of mutant strain. Construction of genetically modified *Trichoderma atroviride* strain SJ3–4 by insertion of *glucose oxidase* (*goxA*) gene derived from *Aspergillus niger* into *Trichoderma atroviride* strain P1 under the control of the homologous chitinase (*nag1*) promotor produced the recombinant with 12–14 copies of *goxA* and thus enhances ISR against *Botrytis cinerea*, *Pythium ultimum* and *Rhizoctonia solani* in bean (Brunner et al. 2005).

6.5 Risk on Environmental Release

For the sustainable production of agriculture-based products, BCAs have played significant role in curbing pests, weeds and diseases. Simultaneously, BCAs reduce the usage of agro-chemicals. Despite all these efforts only few products are available in the market. To launch any BCA in the market, it should be registered and fulfil the guidelines and regulatory status (Alabouvette et al. 2006a, b). To resolve particular

problems associated with the application of microbial BCA in the European Union, the directive 91/414 EEC has set specific regulations before the release of any plant protection products such as plant extracts or microbes-based BCAs (Alabouvette and Cordier 2011). The directive set the principle to identifying the risk or hazards, its toxicity or negative effect on other flora or fauna biodiversity, probability of occurrence of hazard. One of the major reasons for the low acceptance of BCAs in the market is the risk factors associated with their release to the environment, plant health and biodiversity, human and animal health, safety of other living organisms, influence on soil microbial diversity and plant microbial diversity, allergies and vectoring of diseases, on the environment, including effects on non-target organisms. Besides the potency to hinder target pest, the assessment of the BCA unequivocally includes its positive impact on plant, human and environmental health.

The major risk associated with the utilization of microbial BCAs on the environment is that newly entered microbes can proliferate in the ecosystem and turn into a pest. However, Alabouvette and Steinberg (1998) reported that in the deficit of any selection pressure, introduced BCAs deriving from the natural habitat will not turn into dominant when reintroduced in the same habitat. Although no associated hazard can be considered, it is crucial to explore the fate and actions in the environment.

Behaviour of any BCAs is clearly examined in vitro at the lab scale such as plant tissue culture condition, and upon successful establishment at lab scale, they are further monitored in vivo under natural environmental conditions such as pot trail, green house condition and field trail.

BCAs are naturally present in the environment but in a very low density to control any disease and also cannot be detected in such low density. Thus, to monitor the establishment of any BCAs, some tools are designed to develop mutant strain with molecular markers, e.g. antibiotic- or fungicide-resistant gene. Edel-Hermann et al. (2009) taken a UV-irradiated mutant of Fo47 resistant to benomyl to examine their population dynamic in sterile and non-sterile soil condition for a year and found that naturally found microbes re-introduced in the environment from which it has been isolated neither vanish nor multiply beyond the native microbial community. Nevertheless, this strategy employing antibiotic- or fungicide-resistant mutants could only be utilized in a restricted space considering the risk to release mutants in the environment. Additionally, the mutation might have altered the dispersal or survival behaviour of the microbial BCAs.

Another approach to seek the fate of any delivered BCAs consists of designing a SCAR marker for a targeted microbial BCA to distinguish them from other strains of same species in the environment; *Trichoderma atroviride* strain T1 population dynamics studied under sterile and non-sterile soils condition were analogous to that obtained for Fo47. Strain T1 neither vanished nor multiplied in the non-disinfested soils (Cordier et al. 2007). Thus, based on various literature survey, it has been concluded that a soilborne microorganism re-introduced into a soil will survive but will not multiply; it will become part of the native populations of the same species.

Abbey et al. (2019) reported that crop protection industry manufactures microbial formulations containing microorganisms from diverse taxonomic groups including fungi and bacteria. Pertot et al. (2017) observed that long-term application of single microbe-based formulations leads to pathogen resistance and production-related problems. Further they observed that formulations containing microbial consortia from different taxonomic groups with diverse functionality established broad-spectrum activity against multiple pathogens.

In general, there are many ethical aspects linked with genetically modified organisms (GMOs) as biofertilizers or biocontrol agents in agriculture (Glandorf 2019). Few reports have suggested that genetic enhancement of biocontrol agents can bring alteration in the microbial community structure of the rhizosphere (Mark et al. 2006). The speculations related to release of GMOs to the environment is spread of foreign gene across the microbiomes. In addition, limiting the entry of the GMOs to the edible plant parts would need considerate analysis. The intended release of GMOs into the environment is regulated by Council Directive 2001/18/EC, which repeals Council Directive 90/220/EEC in Europe (Mark et al. 2006; Glandorf 2019). Further, genome editing via CRISPR/Cas is an indispensable tool in annotating the mechanisms of plant-microbe interaction (Shelake et al. 2019).

6.6 Conclusions

Increasing awareness regarding the agrochemicals residue among farmers has led to the use of BCAs for plant pathogen suppression. BCAs control plant diseases without harming the environment or non-target organisms. Hence, for sustainable plant protection, more emphasis should be given to search for new BCA and genetic improvement of existing BCAs. Undoubtedly, genetically improved biocontrol agents show high antagonistic activity as compared to parental strains. Expanding knowledge about molecular basis of biocontrol activity as well as the use of biotechnological tools has eased the system to develop superior BCAs. Genetically improved BCA interacts and/or competes with diverse microbial communities which greatly affect the survival and performance of introduced inoculants. Therefore, risk assessment is an important part of regulatory approval for the commercial release of such inoculants. Moreover, monitoring of released genetically modified BCA in the environment is crucial. The characterization of genetic and biological attributes of genetically modified BCA and detailed knowledge about their interaction with other environment components are essential to enhance its efficacy as well as to scrutinize their environmental risks.

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Red Root Rot Disease of Tropical Estate Forests: Pathogen Identification, Dispersal and Management

7

Abdul Gafur

Abstract

The red root rot disease is very prevalent in Southeast Asia's tropical plantation/estate forests. It has been a critical factor in the sustainability of their production. Studies have led to the identification of *Ganoderma philippii* as the main pathogen of the disease. Infection occurs mainly due to physical contact between infected and healthy tissues. Spores also contribute to pathogen dispersal. In general, disease progress remains relatively slow, but infected plants finally die. Plant resistance and biocontrol measures are critical components of the root rot's integrated disease management. A consistent, fast, and effective screening protocol has been developed to identify resistant materials. Similarly, effective biocontrol agents for the disease have been isolated. This chapter discusses pathogen identification, dispersal, and control of the Ganoderma root rot disease in estate forests in the tropics, focusing on the Indonesian experience.

Keywords

Acacia · Disease control · *Eucalyptus* · *Ganoderma* · Plantation forest

7.1 Background

The red root rot disease was in the past considered as one of the most critical factors in the production of estate/plantation forests in the tropics (Table 7.1), especially in Southeast and South Asia (Lee 2000; Old et al. 2000; Gafur 2020). In Indonesia, for

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Table 7.1 Losses caused by *Ganoderma philippii* on *Acacia mangium* at various ages

Place	Age (years)	Loss (%)	Source
Indonesia	3–5 (second rotation)	3–28	Irianto et al. (2006)
Malaysia	14	Up to 40	Lee (2000)
The Philippines	6–10	10–25	Militante and Manalo (1999)
India	9–14	~40	Mehrotra et al. (1996)

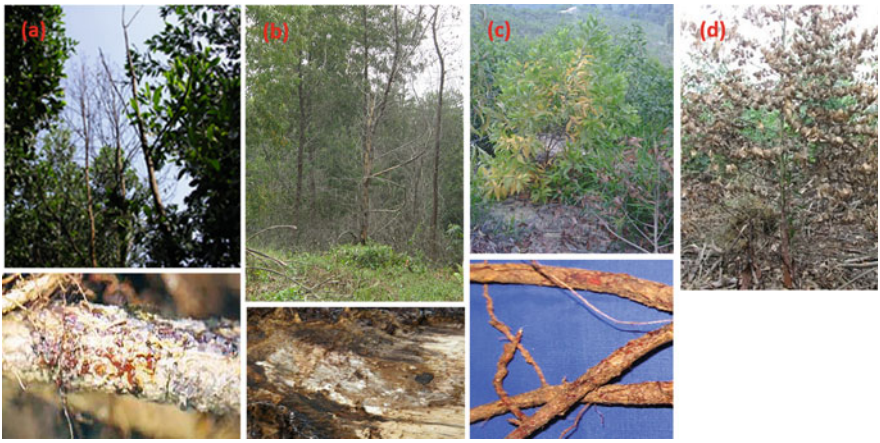

Fig. 7.1 Symptoms at various stages (top) and signs (bottom) of the Ganoderma root rot disease on (**a**) *Acacia mangium*, (**b**) *A. crassicarpa*, (**c**) *A. auriculiformis*, and (**d**) *Eucalyptus pellita* (Gafur et al. 2011a, b, 2012; Rimbawanto et al. 2014)

instance, Irianto et al. (2006) observed that the disease could potentially kill up to 28% of the *Acacia mangium* trees in second-rotation plantations in Kalimantan and Sumatra. Francis et al. (2014) revealed that the root rot disease could kill trees as young as 6 months old in second-rotation *A. mangium* and *A. crassicarpa* plantations. In Malaysia, root rot was responsible for the death of more than 40% of *Acacia* trees aged 9–14 years in heavily infected areas (Lee 2000). Losses due to the same disease were estimated to be as high as 10–25% in *A. mangium* plantations aged 6–10 years in the Philippines (Militante and Manalo 1999). A similar incident was described in India (Mehrotra et al. 1996). The disease has also been observed in commercial eucalypt species in Indonesia although occurred in lower frequencies (Francis et al. 2008; Gafur et al. 2010; Coetzee et al. 2011).

On acacias and eucalypts, symptoms of the red root rot disease include leaf yellowing and crown thinning, which usually leads to slow growth and tree wilting. According to Old et al. (2000), infected trees typically have paler, smaller, and sparse phyllodes. Diseased roots are distinguished by a wrinkled reddish-brown rhizomorph. The underside of the colonized bark has a white mottling pattern (Fig. 7.1). Mortality increases with plant age and planting rotation. Disease progress remains slow but infected plants finally die. No clear relationship between disease

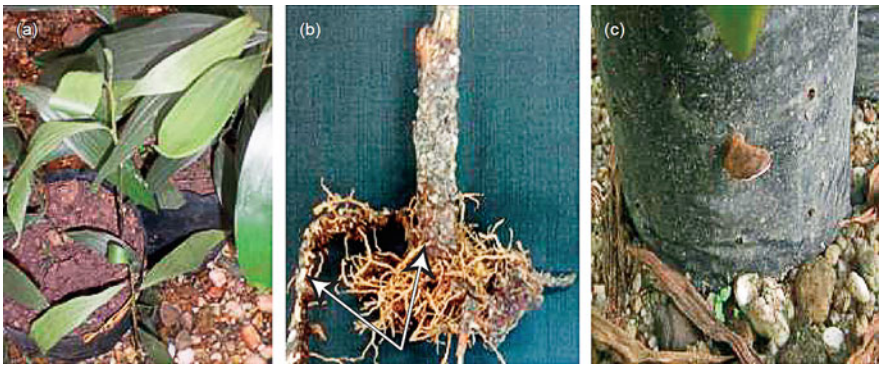

Fig. 7.2 *Ganoderma*-infected *Acacia mangium* seedlings: wilting symptom (**a**), basal stem and roots (**b**), and a fruiting body of the pathogen (**c**) (Gafur et al. 2015a)

incidence and soil type has been recorded. Rainfall does not seem to be critical for disease development (Gafur et al. 2011a, b, 2012).

In potted *A. mangium* seedlings in the greenhouse, wilting symptom is first apparent in infected seedlings 10 weeks after inoculation (Fig. 7.2a). Red rhizomorphs covering the roots and white mycelia under the root bark of diseased seedlings (Fig. 7.2b) are obvious signs of the red root rot disease. Fruiting bodies occasionally appear (Fig. 7.2c). Because all infected seedlings die, healthy ones are considered resistant (Gafur et al. 2014, 2015a, c).

7.2 Pathogen Identification

Ganoderma species are difficult to distinguish. Identification was conventionally based on morphological characteristics of their sporocarps, which are greatly varied both in natural infection in the plantations (Glen et al. 2009) and artificial inoculations in the nurseries (Fig. 7.3). A number of different species of the fungus had been linked to the red root rot-infected acacias and eucalyptus trees in Indonesia. Other observations, however, suggested that a single species might be responsible for the root rot disease in the tropical estate forests. This had led to uncertainty about the disease's primary pathogen. Despite this confusion, however, there was not any serious effort to clarify the issue. Pathogen identification is required to assist the construction of strategy options for its effective management. Thus, an accurate identity of the pathogen was considered very critical.

Considering the situation, Glen et al. (2009) collected newly infected roots from dying *A. mangium* at numerous locations in Sumatra. As the molecular techniques to identify wood rot-associated basidiomycetes had not been utilized for the Ganoderma root rot pathogen on *A. mangium*, they examined variations of the rDNA internal transcribed spacer (ITS) sequences of the collected samples. Data analysis showed that, although other species might also involve, *G. philippii* is the

Fig. 7.3 Fruiting bodies of *Ganoderma philippii* produced in the artificially inoculated *Acacia mangium* seedlings in the nursery

major fungal species recovered from *A. mangium* that stands with the root rot disease symptoms.

In addition to the above-mentioned report, we collected a large number of isolates of *Ganoderma* from the *A. mangium* and *Eucalyptus* roots exhibiting early symptoms of the red root rot disease (Coetzee et al. 2011). As many as 173 isolates were recovered from diseased roots of *A. mangium* trees in the Riau Province and 6 from *Eucalyptus* roots in the North Sumatera Province. The DNA sequence comparisons and phylogenetic analyses (Fig. 7.4) revealed that *G. philippii* is the major pathogen of the red root rot disease on those host trees. Other *Ganoderma* species identified in the disease centers were of less importance. Limited to the *Eucalyptus* plantation in Sumatra, it was the first report of *G. philippii* igniting the red root rot disease on the tree. In other experiments, we also discovered that *G. philippii* was the primary pathogen in citing the red root rot disease in the *A. mangium* and *Eucalyptus* plantations not only in Sumatra but also in Kalimantan (Glen et al. 2014; Yuskianti et al. 2014). Other root rot pathogens isolated included *G. mastoporum*, *G. steyaertanum*, *Phellinus noxius*, and *Rigidoporus microporus*. Based on all these extensive studies, it is therefore concluded that the main pathogen species of the Ganoderma root rot disease in estate forests in Indonesia is *G. philippii*.

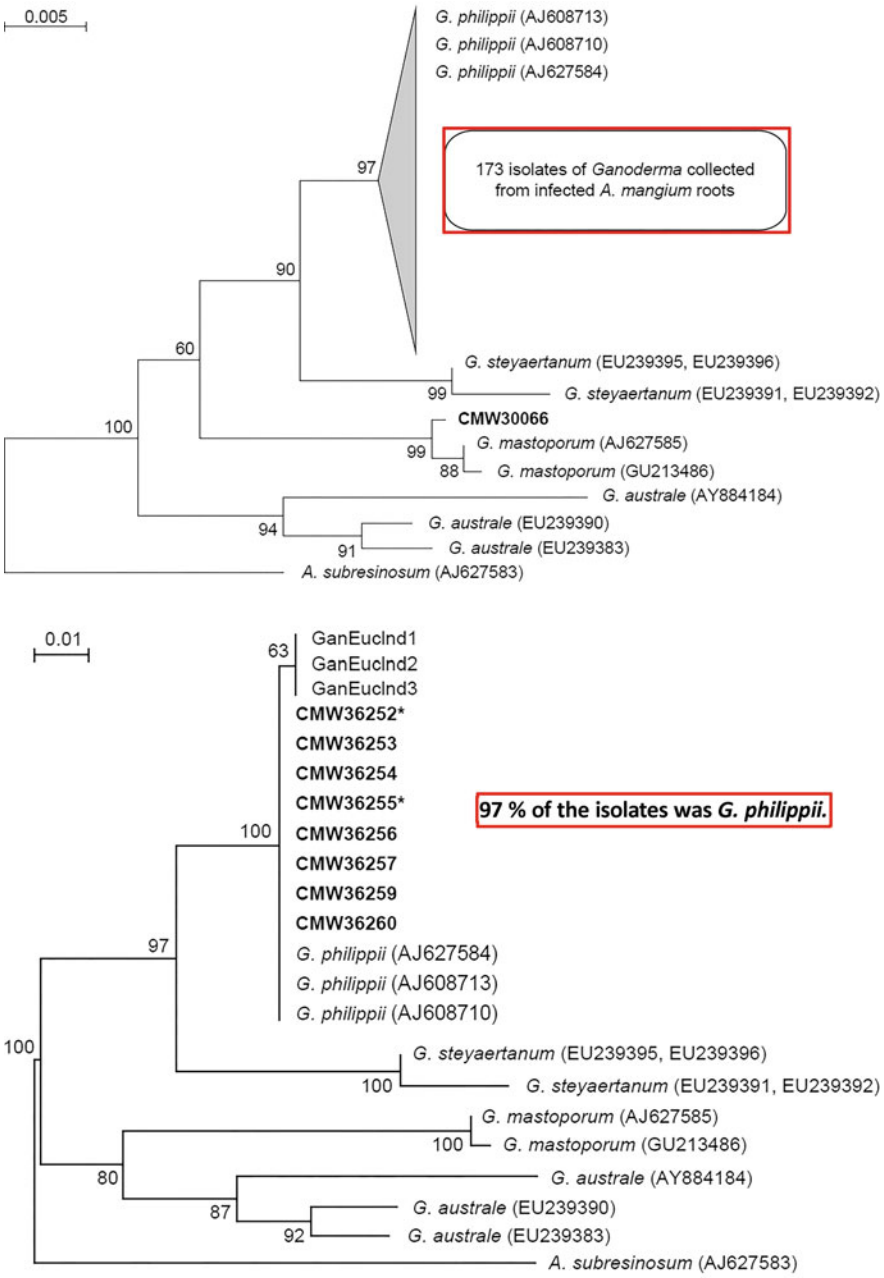

Fig. 7.4 Phylogenetic trees constructed from the data of ITS sequences of *Ganoderma* recovered from infected *Acacia* (top) and *Eucalyptus* (bottom) trees, suggesting that *G. philippii* is the main pathogen of the red root rot disease on the trees (Coetzee et al. 2011)

7.3 Dispersal

The inoculum build up within plantation rotations is very common in root rot pathogens. However, the disease progresses more rapidly in pulpwood plantations due to the shorter rotation (Mohammed et al. 2014). *Ganoderma* survives in woody tissues above and below the soil. The initial infection of healthy plants has been assumed to occur via root contact with diseased tissues deposited in the soil. As shown in Fig. 7.5, the spread to adjacent trees is facilitated by tree-to-tree dissemination through contact of the infected roots with the roots of healthy trees. Spores also contribute to pathogen dispersal (Francis et al. 2014; Page et al. 2020), creating genetic diversity of the pathogen.

Over the course of each rotation, the disease worsens (Page et al. 2020). In a study of Ganoderma root rot disease progression in *A. mangium* stands, Francis et al. (2014) discovered that over time, tree mortality expands approximately in a linear fashion, resulting in the steady coalescence of previously distinct diseased areas. The disease advances at the monthly rate of about 0.3%, and the average time length from pathogen infestation to tree death is about 12 months. Hardie et al. (2017) found that the correlation between soil and topographic variables and mortality causes in *A. mangium* plantations, including Ganoderma root rot disease, is weak and inconsistent. Therefore, Page et al. (2020) suggested that to minimize the occurrence of root rot disease in *A. mangium* plantations, efforts to minimize below ground

Fig. 7.5 Root-to-root contact (left) and basidiospores (right) contribute to the spread of the Ganoderma root rot disease in *Acacia mangium* plantations

dispersal of the pathogen as well as measures to avoid new infestations by the fungal spores must be made.

7.4 Management

As pointed out previously, the red root rot tends to get worse after consecutive rotations because infected woody substances (roots, stumps, and other debris) left in or on the soil consistently add to the inoculum buildup (Gafur et al. 2015a). The severe epidemics and wide spread distribution of root rot pathogens already observed in many *A. mangium* plantations, which imply that the more prevalent and serious outbreaks can be expected in the subsequent planting cycles if the management fails to take actions during or after harvest to minimize inoculum loads and/or to incorporate plant resistance in the disease management strategies (Glen et al. 2009). In either situation, the losses caused by the disease require development of effective control strategies (Francis et al. 2014; Page et al. 2020). As *G. philippii* is the primary pathogen, discussions should therefore be focused on the species.

7.4.1 Resistant Plants

To sustain productivity of *Acacia* plantation forests in the tropics, rapidly growing, disease-resistant plants must become the central focus of integrated management of Ganoderma root rot and other diseases. Incorporation of resistant genotypes into the integrated pest management (IPM) strategy is presumed to be both environmentally and economically attainable to mitigate the risk caused by the red root rot pathogen. However, despite the significant role of resistant plants in IPM, only a small effort had been made for their development. This was primarily due to the absence of consistent and timely screening procedures (unpublished data). In the past, the time length and result inconsistencies had delayed research on the *Acacia* root rot pathosystem. Therefore, a consistent, fast, and practical screening method for identifying and characterizing root rot-resistant *Acacia* has been devised (Gafur et al. 2014, 2015a).

Inoculum substrates used to grow *G. philippii* were fresh wood blocks of rubber (*Heveabrasiliensis* (Willd. ex A. Juss.) Müll. Arg.) with the size of 6 cm × 6 cm × 3 cm. The pathogen inoculum was derived from 3-week-old cultures of the most aggressive *G. philippii* isolate plated on the potato dextrose agar medium. To ensure their complete colonization, the inoculated wood blocks were placed in plastic trays and incubated for 8–9 weeks. In a series of experiments, the *A. mangium* seedlings were then screened. They were planted and inoculated in 30 cm × 25 cm polythene bags. Each experimental unit consisted of ten polythene bags, each comprising five seedlings. A total of 250 plans per family were screened in five replicates (Fig. 7.6) for each series or batch. The inoculated young plants were exposed to synthetic shade net with a light intensity of approximately 80%. To ensure

Fig. 7.6 Screening for *Ganoderma* resistance in *Acacia mangium* (Gafur et al. 2015a)

consistency, the most resistant and the most susceptible families selected in the previous batches were used as controls and baselines in the following series (Gafur et al. 2015a, c).

Seedlings are individually examined. The most obvious symptoms observed are paler and wilted phyllodes, which are linked to red rhizomorph and white hypha in the roots. Because all infected plants die, healthy plants are deemed to be resistant. In all experimental series or batches, significant variations in resistance and/or susceptibility to *G. philippii* were evident within the screened *A. mangium* families. In one of the series, disease incidence ranged between 0.6% (most resistant) and 58.2% (most susceptible) as presented in Fig. 7.7a (Gafur et al. 2015a).

Similar results of significant differences in resistance and/or susceptibility to *G. philippii* within the screened clones and/or families were also reported in different acacias (Gafur et al. 2014, 2015a, c) and eucalyptus (unpublished data) species. In one of the experimental series, disease incidence varied between 3.4% (most resistant) and 23.9% (most susceptible) in *A. crassicarpa* (Fig. 7.7b) and between 2.4% (most resistant) and 16.4% (most susceptible) in the *A. mangium* × *A. auriculiformis* hybrid (Fig. 7.7c). Thus, *A. mangium* and *A. crassicarpa* tested in the current study seemed to be more resistant to *G. philippii* than the hybrid genotypes (Gafur et al. 2015a).

As indicated earlier, *A. mangium* was more susceptible to *G. philippii* than both *A. crassicarpa* and the *A. mangium* × *A. auriculiformis* hybrid. This was consistent with the previous findings in plantations (Gafur et al. 2012). Nonetheless, the existence of differences in the resistance and/or susceptibility levels within each of the three screened species suggests the potential of screening and breeding for resistant materials. This should also provide opportunity to construct molecular marker tools for evaluating and identifying *G. philippii* resistance in tropical forest trees.

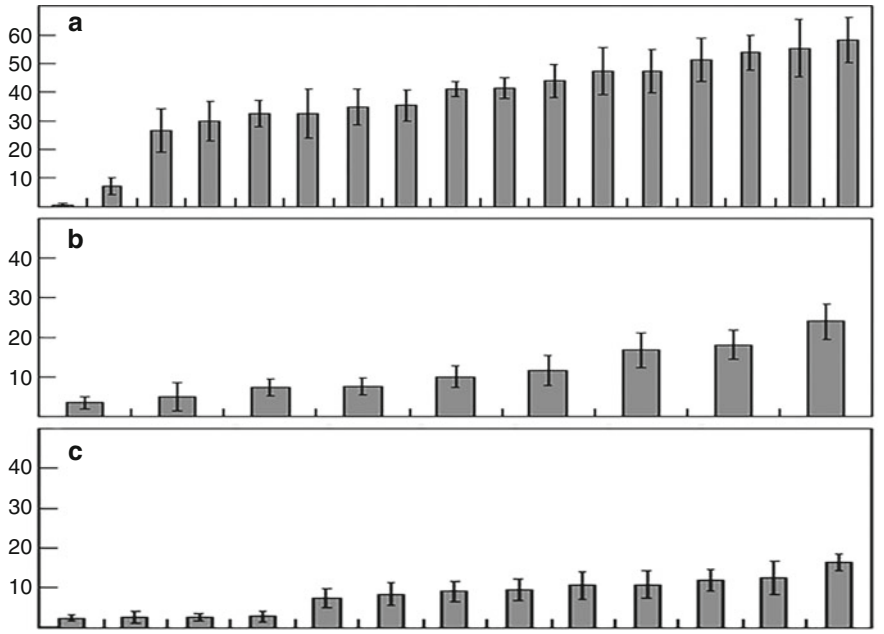

Fig. 7.7 *Ganoderma* incidence on different genotype identities of (**a**) *Acacia mangium*, (**b**) *A. crassicarpa*, and (**c**) *A. mangium* × *A. auriculiformis* hybrid seedlings 19 weeks after inoculation (Gafur et al. 2015a)

7.4.2 Biocontrol Agents

Biocontrol measures contribute significantly to disease control as the critical element of IPM. The role of biocontrol agents (Tjahjono et al. 2009; Gafur et al. 2011a, b, 2015b, 2017a, Gafur 2019a) in managing the red root rot disease in Indonesian estate forests is discussed. *Cerrena*, *Gliocladium*, *Phlebiopsis*, *Trichoderma*, and some other species of white rot basidiomycetes have so far been the antagonists utilized to control the root rot diseases.

Gliocladium and *Trichoderma* are fungal saprophytes that grow fast in a variety of environments. Ecologically, these fungi are highly adaptable and frequently the most ubiquitous culturable soil fungi. These fungi not only occupy roots of plants but they also infect and parasitize other species of fungi. Other known processes used by the antagonists to inhibit other fungal species including pathogens are antibiosis, nutrient and/or space competition, induced resistance, and inhibition of the fungal enzymes. For these reasons, *Gliocladium* and *Trichoderma* are among the most widely used fungi in biological measures of a variety of plant diseases. Table 7.2 lists the various species and/or isolates of the antagonists that have been utilized to control root rot pathogens.

Free-living isolates of *Gliocladium* and *Trichoderma* from various origins and sites have been evaluated in vitro for their efficacy against pathogens of root rot

Table 7.2 Some antagonistic species of *Gliocladium* and *Trichoderma* used to control root rot diseases

Antagonists	Pathogen	Source
Trichoderma harzianum	*Ganoderma lucidum*	Bhaskaran (2000)
Trichoderma harzianum *Trichoderma* spp. *Gliocladium viride*	*Ganoderma boninense*	Dharmaputra et al. (1989) Soepena et al. (2000) Susanto et al. (2005)
Trichoderma spp.	*Ganoderma* spp.	Widyastuti (2006)
Trichoderma viride	*Phellinus weirii*	Nelson et al. (1995)
Trichoderma sp. *Trichoderma harzianum*, *T. polysporum* *Trichoderma hamatum*, *T. harzianum*, *T. viride*	*Armillaria* sp.	Hagle and Shaw III (1991) Berglund and Ronnberg (2004) Raziq and Fox (2006)

Fig. 7.8 Pure culture of *Ganoderma* (G) (left) and *Ganoderma* (G) overgrown by *Trichoderma* (T) in dual culture (right) (Gafur et al. 2011a, b)

diseases such as *Ganoderma* and *Phellinus*. Some of the isolates were successful in outgrowing the root rot pathogens (Fig. 7.8). However, one issue with free-living isolates is their consistency in plantations. Isolates that show excellent inhibitory effects in laboratory tests may not perform well in the field. Furthermore, an isolate that is effective in one environment may not necessarily be potent in another. To exemplify, two trials were built in two different sites, A and B, in the Province of Riau, Sumatera. The trials revealed that *Trichoderma* originated from site A performed the best in site A, lowering *Ganoderma* occurrence by 7.0%. Likewise, *Gliocladium* collected from site B possessed the highest efficacy in site B, with a 10.0% reduction in *Ganoderma* occurrence (Tjahjono et al. 2009; Gafur et al. 2011a, b).

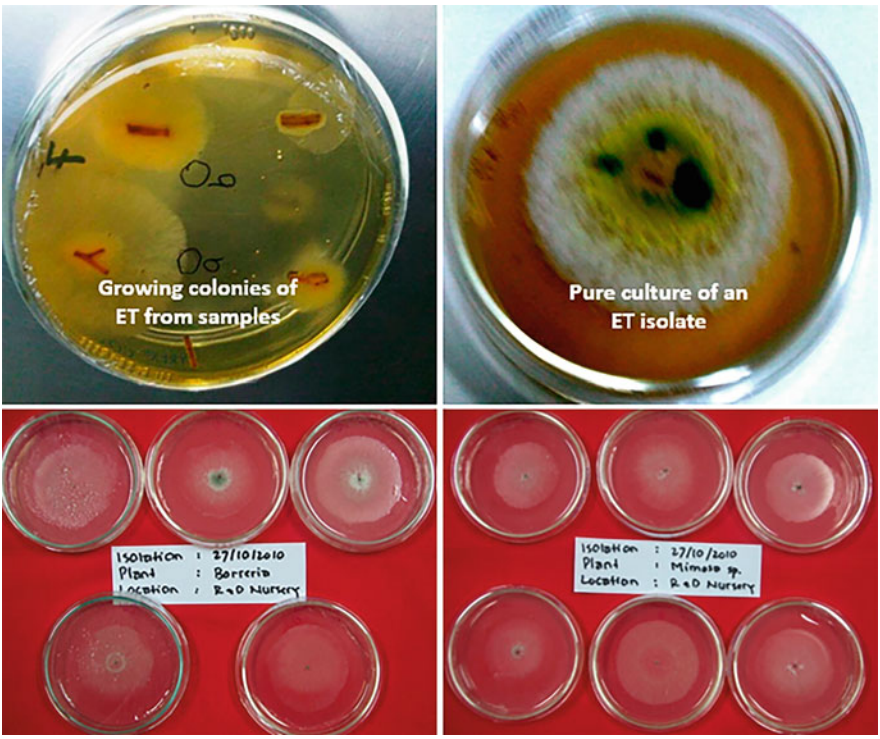

Fig. 7.9 Isolation of putative endophytic *Trichoderma* (Gafur et al. 2017a; Gafur 2019a)

On the other hand, endophytic *Trichoderma* is more stable and adaptive. They can boost plant growth and health while remaining in the root during the rotation of the plants (Hill 2012; Gafur 2021, 2022a; Siregar et al. 2022). We recovered more than 200 presumed endophytic isolates (Fig. 7.9) from different ecological functions and sites in Riau (Gafur et al. 2015b, 2017a, Gafur 2019a) and then evaluated them. Some of the isolates could increase the growth (height and diameter) of *A. mangium* seedlings and decrease considerably incidence of the Ganoderma root rot disease (Figs. 7.10 and 7.11).

Other antagonists frequently developed to control root rot pathogens include non- or weakly pathogenic white rot basidiomycetes. These fungi can degrade wood debris quicker than the pathogen, compete for space and nutrient, generate inhibitory secondary metabolites, and parasitize the pathogen (Eyles et al. 2008; Peterson 2006). In the northern hemisphere, the commercially accessible *Phlebiopsis gigantea* is widely employed to manage *Heterobasidion annosum*, another root rot pathogen. In spite of this, however, it was only recently that white rot basidiomycetes capable of competing for resources with *G. philippii* or *P. noxius* had been properly investigated in Indonesia. *Cerrena* and *Phlebiopsis* suppress hyphal growth of *G. philippii* and *P. noxius*. The two species compete for space and nutrients with the root rot pathogens. In vitro trials have previously shown that

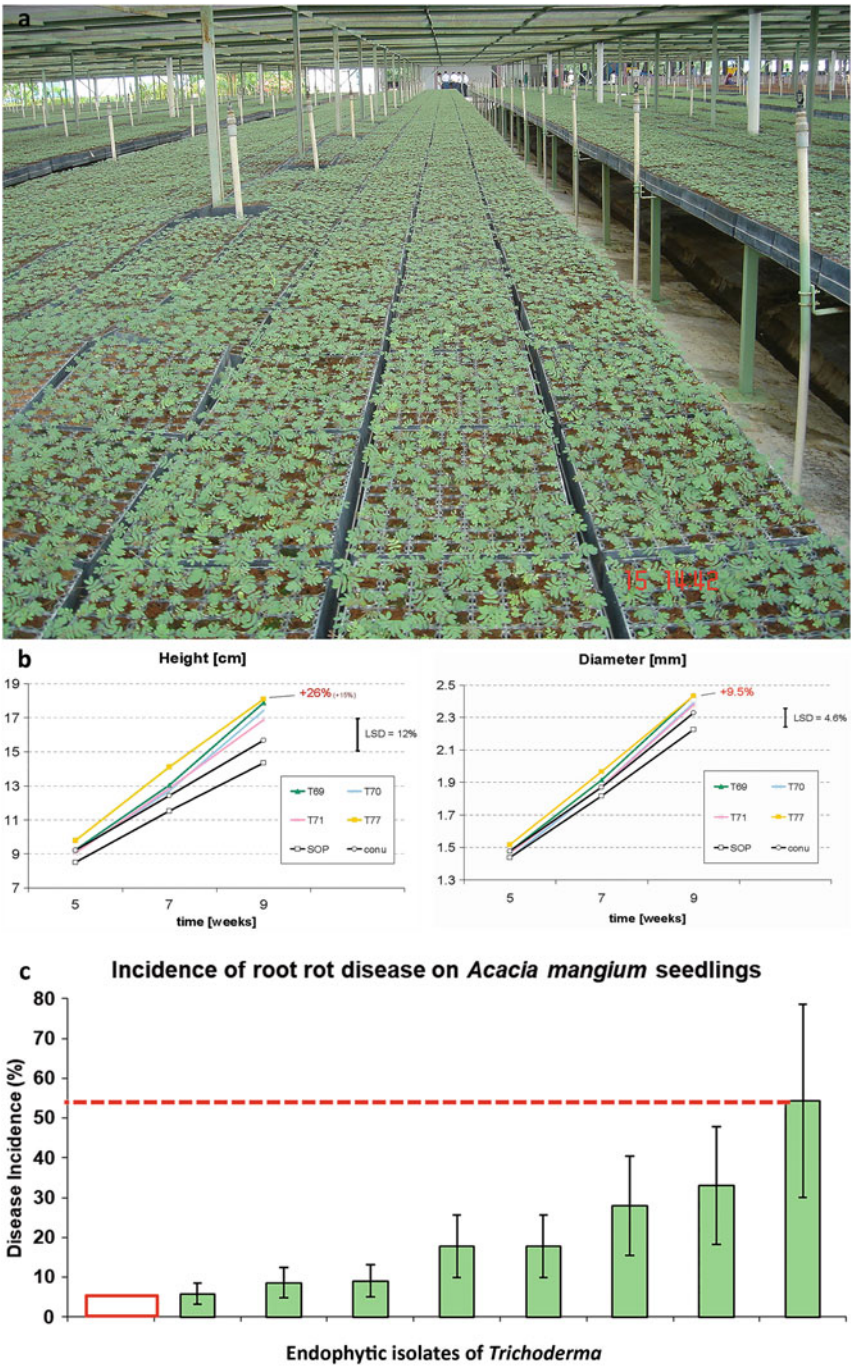

Fig. 7.10 Nursery screenings of endophytic *Trichoderma* on *Acacia mangium* seedlings. One of the large-scale trials (**a**). Some isolates could increase the seedling growth rate (**b**) and decrease or even abolish the Ganoderma root rot incidence (**c**) (Gafur et al. 2015b, 2017a)

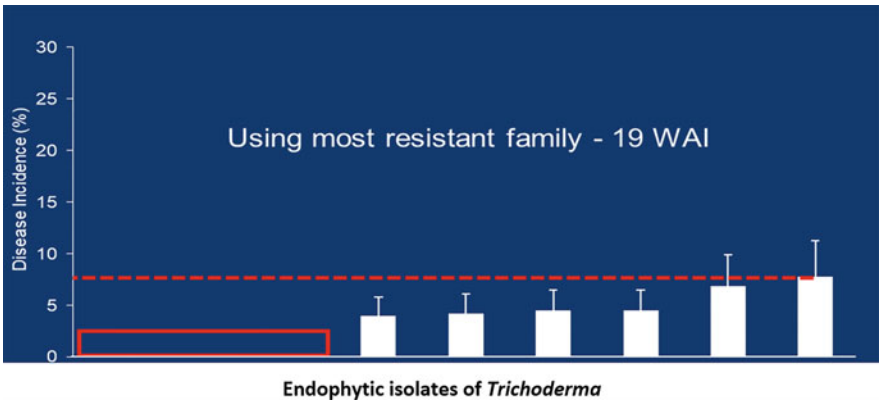

Fig. 7.11 Using the most resistant *Acacia mangium* family, more *Trichoderma* isolates are able to further reduce or eliminate occurrence of Ganoderma root rot disease (Gafur 2019a)

they are antagonistic to the pathogens. We also investigated how to apply the antagonists to effectively manage the red root rot pathogen in plantations (Glen et al. 2006; Puspitasari et al. 2014, 2017; Gafur 2015; Gafur et al. 2017b; Hidayati et al. 2017, 2019; Indrayadi et al. 2017; Nurrohmah et al. 2019). The antagonists were inoculated into stumps to prevent pathogen infections and colonization (Fig. 7.12).

Besides *Cerrena* and *Phlebiopsis*, we sampled 107 specimens of other white-rot basidiomycetes from plantation forests in Riauto to explore their possibilities as antagonists of root rot pathogens. The fungi were collected from rotten wood, which included trunks and twigs, as well as fungal fruiting bodies (Sitompul et al. 2011). Of the 107 specimens obtained, 28 were taken from rotten woods and 51 were from fruiting bodies. Isolated fungi were screened on wood block, wood disc, and malt extract agar added with wood powder. The results of the three-step screenings revealed that two isolates, WFA033 and WFA068 (Fig. 7.13), have the possibility of being used as antagonists against *G. philippii*, the red root rot causal organism.

7.4.3 Other Control Measures

In addition to plant resistance and biocontrol measures, work on field control of the Ganoderma root rot disease in *Acacia* plantation forests in Indonesia has also included reduction of pathogen inoculum, implementation of appropriate silviculture practices, and planting of alternative species (Fig. 7.14), although with limited success (Gafur et al. 2011a, b, 2012). Urea stump application, de-stumping, and alternative species were treatments evaluated in the experiments. In addition, we also noticed that in naturally regenerated *A. mangium* plots, the *Ganoderma* occurrence was lower than in planted plots. However, the growth (height and diameter) of the naturally regenerated stands was much slower than that of planted trees.

Fig. 7.12 Stump treatment with *Cerrena* spp. and *Phlebiopsis* spp. to avoid invasion by root rot fungal pathogens (Hidayati et al. 2019; Nurrohmah et al. 2019)

The need to incorporate different groups of plant growth promoting microbes (PGPM) into strategies to control a variety of diseases (Arora et al. 2021; Hamid et al. 2021; Kapadia et al. 2022a; Lahiri et al. 2022) and to improve productivity (Jabborova et al. 2021; Saboor et al. 2021; Sarkar et al. 2021; Gowtham et al. 2022; Kapadia et al. 2022b; Mir et al. 2022) of different crops has become more apparent in recent years. This is also true with the management of health and growth of estate forests in the tropics (Gafur 2019a, 2022b; Zul et al. 2022a, b). Within this scenario, nursery evaluation of a biofertilizer consortium product comprised *Bacillus*, *Brevibacillus*, *Brevundimonas*, *Burkholderia*, *Microbacterium*, *Ochrobactrum*, *Pseudochrobactrum*, and *Pseudomonas* (LIPI 2015; Antonius et al. 2021) on its ability to improve resilience of acacia and eucalypt seedlings was recently initiated. The treated seedlings were in turn expected to become more resistant trees against abiotic and biotic factors including the red root rot disease when later planted commercially in plantations (Gafur 2019b; Arifin et al. 2022; Syaffiary et al. 2022).

7.5 Conclusion

The red root rot disease is likely to remain one of the most important issues in the sustainability of plantation forest production in the tropics, particularly in Indonesia. The disease is primarily caused by *G. philippii*. Root-to-root contact and

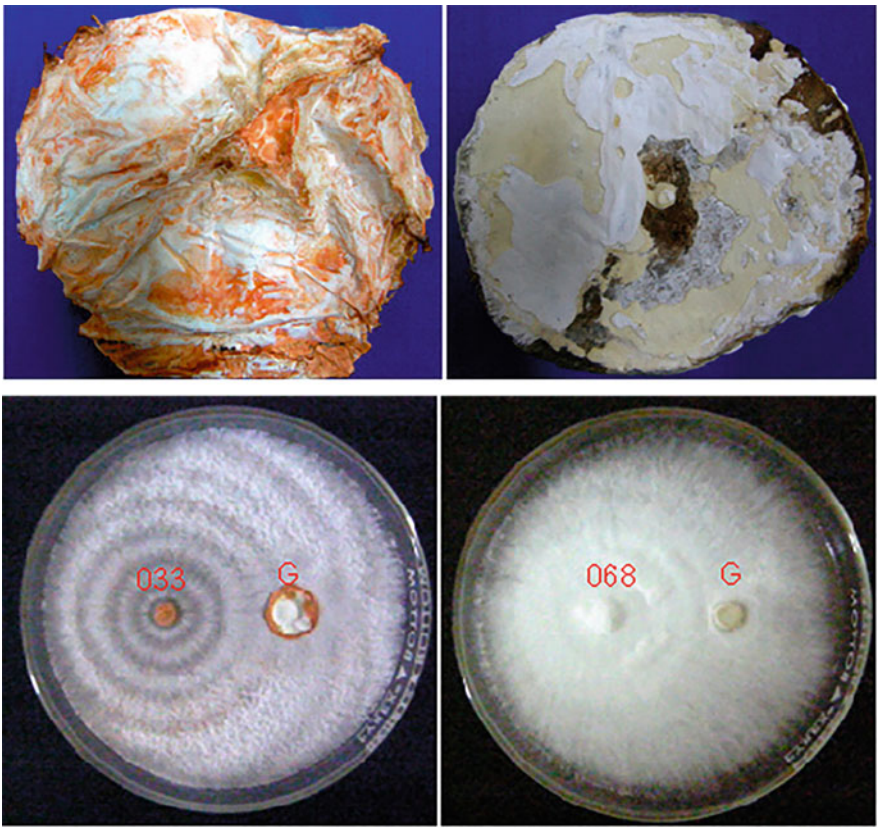

Fig. 7.13 WFA033 (left) and WFA068 (right) isolates were cultured together with *Ganoderma philippii* on a wood disc (top) and on MEA-WP media (bottom). The white rot basidiomycetes over-colonized and inhibited the pathogen (Sitompul et al. 2011)

basidiospores facilitate disease dispersal within *A. mangium* plantations. Despite these challenges, there are enormous possibilities for integrated management of the disease using continuously developing technologies and innovations. Whenever available, plant resistance should serve as the core of the integrated pest management (IPM) of pests and diseases of estate forests, including the red root rot disease. Considering that biocontrol measure is one of the IPM key components, development of biocontrol agents is very critical. Biofertilizers, bio-stimulants, and other PGPM groups should also be prioritized in the future efforts of the red root rot disease management.

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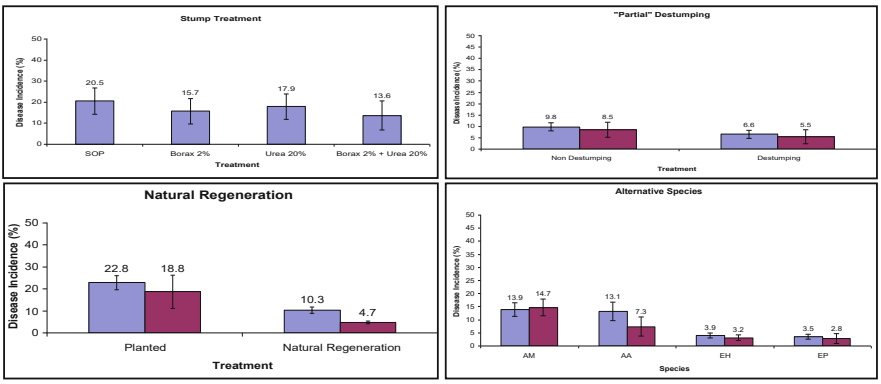

Fig. 7.14 Different field management strategies of the red root rot disease have also been explored although with a limited success (Gafur et al. 2011a, b, 2012)

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Health Management of Rhizospheric Microbiome

8

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Abstract

The contemporary situation of climate change and population burst has rendered enormous pressure on food security. Sustainable management of ecosystem and food production enhancement must take into account the maintenance and augmentation of soil fertility and productivity. In this context, rhizospheric microbiome has an indispensable role to play. The rhizosphere is the area of soil where plant roots are active, harboring diverse microbial populations being the hot spot for microbial activities. Microbes are suggestively a biological indicator to determine rhizospheric health. They are pivotal for soil and plant health, thus helping plants to mitigate biotic and abiotic stresses as upcoming strategy to maintain health in a sustainable way. On the other hand, conventional agricultural approaches and non-judicious use of agrochemicals can potentially bring antagonistic changes in rhizospheric microbiomes. This alteration is further aggravated with climate change, pressurizing soil microbiome and interfering with ecological processes in terms of diversity, structure, and functions, which are associated with a loss in soil biodiversity and soil organic matter. Increasing

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urbanization has emerged as a major source of soil degradation. Protecting and restoring soil microbiome has both economic and environmental benefits. A combination of soil amendments, genetic modification, and targeted microbiome engineering can be beneficial in reducing additional input of agrochemicals. In recent decades, incorporation of molecular techniques has refined our understanding of microbial ecology that could be crucial in drafting future strategies. This chapter summarizes the current knowledge related to rhizospheric microbiome composition and its functions in soil and plant health for sustainable production system, besides reviewing the strategies to enhance its own overall health.

Keywords

Microbiome · Plant health management · Rhizosphere · Soil-borne pathogen · Stress

8.1 Introduction

The contemporary projections for climate change are expected to impose stress on high-quality agricultural lands worldwide (Vati and Ghatak 2015). Significant land areas are likely to be rendered to the rising sea levels, soil erosion, increased salinity, and expanding desertification (IPCC 2014). Consequently, crop yields must be maintained under more stressful conditions in order to ensure food security for the growing global population. Sustainable management of ecosystem and food production enhancement must take into account through the maintenance and augmentation of soil fertility and productivity (Ganguly et al. 2021).

Various biotic elements are known to exist in association with plant structures, namely flowers, fruits, stems, leaves, and roots, and form the phytomicrobiome (Berg et al. 2016). The diverse microbial community (including bacteria and fungi) associated with the plant roots constitute the rhizomicrobiome. Among all biomes associated with higher plants, rhizomicrobiome is extolled as the most diverse and populous. The nitrogen-fixing rhizobia associated with legumes are the best understood and characterized examples (Gray and Smith 2005). Plants intensely influence the microbial community in their root surface vicinity known as the rhizosphere, the term originally coined by Hiltner (1904). According to the recent terminologies, endophytic microbes inhabit inside the roots, while the rhizoplanic microbes acquire the root surface (Gray and Smith 2005; Zhang et al. 2017a, b).

Plants have evolved to precisely control the composition and activity of the rhizomicrobiome (Zhang et al. 2017a, b). Root exudates of varied compositions are synthesized and secreted by plants, which can be more suitable to some microbes than others (Chaparro et al. 2012; Trabelsi and Mhamdi 2013). Plants produce specific signal compounds that can up- or downregulate the genetic and biochemical activities of target microbial species (Nelson and Sadowsky 2015; Smith et al. 2017). Besides this, the rhizomicrobial community in itself takes charge of various aspects

as well (Leach et al. 2017). When conditions warn of a collective physiological shift or stress, the microbes can produce quorum-sensing signal compounds to communicate based on population gradient (Chauhan et al. 2015). In response to this, plants produce analogs of microbial quorum-sensing compounds for the next level regulation over the rhizomicrobiome (Ortiz-Castro et al. 2009). Both beneficial and harmful microorganisms are found in rhizosphere of a plant. The microbes of the rhizomicrobiome plays an inevitable role in both acquisition as well as in assimilation of soil nutrients via the biogeochemical cycling of organic matter and minerals, better soil texture, modulating the secretion of hormones, secondary metabolites, antibiotics, and signal compounds. All these factors facilitate the plant growth and vigor, providing defense against several pests and diseases and helping plants to mitigate abiotic stresses (Table 8.1).

The Green Revolution has resulted in a global increase in food production that has never been seen before. Deployment of agrochemicals (pesticides, herbicides, and chemical fertilizers) and crop improvement through genetic manipulations and targeted breeding have been the two main pillars of this advancement. However, the achievements associated with intensive agriculture bear heavy environmental repercussions. The conventional agricultural approaches, including intensive tillage, recurrent cropping, and non-judicious use of agrochemicals, can potentially bring about antagonistic changes in the rhizospheric microbiomes. This alteration is further aggravated with climate change, pressurizing the soil microbiome and interfering with ecological processes. Intensive agriculture practices affect the diversity, structure, and function of the rhizosphere microbial community. These practices are associated with a loss in soil biodiversity and loss of soil organic matter. Increasing urbanization has also emerged as a major source of soil degradation, preventing normal soil functions. Protection and restoration of the soil microbiome has both economic and environmental benefits. A combination of soil amendments, plant breeding or genetic modification, and targeted microbiome engineering can be beneficial in reducing the additional input of agrochemicals. Restoration approaches will need to be deliberated on a site-specific basis, pertaining to varied soil distribution. New approaches such as the application of biological alternatives like inoculants and microbially produced compounds, as well as the planting of improved crops, are urgently required (Timmusk et al. 2017). Tremendous potential underlies in exploiting the rhizomicrobiome community to increase crop production across the globe (Barea 2015; Nehra and Choudhary 2015).

8.2 Rhizosphere Microbiome Composition and Their Roles

Plants, soil, and microbes interact in a complex network in agriculture. Thus, a thorough understanding of microbial diversity and its role in agriculture is critical, as these organisms can serve as indicators of plant productivity as well as soil quality and health. The rhizosphere microbiome is necessary for plant growth and health because it serves as the first line of defense against a variety of soil-borne pathogens that cause root infections (Ghatak et al. 2010). In addition, soil microorganisms

Table 8.1 Effect of rhizospheric microbiome on abiotic stress management

Abiotic stress	Microorganism	Host plant	Reference
Drought stress	*Glomus intraradices*	Rice	Ruiz-Sánchez et al. (2010)
	Achromobacter piechaudii ARV8	Tomato	Mayak et al. (2004a, b)
	A. piechaudii ARV8	Pepper	Mayak et al. (2004a, b)
	Burkholderia phytofirmans strain PsJN	Maize	Naveed et al. (2014)
	B. pumilus strain DH-11 and *B. firmus* strain 40	Potato	Gururani et al. (2013)
	Mixture of *Pseudomonas putida* KT2440, *Sphingomonas* sp. OF178, *Azospirillum brasilense* Sp7, and *Acinetobacter* sp. EMM02	Maize	Molina-Romero et al. (2017)
Salt stress	*Piriformospora indica*	Barley	Waller et al. (2005)
	B. subtilis GB03	*Arabidopsis*	Zhang et al. (2008)
	T. harzianum	Rice	Rawat et al. (2012)
	T. asperellum Q1	Cucumber	Qi and Zhao (2013)
	T. Asperelloides T203	*Arabidopsis thaliana*	Brotman et al. (2013)
	B. pumilus strain DH-11 and *B. firmus* strain 40	Potato	Gururani et al. (2013)
	Streptomyces sp. strain PGPA39	Tomato	Palaniyandi et al. (2014)
	Pseudomonas strains PF1 and TDK1	Rice	Sen and Chandrasekhar (2014)
	B. phytofirmans strain PsJN	*Arabidopsis*	Pinedo et al. (2015)
	B. amyloliquefaciens SQR9	Maize	Chen et al. (2016a, 2016b)
	Enterobacter sp. UPMR18	Okra	Habib et al. (2016)
	T. longibrachiatum T6	Wheat	Zhang et al. (2016)
Cold stress	*B. phytofirmans* PsJN	Grapes	Barka et al. (2006)
	B. phytofirmans PsJN	Grapes	Theocharis et al. (2012)
	B. amyloliquefaciens and *Brevibacillus laterosporus*	Rice	Kakar et al. (2016)
	G. mosseae	Blueberry	Xiao et al. (2017)

(continued)

Table 8.1 (continued)

Abiotic stress	Microorganism	Host plant	Reference
	R. irregularis	Cucumber	Ma et al. (2018)
	G. versiforme and *R. irregularis*	Barley	Hajiboland et al. (2019)
	R. irregularis	Cucumber	Ma et al. (2019)
Heat stress	*Serratia proteamaculans*	Soybean	Zhang et al. (1995)
	S. Proteamaculans	Soybean	Zhang et al. (1996)
	T. harzianum	*Arabidopsis*	Montero-Barrientos et al. (2010)
	P. putida	Wheat	Grover et al. (2011)
Heavy metal stress	*B. subtilis* SJ-101	*Brassica juncea*	Zaidi et al. (2006)
	Agrobacterium radiobacter	*Populus deltoides*	Wang et al. (2011)
	B. pumilus strain DH-11 and *B. firmus* strain 40	Potato	Gururani et al. (2013)
	Lewia sp.	*Festuca arundinacea*	Cruz-Hernandez et al. (2013)
	B. licheniformis, *Micrococcus luteus*, and *P. fluorescens*	Grapes	Pinter et al. (2017)
	AM fungi	Pepper	Ruscitti et al. (2017)
	Bacterial genera *Pseudomonas*, *Cupriavidus*, *Bacillus*, and *Acinetobacter*	*Boehmeria nivea*	Jiang et al. (2017)
	Rhizobium spp.	Pulses	Rangel et al. (2017)

produce hydrogen cyanide (HCN), IAA (indole-3-acetic acid), nitrogen fixation, and nutrient solubilization (P, K, and Zn), as well as siderophore production, all of which promote plant growth (Nehra and Choudhary 2015). Plant growth-promoting microorganisms (PGPMs) aid in the efficient solubilization of nutrients in the soil as well as the facilitation of absorption by the plants, resulting in increased plant growth and yield. Furthermore, PGPMs can be used to maintain soil health, soil fertility, and nutrient mobilization in sustainable agriculture.

8.2.1 Composition of Rhizospheric Microbiome

The rhizosphere is a small area of soil that surrounds the roots and is rich in microbial diversity. The community structure of bulk soil and the rhizosphere, however, is

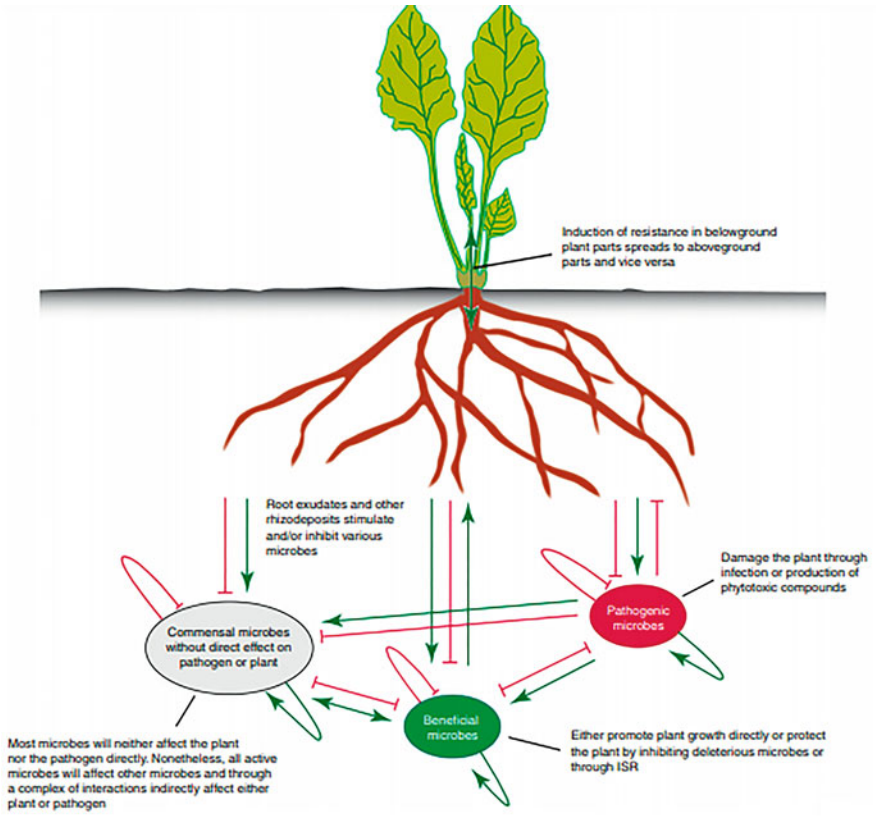

Fig. 8.1 Schematic representation of plant microbial association

found to be different (Reinhold-Hurek et al. 2015). As a result, it can also be defined as the biologically active zone of the soil, which includes bacteria and fungi as soil-borne microbes. Moreover, the rhizosphere serves as an infection court for soil-borne pathogens in order to establish a parasitic relationship with the plant. However, pathogens must compete with members of the rhizosphere microbiome for available nutrients as well as microsites in order to infect the tissues of the roots (Chapelle et al. 2016). The totality of microorganisms, their genomes, and their interactions in the rhizosphere are referred to as the rhizosphere microbiome (Mendes et al. 2013; Philippot et al. 2013; Berg et al. 2014). It harbors diverse microbes, which benefit plants as they help in inhibiting pathogenic infection as well as plays a crucial role in assisting soil nutritional acquisition (Fig. 8.1).

The rhizosphere is home to bacteria, fungi, oomycetes, viruses, nematodes, algae, protozoa, archaea, and arthropods (Lynch 1990; Meeting Jr 1992; Bonkowski et al. 2009; Buee et al. 2009; Raaijmakers et al. 2009). The vast majority of rhizosphere microbiome members are part of a sophisticated food web that consumes a large amount of plant nutrients. Exudates, border cells, and mucilage are all examples of

rhizodeposits that play a role in the regulation of microbial diversity and activity on plant roots. Plants have the ability to manipulate the rhizosphere microbiome to their advantage, according to Cook et al. (1995), by selectively stimulating microbes with rich in beneficial health and growth traits. Rhizodeposits are important regulators of symbiotic and protective relationships between plants and soil microorganisms (Farrar et al. 2003; Hirsch et al. 2003; Moore et al. 2003). Their roles in regulating other interactions, on the other hand, are not well understood (Paterson 2003).

Furthermore, understanding the genetic makeup, taxonomic, and functional components of the microbial community is also important for long-term crop production (Mendes et al. 2011; Busby et al. 2017). In some crop plants, such as *Arabidopsis* (Bulgarelli et al. 2012; Lundberg et al. 2012), significant work has been done to characterize rhizosphere microbiomes (Edwards et al. 2015), millet (Jin et al. 2017), soybean (Mendes et al. 2014), populous (Gottel et al. 2011), grapevine (Marasco et al. 2018), corn (Walters et al. 2018), sugarcane (Hamonts et al. 2018), cucumber (Ofek-Lalzar et al. 2014), barley (Bulgarelli et al. 2015), wheat (Donn et al. 2015), and citrus (Zhang et al. 2017a, b) by exploring their structure, functional genes, and the factors that determine the assembly of microbiome (Table 8.2).

Numerous other elements, such as the variability in microorganisms including pathogens, characteristics of the soil, the surrounding environment, and the background microbial composition, have an impact on the makeup of the rhizosphere microbiome community (Qiao et al. 2017; Kumari and Ghatak 2018; Kumar et al. 2020). Because different plant species support diverse microbial communities even when cultivated in the same soil, plants have the ability to change the rhizosphere microbiome (Aira et al. 2010; Berendsen et al. 2012; Bazghaleh et al. 2015).

8.2.2 Role of Rhizospheric Microbiome

Stress tolerance, nutrient acquisition, and protection against soil-borne pathogens are functions that microorganisms in the rhizosphere provide for the host plant (Mendes et al. 2011, 2014; Perez-Jaramillo et al. 2016). The main reservoir of microbial species colonizing the rhizosphere is the bulk soil (Jones et al. 2009; Mendes et al. 2013). The bulk soil is the primary source of microbial species that colonize the rhizosphere (Jones et al. 2009; Mendes et al. 2013). Therefore, the key determinants of the makeup and function of the rhizosphere microbiome are plant species, cultivars, and soil type (Marschner et al. 2001; Berg and Smalla 2009; Bulgarelli et al. 2012, 2015; Inceoglu et al. 2012). Furthermore, rhizosphere microbes play an important role in phytoremediation; as they are in close proximity to the root, any chemical or physical changes in the rhizosphere environment can easily affect heavy metal uptake by plants. The role of plant growth-promoting rhizobacteria (PGPR) as a phytoremediation tool has been recognized by researchers (Backer et al. 2018). Additionally, through rhizospheric processes, plants can bioconcentrate (phytoextraction) and bioimmobilize heavy toxic metals. Root-associated microbes that produce large amounts of the glycoprotein glomalin (which helps to stabilize soil aggregates) have been identified as having a significant role in maintaining soil

Table 8.2 Different mechanisms for biotic stress management through rhizospheric microbiome

Mechanisms	Rhizospheric microbes	Pathogens/insect pests/ nematodes	Reference
Antibiosis	*Bacillus subtilis* IFS-01	*Listeria monocytogenes*, *Stretomyces aureus*, *Erwinia carotovora*, *Pseudomonas syringae*, and *Xanthomonas campestris*	Foldes et al. (2000)
	B. subtilis AU195	*Aspergillus flavus*	Moyne et al. (2001)
	B. amyloliquefaciens FZB42	*Fusarium oxysporum*	Koumoutsi et al. (2004)
	Lysobacter sp. strain SB-K88	*Aphanomyces cochlioides*	Islam et al. (2005)
	B. subtilis BBG100	*Pythium aphanidermatum*	Leclere et al. (2005)
	P. fluorescens isolates	*F. oxysporum* and *Aspergillus* sp.	Showkat et al. (2012)
	Streptomycetes cacaoi strain M-20	*F. oxysporum*	Janaki (2017)
	B. pumilus strains	*Phytopthora infestans*	Caulier et al. (2018)
Competition	*B. subtilis*	*F. oxysporum*	Yu et al. (2011)
	Bacillus spp.	*Ralstonia solanacearum*	Kesaulya et al. (2018)
	B. amyloliquefaciens (UQ154), *B. velezensis* (UQ156), and *Acinetobacter* sp. (UQ202)	*Phytophthora capsici*	Syed-Ab-Rahman et al. (2018)
	Bacillus and *Pseudomonas* spp. strains	*P. infestans*	Caulier et al. (2018)
	Pseudomonas spp. Strain PICF141	*Verticillium dahliae*	Gómez-Lama Cabanás et al. (2018)
	Pseudomonas sp. MSSRFD41	*Pyricularia grisea*	Sekar et al. (2018)
	Ralstonia mannitolilytica QL-A2, *R. mannitolilytica* QL-A3, *R. taiwanensis* QL-117, and *R. pickettii* QL-140	*R. solanacearum*	Gu et al. (2020)
	Penicillium chrysogenum	*R. solanacearum* and *X. oryzae* pv. *oryzae*	Chowdappa et al. (2020)
Hyperparasitism	*Hypoviruses*	*Cryphonectria parasitica*	Tjamos et al. (2010)
	Acremonium alternatum, *Acrodontium crateriforme*,	*Erysiphe* spp.	

(continued)

Table 8.2 (continued)

Mechanisms	Rhizospheric microbes	Pathogens/insect pests/ nematodes	Reference
	Ampelomyces quisqualis, *Cladosporium oxysporum*, and *Gliocladium virens*		Heydari and Pessarakli (2010)
	Trichoderma spp.	*R. solani*	Sharma and Bhat (2011)
	Pochonia chlamydosporia	Genus *Globodera*, *Heterodera*, *Meloidogyne*, *Nacobbus*, and *Rotylenchulus* nematodes	Manzanilla-López et al. (2013)
	P. Chlamydosporia	*Meloidogyne javanica* eggs	Escudero et al. (2016)
	Trichoderma gamsii YIM PH30019	*Panax notoginseng*	Chen et al. (2016a, 2016b)
	Beauveria bassiana	*Planococcus ficus* and *Empoasca vitis*	Rondot and Reineke (2018)
	Metarhizium brunneum	*Myzus persicae*	Jaber and Enkerli (2016)
	B. bassiana, *Isaria fumosorosea*, and *Lecanicillium lecanii*	*Tetranychus urticae*	Dash et al. (2018)
Disease resistance induction	*Piriformospora indica*	*F. culmorum*	Waller et al. (2005)
	Serratia liquefaciens MG1 and *Pseudomonas putida* IsoF	*Alternaria alternata*	Schuhegger et al. (2006)
	Mild variants of the *Pepino mosaic virus*	*Pepino mosaic virus*	Schenk et al. (2010)
	P. fluorescens	*P. syringae*	Weston et al. (2012)
	Bacillus spp.	*Spodoptera exigua*	Zebelo et al. (2016)
	Paenibacillus lentimorbus B-30488	*Scelerotium rolfsii*	Dixit et al. (2016)
	Trichoderma harzianum T34	*F. oxysporum* f.sp. *lycopersici* race 2 and *Botrytis cinerea*	Rubio et al. (2017)
	Trichoderma harzianum strain M10	*Rhizoctonia solani*	Manganiello et al. (2018)
	Pseudomonas sp. 23S	*Clavibacter michiganensis* subsp. *michiganensis* and *P. syringae* pv. *tomato*	Takishita et al. (2018)
	Rhizosphagus irregularis	*Xanthomonas campestris*	Smigielski et al. (2019)
		R. solani AG1-IA	

(continued)

Table 8.2 (continued)

Mechanisms	Rhizospheric microbes	Pathogens/insect pests/ nematodes	Reference
	Bacterial genera *Bacillus*, *Staphylococcus*, *Ochrobactrum*, *Pseudomonas*, *Lysinibacillus*, *Micrococcus*, *Leifsonia*, *Exiguobacterium*, and *Arthrobacter*		Bhattacharyya et al. (2020)

structure and aggregate stability (Sen 2003). However, biotic interactions occurring below ground play a significant role in determining plant diversity above ground by providing direct feedback on host growth and indirect effects on competing plants (Bever 2003). Furthermore, root exudation is an important process in the rhizosphere for carbon transfer into the soil, affecting the role of soil microbial communities in decomposition of organic materials and nutrient cycling (Baudoin et al. 2003; Ostle et al. 2003). The biomass and activity of soil microorganisms and fauna in the rhizosphere are positively influenced by root exudates (Butler et al. 2003). Plant carbon is required by soil microorganisms, which in turn provide nitrogen (N), phosphorus (P), and other minerals to plants through the decomposition of soil organic matter.

Rhizosphere microorganisms use a variety of mechanisms to promote plant growth and protect them from pathogen attack (Lugtenberg and Kamilova 2009; Raaijmakers et al. 2009)—biofertilization, plant root stimulation, rhizoremediation, abiotic stress control, and disease control are among the mechanisms. Proteobacteria and Firmicutes rhizobacteria, such as *Pseudomonas* and *Bacillus*, as well as Deuteromycetes fungi, such as *Trichoderma* and *Gliocladium*, are well-documented mechanisms (Kogel et al. 2006; Ghatak et al. 2010; Qiang et al. 2012).

8.3 Effect of the Rhizospheric Microbiome on Soil and Plant Health

Soil is a moist milieu that harbors extensive soil microbial communities. The microbiome also plays an important role in the maintenance of soil health by improving its physical, chemical, and biological properties. The rhizospheric microbiome is crucial for the growth and health of the plant, and its influence increases with decreasing distance from plant roots (Backer et al. 2018). The rhizospheric microbiome generally consists of fungi and bacteria that are either beneficial as nitrogen-fixing bacteria, growth-promoting rhizobacteria like PGPR, mycorrhizal fungi and bioagents, or deleterious like plant pathogenic microorganisms (Mohanram and Kumar 2019). These assemblies of plants and microbes detect and respond to the environmental stimulus, thus changes plant

growth and development (Hu et al. 2018). The overall fitness of plants greatly depends on these associations, which include biofertilization and protection from abiotic and biotic stresses (Gopal and Gupta 2016; Balodi et al. 2017).

8.3.1 Effect of the Rhizospheric Microbiome on Soil Health

Soil physical properties comprise soil color, soil texture, soil moisture, bulk density, electrical conductivity (EC), and organic matters and these properties are greatly affected by the presence of rhizospheric microbiomes. Bhatti and Qureshi (2005) conducted a study to evaluate the effect of mixed cultures of beneficial naturally occurring microorganisms such as photosynthetic bacteria, actinobacteria, lactic acid-producing bacteria, *Saccharomyces* fungi, and fermenting fungus on soil physical properties and found that soil color transformed from brown to dark brown because of increase in soil organic matter contents. It also increased the soil bulk density, soil organic matter, and soil moisture (by increasing water-holding capacity due to increased pore space through replacement of salts from soil particles) while decreased the soil pH and soil EC. However, no significant effect of microorganisms was reported on soil texture. Till date, little study has been done in this context. Hence, it is difficult to infer the exact influence of microorganisms on the physical properties of soil (Fig. 8.2).

Soil microorganisms solely serve as a source of soil organic carbon. Nannipieri et al. (2003) revealed that several rhizospheric microbes helped in the production of a large amount of organic carbon in the soil through carbon cycling. Besides *Rhizobium*, there are several free-living diazotrophic bacteria such as blue-green algae, *Azotobacter*, *Azospirillum*, and *Pseudomonads* that can convert atmospheric nitrogen into an available form of nitrogen in the soil (Kahindi et al. 1997). The nitrogen, phosphorus, and potassium content of soil can also enhance with the application of effective microorganisms with compost (Jusoh et al. 2013; Setiawati 2014). These microorganisms are found to lower the soil pH of calcareous soil as compared to the untreated soil and effectively reduce the micronutrient deficiency (Bhatti and Qureshi 2005) due to the presence of beneficial microorganism such as *Agromyces*, *Acremonium*, *Bacillus*, *Bradyrhizobium*, *Chaetomium*, *Lysobacter*, *Mesorhizobium*, *Micromonospora*, *Microvirga*, and *Pseudonocardia* (Wang et al. 2017). Besides this, supplying nutrients such as nitrogen and phosphorous at the same time promotes the growth of native beneficial microorganisms in the polluted soil, thus aiding bioaugmentation. *Acinetobacter* SZ-1 strain KF453955 had a positive effect on total petroleum hydrocarbon degradation (Wu et al. 2016).

Extensive use of artificial inoculums has been shown to improve soil health for disease control and degradation of pollutants. Microbes that are isolated from indigenous soil are indispensably better performers than alien inoculants pertaining to their adaptive advantages in their microenvironment. Recently, work regarding isolation of the pollutant tolerant as well as degrading microbes has been done. The soil inoculation of *Delftia* sp. B9, a Gram-negative bacterium, in cadmium-contaminated soil resulted in a safe level of cadmium content in rice grain (Liu

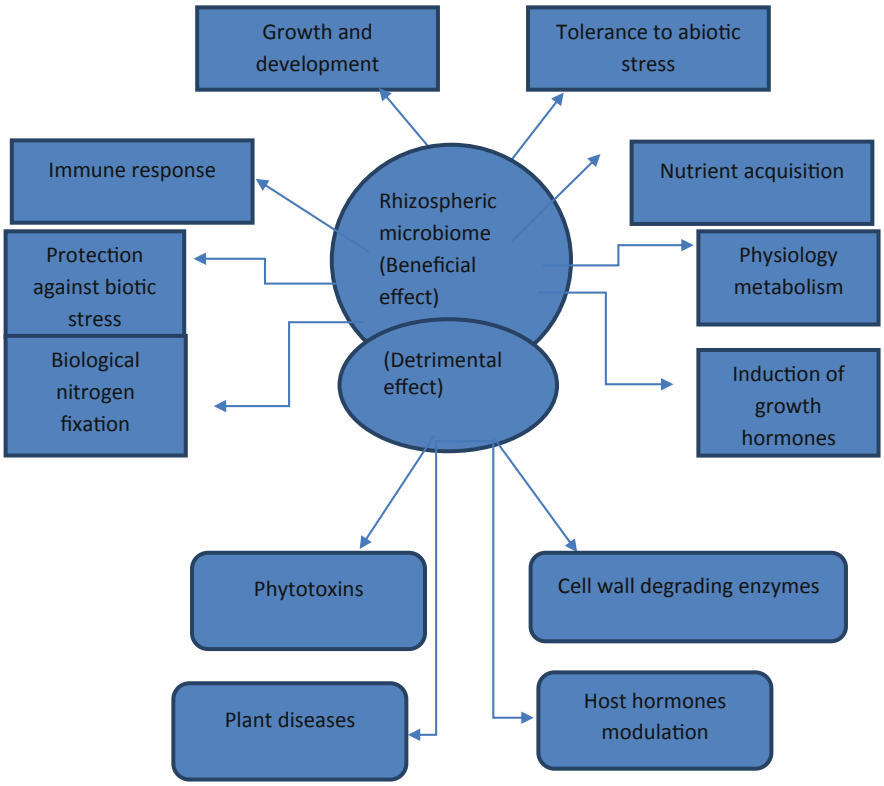

Fig. 8.2 Interactions in the rhizosphere. Plants are able to influence the composition and activation of their rhizosphere microbiome through exudation of compounds that stimulate (green arrows) or inhibit (red blocked arrows). Vice versa, a wide range of soil-borne pathogens are able to affect plant health. Prior to infection, these deleterious microbes are in competition with many other microbes in the rhizosphere for nutrients and space. In these battle resources, beneficial microbes will limit the success of the pathogen through production of biostatic compounds, consumption of (micro)nutrients or by stimulation of the immune system of the plant. Most microbes might neither affect the plant nor the pathogen directly because they occupy different ecological niches (commensal microbes), but are likely to affect every other organism to a certain extent through a complex network of interactions. *IST* induced systemic resistance. Beneficial and detrimental effects of plant microbial interaction. [Roeland L, Berendsen, Corne MJ, Pieterse, Peter AHM, Bakker (2012) The rhizosphere microbiome and plant health. Trends in Plant Science, 17 (8):478–486]

et al. 2018a, b). Efficient and durable effects on disease reduction or contamination elimination may be achieved via appropriate assemblages of different complementary or synergistic microorganisms. However, the establishment and survival of inoculum in the soil remains a major constraint in the application of bioremediation or bioagents (Raaijmakers and Mazzola 2016).

8.3.2 Abiotic Stress Management

Abiotic stress refers to the negative effect of specific environmental conditions on living organisms. It comprises several factors such as low/high temperature, drought/submergence, salinity/acidic conditions, and nutrient deficiency (Meena et al. 2017). Rhizosphere microorganisms are capable to reduce abiotic stresses in plants through their intrinsic metabolic and genetic effects (Gopalakrishnan et al. 2015). Several genera of rhizospheric microorganisms, e.g., *Achromobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Methylobacterium*, *Pantoea*, *Pseudomonas*, *Rhizobium*, and *Trichoderma*, have been widely known for promoting the growth of plant by mitigating numerous types of environmental stresses (Omar et al. 2009; Atieno et al. 2012; Ahmad et al. 2015; Sorty et al. 2016; Singh et al. 2021).

A drought-tolerant *Pseudomonas putida* strain MTCC5279, which was isolated from the rhizosphere of a drought-stressed chickpea, was able to reduce drought stress by producing an exo-polysaccharide with a unique water-retentive capacity and by modifying the gene expression of compounds involved in the biosynthesis of ethylene, the activation of jasmonates transcription, the production of salicylic acid, and the biosynthesis of antioxidant enzyme (Tiwari et al. 2016). On the other hand, a thermostable *P. putida* strain NBRI0987 isolated from chickpea rhizosphere overexpresses the stress sigma factor by the production of alginate to mitigate high-temperature stress (Srivastava et al. 2008). Under water-deficient conditions, an increase in photosynthesis, high chlorophyll content, and grain yield was reported in wheat treated with *B. phytofirmans* PsJN (Naveed et al. 2014). Similarly, modification in root structure, increase in biomass of root and its nodule, length of root, abscisic acid in the root, and content of total nitrogen were observed in soybean crop under drought conditions upon application of thuricin 17 (chemical produced by *B. thuringiensis* NEB17) (Prudent et al. 2015). While Etesami et al. (2014) witnessed increased root elongation through lowering ethylene production in rice under submerged when seedlings of rice were inoculated with an ACC deaminase-producing *P. fluorescens* strain REN1.

In a study, Fernandez et al. (2012) found that inoculation of *B. phytofirmans* PsJN successfully modified the carbohydrate metabolism in grapevine to reduce chilling damage occurring due to low-temperature stress. Similarly, Subramanian et al. (2015) found that inoculation of *P. vancouverensis* strain OB155 and *P. frederiksbergensis* strain OS261 enhanced the expression of cold acclimatization genes and activity of antioxidant in the leaf tissues of tomato plant under cold stress, thus mitigated the stress in the plant. On the other hand, *Serratia nematodiphila* (a gibberellin-producing PGPR) increased the growth of pepper under low-temperature stress conditions by increasing gibberellic acid and abscisic acid content and reducing salicylic acid and jasmonic acid contents (Kang et al. 2015). Meena et al. (2015) isolated PGPR from the root nodules of pea plants growing under low temperature and found efficient biofertilizing ability in them, which can mitigate the low-temperature stress condition. Ghorbanpour et al. (2018) studied the positive effect of *T. harzianum* in tomato under cold stress, citing the reduced rate of

lipid peroxidation and electrolyte leakage, whereas increased the leaf water content and proline accumulation as the cause. Mukhtar et al. (2020) studied the potential of heat-tolerant PGPR *B. cereus* in improving tomato plant growth under heat stress conditions and found that this bacterium was capable of reducing the effect of heat on the plant by the production of ACC-deaminase and EPS. It also promoted growth and plant health through the production of auxin and the solubilization of phosphate. Similarly, Khan et al. (2020) also discovered a thermotolerant *B. cereus* SA1 bacterium, which was able to mitigate heat stress in soybean plants by reducing ABA content, as well as increasing SA content in plant and also improves biomass and chlorophyll content of plant by the production of gibberellic acid, auxin, and organic acids.

Another study was performed by Ahmad et al. (2015) on Indian mustard to ameliorate salinity stress in the crop with the inoculation of fungus, *Trichoderma harzianum*, that enhanced the uptake of essential nutrients, antioxidants, and osmolytes accumulation and reduced the sodium uptake under brackish conditions. Wang et al. (2016) inoculated the pea plant with *Variovorax paradoxus* 5C-2 to mitigate the effect of salt by the production of enzyme ACC deaminase. Under salt stress at 70 and 130 mM NaCl, it boosted root biomass, increased electron transport, balanced ion homeostasis through increased potassium absorption by shoots and sodium deposition in roots, and decreased stomatal resistance and xylem balancing pressure in the plant. Due to the inoculation of wheat with the halotolerant *Dietzia natronolimnaea*, genes implicated in the ABA-signaling cascade, ion transporters, the excessively salt-sensitive pathway, and the synthesis of antioxidant enzymes were all activated (Bharti et al. 2016). Besides these, Panhwar et al. (2014) found that phosphate-solubilizing bacteria, i.e., *B. thailandensis*, *B. seminalis*, and *Sphingomonas pituitosa* effectively mitigated the acidity of soil and also enhanced root volume and dry seedling weight of rice.

Rhizobium and *Bradyrhizobium* present in the root nodules of pulse crops convert the free atmospheric N_2 into an available form, which is exploited by the plants for their benefit (Sharma et al. 2011), while *Bacillus pumilus* S1r1 inoculation gave an alternative approach of delaying nitrogen remobilization of naturally fixed atmospheric nitrogen in maize plant resulted in higher yield with less utilization of nitrogenous fertilizers (Kuan et al. 2016). Bakhshandeh et al. (2015) affirmed the phosphate solubilization activities of *Pseudomonas*, *Penicillium*, *Bacillus*, *Micrococcus*, *Sclerotium*, *Flavobacterium*, *Aspergillus*, and *Fusarium* that enhance growth and yield of the crop. In a study by Zhou et al. (2016), it was discovered that plants inoculated with *Paenibacillus polymyxa* BFKC01 activated iron acquisition machinery to boost iron assimilation, which increased the plant's photosynthetic efficiency and growth. *P. aeruginosa*, *P. fluorescens*, *Mycobacterium* spp., *Haemophilus* spp., *Rhodococcus* spp., and *Paenibacillus* spp. are just a few of the rhizospheric microbiomes that can break down polyaromatic hydrocarbons that are present there (Bisht et al. 2015). Besides these, rhizobacteria such as *Actinobacteria*, *Microbacterium*, and *Verrucomicrobia* and fungi such as *Lewia* spp. and mycorrhizal (VAM) fungi are also probable candidates of rhizospheric remediation as they

change the movement and bioaccessibility of metals, thus increases their uptake via plants (Cruz-Hernandez et al. 2012; Kawasaki et al. 2012; Yang et al. 2016).

8.3.3 Biotic Stress Management

Many diseases are being controlled using biocontrol agents. Mitigation of plant stress can be resolved through the intervention of microbes. This has been demonstrated in various pathosystems, e.g., pulse pathosystems (Ghatak et al. 2010) and vegetable pathosystems (Ghatak 2020). Moreover, biofumigation of the fields can render in satisfactory inhibition in soil-borne disease problems (Srivastava and Ghatak 2017). The nonchemicals have been proved to be good in postharvest storage systems (Prakash et al. 2016; Kumar et al. 2018).

The rhizosphere provides a platform for both the combat zone to the roots of plant against soil-borne pathogenic microorganisms (Cook et al. 1995) and an outbreak of the disease amid plants through establishing a plant–parasitic relationship (Raaijmakers et al. 2009). Soil-borne pathogens exert a negative effect on crop production by producing several diseases (Bruehl 1987). Induction in the stress due to plant pathogenic microbes in the community of the rhizosphere can lead to variations in the composition of the microbiome and antagonistic effects of the beneficial microbiome. Thus, positive interactions among the plants, pathogens, and the rhizospheric microorganisms lead to the development of a protective plant microbiome (Chapelle et al. 2016). The rhizosphere microbiome members can restrict the pathogens born in the soil both before and throughout primary or secondary infection in the tissues of root (Mendes et al. 2013). Antibiosis, rivalry for space and nutrients, parasitism, and induction of systemic resistance are the main mechanisms by which rhizospheric microorganisms discourage the infection of plant pathogens (Sehrawat and Sindhu 2019). Most rhizobacteria and some rhizosphere fungi produce antibiotic metabolites to keep a check on the growth or development of pathogenic organisms (Hoffmeister and Keller 2007; Brakhage and Schroeckh 2011). Various antibiotics were produced by different strains of *Trichoderma* such as *T. viride* (trichotoxins, trichodecenins, trichorovins, and trichocellins), *T. harzianum* (trichorzianins, trichorzins, HA, and MA), *T. longibrachiatum* (tricholongins), *T. koningii* (longibrachins and trichokonins), and *T. atroviride* (atroviridins and neoatroviridins). Additionally, several other antifungal and antibacterial metabolites, i.e., koningins, viridin, dermadin, trichoviridin, lignoren, and koningic acid, were isolated from the cultures of different *Trichoderma* strains (Reino et al. 2008). Numerous rhizobacteria belonging to the genera *Bacillus*, *Serratia*, *Pantoea*, *Agrobacterium*, *Pseudomonas*, *Stenotrophomonas*, and *Streptomyces* produce several broad-spectrum antimicrobial metabolites (Kohl et al. 2019). Several lipopeptides (iturin, surfactin, and fengycin) and antibiotics (DAPG, pyrrolnitrin, and phenazine) have been produced by *Bacillus* and *Pseudomonas* species, respectively (Ongena and Jacques 2008; Raaijmakers and Mazzola 2012). *Pseudomonas fluorescens* produces an antibiotic 2,4-diacetylphloroglucinol (DAPG) that suppresses soil-borne pathogens like *Meloidogyne incognita*,

Fusarium oxysporum (Meyer et al. 2016), *Rhizoctonia solani* (Mazzola and Gu 2002), and *Gaeumannomyces graminis* var. *tritici* (Mazzola 2002; Weller et al. 2002). Most of the strains of biocontrol microbes produce numerous antibiotic compounds with a variable magnitude of antimicrobial activity. Agrosin 84, a bacteriocin secreted via *Agrobacterium radiobacter* (Kim et al. 2006), showed antibiotic activities to its closely linked strains or genus, although polyketide and non-ribosomal peptides antibiotics display its broad-spectrum nature (Raaijmakers et al. 2010). Gliotoxin and gliovirin antibiotics produced by P and Q group strains of *Trichoderma* were found effective against *Pythium ultimum* and *R. solani* (Howell et al. 2000). Similarly, Dunlop et al. (1989) found an antimicrobial substance (Koninginin D) produced by *T. koningii* that suppresses the pathogens of soil such as *Bipolaris sorokiniana*, *F. oxysporum, G. graminis* var. *tritici*, *Phytophthora cinnamomi*, *P. middletonii*, and *R. solani*. Harzianic acid synthesized by *T. harzianum* showed its antibiotic activities against *Sclerotinia sclerotiorum*, *P. irregular*, and *R. solani* (Manganiello et al. 2018), whereas viridins derived from *T. koningii*, *T. viride*, and *T. virens* delimits spore germinations in *Aspergillus niger*, *Botrytis allii*, *Colletotrichum lini*, *F. caeruleum*, *Penicillium expansum*, and *Stachybotrys atra* (Singh et al. 2005). When antibiotic activities are combined with lytic enzyme activities, additional antagonistic effects are produced. This was observed by Howell (2003) in *B. cinerea* and *F. oxysporum* where an initial disintegration of cell walls boosted the penetration of antibiotics into the hypha of fungus. High concentrations of antibiotics act as a growth inhibitor, while at low concentration it acts as a mediator of intercellular signaling that shows its function in a concentration-dependent manner (Romero et al. 2011). *Enterobacter cloacae* suppressed *P. ultimum* by competing for nutrients in the spermosphere (Van Dijk and Nelson 2000). Kloepper et al. (1980), first reported pseudobactin, a type of siderophore produced by *P. fluorescens* against *Erwinia carotovora*. The production of siderophores by *B. subtilis* against *F. oxysporum* was also reported by Yu et al. (2011). Several biocontrol agents such as *A. niger*, *P. citrinum*, and *T. harzianum* are also found to produce siderophores that reduce diseases and enhance the growth of chickpea crops (Yadav et al. 2011).

Hyperparasitism is a process, where one fungus parasitizes another one or a direct competition among two parasites where one gains benefits over the other. It is most common in fungus rather than bacteria. *Bdellovibrio bacteriovorus* is a predatory bacterium, and its specific strains parasitize different plant pathogenic bacterial genera such as *Agrobacterium*, *Xanthomonas*, *Erwinia*, *Pseudomonas*, and *Burkholderia* (McNeely et al. 2017).

Induced systemic resistance (ISR) is a mechanism in which resistance hostile to infection is established by increasing either physical or chemical barriers, or both in the host plant. Rhizospheric bacteria can modify the plant immune system by inducing ISR in plants and the ISR is regulated via either jasmonic acid, ethylene, or salicylic acid pathway (Zamioudis and Pieterse 2012; Van de Mortel et al. 2012) depending on strains, while some other rhizobacteria such as *B. cereus* strain AR156 can produce resistance in the plant systemically via triggering both the signaling pathways (Niu et al. 2011). The siderophores (pyoverdine and pyochelin) produced

by *P. aeruginosa*, and its originator salicylic acid, can generate resistance in opposition to the pathogen-causing gray mold of bean and tomato, anthracnose of beans, and tobacco mosaic virus disease of tobacco (Bigirimana and Hofte 2002; Hofte and Bakker 2007). Srivastava et al. (2016) also found that *B. amyloliquefaciens* (SN13) enhances tolerance against *R. solani* by modulating phytohormones (JA/ET/SA) signaling, maintaining elicitors, producing specialized metabolites, and ROS scroungers, thus balancing reactive oxygen species. Similarly, in cucumber, resistance against *C. orbiculare*, *F. oxysporum*, *Cucumber mosaic virus*, *P. syringae*, and *E. tracheiphila* has been induced via the production of catechol-type siderophore through *Serratia marcescens* 90–166 (Press et al. 2001). Besides PGPR bacteria, fungi such as mycorrhizal fungi (Pozo and Azcon-Aguilar 2007), *Trichoderma* spp. (Segarra et al. 2009) and other fungal biocontrol agents (Shoresh et al. 2010) have also been seen to induce ISR. Different modifications such as the solidification of epidermal and cortical cell walls through callose, lignin, and phenolics deposition; enhanced amount of enzymes such as chitinase, phenylalanine ammonia-lyase, peroxidase, and polyphenol oxidase; improved phytoalexin production; and expression of stress-related genes have been seen in resistance-induced plants (Heil and Bostock 2002; Whipps 2001a, b; Yi et al. 2013). According to Li et al. (2016), *Enterobacter asburiae* BQ9 was found to induce tomato yellow leaf curl virus resistance by enhancing the expression of defense-related genes and antioxidant enzymes, together with PAL, catalase, and superoxide dismutase. Similarly, *Paenibacillus lentimorbus* B-30488 induces resistance against cucumber mosaic virus by increasing expression of the pathogenesis-related gene and antioxidant enzyme activity in the plant (Kumar et al. 2016).

Nematodes and soil-borne insect pests are also the uttermost severe crop delimiting factors existing in soil and their interactions with the rhizosphere microbiome offer several opportunities to know the effect of the rhizosphere microbiome on these organisms. Rhizosphere microorganisms may balance the growth and reproduction of several nematodes and insect pests using suppressive soils (Gine et al. 2016). However, because of their high fecundity in the availability of a suitable host plant, monoculture practices may alter this balance. Kerry (2000) found that *Verticillium chlamydosporium* was also able to infect eggs and female cyst nematode. Son et al. (2009) revealed that *Paenibacillus polymyxa* and *P. lentimorbus* showed potent antifungal activities, thus interferes using the interaction amid *M. incognita* and *F. oxysporum* and associated nematode invasion in plants of tomatoes. Similarly, both *B. pumilus* isolates ZHA90 and *P. castaneae* isolates ZHA296 and ZHA178 affected the root galling in treated plants while isolates ZHA296 and ZHA178 of *P. castaneae* affected only egg masses of *M. incognita* (Cetintas et al. 2018). Manzanilla-López et al. (2013) discovered that *Pochonia chlamydosporia* acts as a parasite of nematode eggs. Nematophagous fungi such as *Arthrobotrys oligospora* and *A. dactyloides* can trap motile nematodes in the rhizosphere of the plant by using specialized hyphal organs (Nega 2014). Elhady et al. (2018) examined the effect of soil microbiome on infestation and multiplication of root-knot nematode and the root-lesion nematode and found that maize or soybean microbiome affected the root invasion by *P. penetrans* while in

both *P. penetrans* and *M. incognita*, root invasion got affected by maize and tomato rhizosphere microbiomes contrary to soybean or bulk soil. Additionally, root-knot nematodes were highly inhibited in the tomato rhizosphere microbiome. *Klebsiella pneumoniae* induced systemic resistance in treated plants by enhancing the expressions of a few defense-related genes, including pathogenesis-related genes PR1, PR2, and PR5 or plant defensin gene pdf1.2 (Liu et al. 2018a, b). Imperiali et al. (2017) revealed that *Pseudomonas* and arbuscular mycorrhizal fungi (AMF) significantly antagonized the infestation of fruit fly (*Oscinella frit*) in the wheat crop when applied alone or in combination at wheat seedlings. A common soil-inhabiting actinomycete, *Streptomyces avermitilis*, was revealed to produce avermectins molecules derived from lactones, effective against several arthropods and nematodes by acting on their outer nervous system through affecting the γ-aminobutyric acid (GABA) receptors, thus inciting paralysis in them (Vurukonda et al. 2018). The entomopathogenic nematodes, *Steinernema* and *Heterorhabditis*, can manage soil-dwelling insects by infecting them naturally (Barbercheck 2019). Glare and O'Callaghan (2000) reported different species of *B. thuringiensis* to affect diverse insect pests such as *B. thuringiensis* subsp. *kurstaki*, *B. thuringiensis* subsp. *aizawai* strain ABTS-1857 (armyworms and diamondback moth larvae), *B. thuringiensis* subsp. *israelensis* and *tenebrionis* (coleopteran larvae). On the other hand, different entomopathogenic bacteria, *Brevibacillus laterosporus*, strains have been found effective against insects of several orders (Diptera, Lepidoptera, and Coleoptera), mollusks, nematodes, phytopathogenic bacteria, and fungi (Ruiu 2015). Besides this, He et al. (2014) reported that whole-cell broth cultures of *B. laterosporus* strain A396 were toxic to *Spodoptera exigua* and *Tetranychus urticae*.

Therefore, the interaction of more than one microbe inside the rhizosphere offers greater pathogen antagonisms, besides modifying the immune system of the plant. As there are numerous antagonists present in the soil at the same time, it is commendable to check the combined application of these microorganisms that may produce collaborative effects. The data from the experiment indicate this, but it is difficult to witness in normal situations.

8.4 Interaction Between Plant and Rhizospheric Microbiome

With the world's population growing at an ever-increasing rate and diminishing food resources, producing enough food in a sustainable manner has become a major challenge (Ganguly et al. 2021). A wide range of biotic stresses, such as pathogens and herbivores, also impede food plant production (Iriti and Faoro 2009; Gust et al. 2010; Thakur and Sohal 2013). Innate immunity is how plants defend themselves. In the rhizosphere, there is a complex interaction between plants, soil microbes, and soil (Van Dam and Bouwmeester 2016). The root-associated soil's physical, chemical, and biological characteristics have an impact on the variety (population and activity) of microorganisms in this region (Barea et al. 2002). These microbes provide the plant with a variety of services and benefits in exchange for the plant, providing reduced carbon and other metabolites to the microbial community.

A healthy colony of microorganisms is always present around a plant that is growing in the field (Turner et al. 2013; Lebeis 2014; Bulgarelli et al. 2015). This community is known as the phytomicrobiome; the halobiont is made up of both the phytomicrobiome and the plant (Berg et al. 2016; Theis et al. 2016; Smith et al. 2017). All eukaryotic and multicellular creatures most likely share a microbiome. In fact, they probably existed before the plants colonized the continent (Berg et al. 2014). Since the beginning of time, this microbial community has been linked to terrestrial plants, assisting early land plants in overcoming the challenges such as nutrient availability, novel and frequently stressful conditions, and pathogens (Smith et al. 2017).

Plant physiology and development are influenced by microbial communities, which play a vital role in their functioning. Plant-associated microorganisms promote plant growth and influence crop quality and yield by mobilizing as well as by transporting nutrients. As a result, it appears that the rhizosphere microbiome is one of the most important determinants of plant productivity and health. Plant roots can influence the rhizosphere microbiome by creating chemical niches in the soil, which is mediated by the release of phytochemicals (i.e., root exudates) and is influenced by genotype, nutritional status, soil properties, and climatic conditions. Both harmful and beneficial microbes live in the rhizosphere, which can have a significant impact on crop yield and plant growth (Beneduzi et al. 2012; Vacheron et al. 2013; Garcia-Fraile et al. 2015). Microbes that increase nutrient availability and reduce soil-borne diseases include symbiotic bacteria, free-living bacteria, actinomycetes, and mycorrhizal fungi (Garcia-Fraile et al. 2015). While many rhizosphere microbiome members are capable of stimulating growth, plant pathogenic microbes penetrate the rhizosphere to spread illness by breaching the protective microbial shield and avoiding the inherent plant defense systems. Plant growth-promoting rhizomicrobes/bacteria (PGPR) elicit plant resistance or defense priming while their roots engage in intricate chemical communication with the microbes in the rhizosphere, leading to the creation of helpful microbe biofilm. Beneficial microbes penetrate plant roots in the rhizosphere, triggering an ISR (induced systemic response) and enhancing the plant's defense mechanisms against diseases that infect the leaves (Hilker et al. 2015).

8.4.1 Impact of Plant and Microbiome Interaction on Plant Health, Growth, and Disease

Rhizosphere bacteria have an impact on the composition and productivity (i.e., biomass) of natural plant communities both directly and indirectly (van der Heijden et al. 1998, 2006, 2008; Schnitzer et al. 2011). It has been proposed that the variety of below-ground microbial species can be utilized to predict the variety and productivity of above-ground plants (De Deyn et al. 2004; van der Heijden et al. 2008; Lau and Lennon; Wagg et al. 2011). According to Wagg et al. (2011), the below-ground diversity may serve as a type of insurance for maintaining plant productivity in a range of settings.

Due to their sensitivity to minute changes in abiotic circumstances, such as environmental stress and perturbation, soil and rhizosphere bacteria are regarded as bioindicators of soil quality. These microbes help plants grow and defend them against pathogens in a number of different ways (Lugtenberg and Kamilova 2009; Raaijmakers et al. 2009). Biofertilization, root growth stimulation, rhizoremediation, abiotic stress management, and disease management are a few of these. Deuteromycetes fungi, *Trichoderma* and *Gliocladium*, as well as Proteobacteria and Firmicute rhizobacteria, such as *Pseudomonas* and *Bacillus*, have well-researched mechanisms (Kogel et al. 2006; Qiang et al. 2012).

8.4.2 Rhizosphere Microorganisms and Acquisition of Plant Nutrient

In the laboratory assay, it has been demonstrated that the growth of *Magnaporthe* isolates originating from rice and finger millet is determined by the kind of nutrition (Balodi et al. 2015). The microbiome of the rhizosphere has a substantial impact on plant nutritional status. Rhizobia that fix nitrogen and mycorrhizal fungi that help the uptake of phosphorus by plants are two well-known examples (Hawkins et al. 2000; Richardson et al. 2009; Miransari 2011). For instance, mycorrhizal fungi are well-known and well-documented for their roles in the soil's physical structuring and formation of stable soil aggregates (Degens et al. 1996; Miller and Jastrow 2000), the transfer of nutrients and minerals from the soil to plants (Gianinazzi et al. 2010; Adeleke et al. 2012), and the suppression of soil-borne plant pathogens (Whipps 2001a, b; Pozo (Smith and Read 1997; Kapulnik and Douds Jr 2000; Brundrett 2002; Salvioli and Bonfante 2013). Rhizobacteria produce or exude chemicals that are helpful to plants and can cause particular alterations or adjustments to the plant transcriptome in order to form a symbiotic relationship. Plant-produced phytohormones, such as auxins, gibberellins, and cytokinin, are growth and defense regulators, and PGPR can produce these compounds as well (Fahad et al. 2015). PGPR produces volatile chemicals that help to maintain soil health, modulate plant growth, and induce resistance (Wei-wei et al. 2008; Kai et al. 2009). *Bacillus* species dominate several PGPR genera, with *Pseudomonas* being the most preferred beneficial group due to its many features such as plant growth stimulation, disease control, and bioremediation. PGPR may reduce soil-borne pathogen infections either directly (through metabolism inhibition) or indirectly (through competition). Some PGPR (*Bacillus* and *Pseudomonas* spp.) produce antibiotics, lytic enzymes that inhibit soil-borne pathogen growth, toxins against insect pests, and siderophores.

Plants benefit from siderophore synthesis because it provides direct iron to them and is linked to the suppression of soil-borne disease (by lowering pathogen competitiveness) (Tank et al. 2012). Thus, plant diseases may be prevented by PGPR in the rhizosphere in a variety of ways, including by competition for available nutrients, preventing pathogen–plant root contact, and by interfering with the mechanisms that cause plant infection (Saraf et al. 2005, 2014). In addition to *Rhizobium* and *Bradyrhizobium*, several other genera of nitrogen-fixing bacteria

have also been identified in the rhizosphere (Zehr et al. 2003; Gaby and Buckley 2011).

Some trace metals, including iron, are also helped to be assimilated by microorganisms in the rhizosphere. Although there is a lot of iron in soil, it is generally insoluble as ferric oxide under neutral to alkaline conditions, which prevents microbial development. Because free iron is poisonous at high quantities and is extremely scarce in many microbial habitats, bacteria use a variety of processes to control the levels of iron inside their cells, including the release of siderophores (Lindsay and Schwab 1982; Andrews et al. 2003; Buckling et al. 2007; Hider and Kong 2010).

8.4.3 Metabolomics: The Plant–Rhizomicrobes Interactions

Metabolomics is a data-driven, hypothesis-generating scientific method that is well suited to the examination of complex interactions. Each analysis can discover and quantify hundreds of compounds (Lloyd et al. 2015). Furthermore, it greatly simplifies the modeling of reciprocal responses between plants and other rhizosphere-dwelling species. This novel strategy for metabolomics studies of the host–pathogen interaction will help us to understand both the autonomous metabolism of the pathogens and the metabolic cross talk that constitutes the interactome. This endeavor has been made easier by recent advances in excellent selectivity, precision, and robustness in analytical instrumentation and analysis as well as software developments for data processing and the availability of public databases. As a result, owing to these developments, researchers may now look into how a biological system interacts with its environment as well as just one part of it (Rochfort 2005; Lloyd et al. 2015; Tenenboim and Brotman 2016; Van Dam and Bouwmeester 2016).

8.5 Strategies for Health Management of Rhizospheric Microbiome

The rhizospheric microbiome is diverse in nature and has a great impact on the physiology and development of plants, leading to affect health of the consumer. These microbial communities often get affected by multiple factors such as abiotic stress, host genotypes, and microbes interactions (Mendes et al. 2013; Foo et al. 2017). For this reason, it draws the interest of researchers to develop strategies for improving or reshaping the rhizospheric microorganisms, thereby ensuring an enhancement in crop productivity and plant as well as human health. The comprehension of the underlying actions and plant–rhizomicrobe interactions involved proposes vivid strategies through which the rhizosphere can be structured for better health of both plant and soil (Ryan et al. 2009). A number of these means of health management or improvement of rhizomicrobiomes have been nattered in the following subsections.

8.5.1 Cultural Practices for Health Management of Rhizospheric Microbiome

The use of organic amendments improves soil quality and fertility. Bausenwein et al. (2008) reported an increase in chemotrophic bacterial populace and soil carbon due to the incorporation of *Prunus dulcis* shells. The populations of either total and spore-producing bacteria or rod-shaped branched bacteria increased by incorporation of animal manure in the soil but did not relate to organic carbon matter of the soil. Small additions of compost can have lasting benefits (Ryals et al. 2014).

Intercropping is an influential approach to encourage a more expanded group of plants in the field, thereby permitting harmonizing and facilitative associations. It is generally assisted by an increased number of microbes in the soil, and therefore enhanced enzymatic activities of soil (Chai et al. 2005). The plant could get nutrients through the microbial breakdown of the crop refuses. The composition and populations of soil microbes were found to be high in sugarcane–soybean cropping in comparison to either sugarcane or soybean cropping in an area.

Cover cropping and plasticulture affect both soils and rhizospheric microbial populations. This activity changes the soil environment to some extent in terms of altering the moisture and temperature of the soil. Positive impacts of the cover crop on microorganism biomass and population structure were seen in the tomato cropping system (Buyer et al. 2010). In another study, increased microbial biomass of soil due to integration cover crop has been reported (Doran et al. 1987; Wang et al. 2007).

Soil health is dependent on the input used in the farm for crop production. Agrochemicals (pesticides and fertilizers) play a major role in modern farming in terms of obtaining higher productivity. However, improper and adequate use of agrochemicals may increase the risk of contamination in environmental components (soil, water, and air) (Ramwell et al. 2004). Soil and water are very prone to contamination on the use of agrochemicals. Soil ecosystem contains a large number of living organisms, which includes macro- and microorganism. Microorganism maintains soil health and supports the plant system for its growth.

The widespread use of agrochemicals in crop production activities has negative effects on the surroundings, including challenges to food safety, increased cost-effectiveness, energy conservation and emission, pesticide resistance impacts on beneficial invertebrates, and agricultural non-point source pollution (Klaine et al. 2010; Velasco et al. 2012; Kriti and Ghatak 2021). The integration of clothianidin or organic fertilizer on the diversity, structure, and function of the rhizospheric bacterial population was shown by Huang et al. (2020), who investigated how a pesticide-fertilizer affects soil environment of sugarcane. When a bacterial population was exposed to the pesticide clothianidin–fertilizer, it showed more variety than the control.

8.5.2 Microbial Inoculation in Soil

Microbial inoculation is the most beneficial approach to diversify and improve the health of the rhizospheric microbiome. In this strategy, the introduction of beneficial rhizospheric microorganisms has been done at deficient or degraded sites, with the aim of restoring or enriching those environments (Wubs et al. 2016), and the best approach of restoration is the introduction of disease-suppressive soil as well as the direct introduction of microorganisms in the soil. Mixing disease-suppressive soil to conducive soil is an effective way of introducing beneficial rhizospheric microorganisms to the disease-prone area, to reduce the disease, and to enrich the rhizospheric microbiome diversity and also its health. Induced disease suppression in the take-all disease of wheat and the take-all patch of turfgrass is widely known by using root-colonizing *Pseudomonas* bacteria-enriched suppressive soil (Sarniguet and Lucas 1992; Weller et al. 2002). Kyselkova et al. (2009) used 16S rRNA taxonomic microarray to discern the rhizospheric microbiome community of suppressive soil of *Thielaviopsis basicola*-mediated tobacco black root rot and found taxa *Azospirillum*, *Gluconacetobacter*, *Burkholderia*, *Comamonas*, and *Sphingomonadaceae*, along with fluorescent *Pseudomonas*, which are widely known for containing plant-beneficial properties. Similarly, Mendes et al. (2011) used *R. solani* suppressive soil against the conducive ones in 1:9 ratio to prevent its infection in the sugar beet plant. Metagenomic analysis by using PhyloChip showed the presence of 17 different bacteria communities belonging to the phylum Proteobacteria, Firmicutes, and Actinobacteria. The comparative difference has been reported to occur between the diversity of microbial community present in potato scab disease-suppressive soil and the conducive soil. A higher magnitude of relative abundance of genus *Bacillus* was found to exist in disease-suppressive soil (Rosenzweig et al. 2012). Siegel-Hertz et al. (2018) analyzed the suppressive and non-suppressive soils from different sites in France to determine the taxonomic diversity of fungal and bacterial communities using the comparative metabarcoding analysis technique. They found 10 different fungal genera (*Acremonium*, *Penicillium*, *Cladosporium*, *Fusarium*, *Clonostachys*, *Chaetomium*, *Mortierella*, *Verticillium*, *Ceratobasidium*, and *Scytalidium*) and 11 bacterial genera (*Adhaeribacter*, *Arthrobacter*, *Amycolatopsis*, *Geobacter*, *Massilia*, *Microvirga*, *Paenibacillus*, *Rhizobium*, *Rhizobacter*, *Rubrobacter*, and *Stenotrophomona*) in suppressive soil, and several genera among them have been known to be effective in managing *F. oxysporum*. Ou et al. (2019) found the presence of bacterial genera *Chryseolinea*, *Terrimonas*, and *Ohtaekwangia* as a major group in the suppressive soil of banana field that confers suppressiveness against *Fusarium* wilt of banana. Besides this, the introduction of microorganisms can be done directly into the soil or through the seed/seedling treatment to maintain the health of the rhizospheric microbiome through reducing harmful microbes and increasing the nutrients composition in the rhizosphere. The introduction of several beneficial fungal strains along with rhizobacterial strains having different traits into the soil or onto seeds or planting materials could uplift the performance of the plant (Bhattacharyya and Jha 2012). For instance, *Streptomyces* isolates cultured from the rhizosphere of apple

plants were found to be efficient in managing leaf spot disease in the oil palm seedlings when used as a biocontrol agent (Sunpapao et al. 2018). Numerous nitrogen-fixing endophytic bacterial genera such as *Rhizobium* and *Bradyrhizobium* as well as phosphate-solubilizing bacteria such as *Pseudomonas* and *Bacillus* and fungi such as *Aspergillus* and *Penicillium* have been found to have positive effects on crops and the rhizosphere by increasing both above- and below-ground biomass (Mohanram and Kumar 2019). The use of combinations of different microbial strains has been advantageous for the rhizosphere because it enhances the diversity of root zone microorganisms by limiting the struggle among them and also promotes the growth of plants by mitigating stress conditions and providing better nutrition to them. A study, where *Bradyrhizobium japonicum* was inoculated with *Azospirillum brasiliense*, supported the phytostabilization of arsenic (Armendariz et al. 2019). Similarly, when *Rhizobium tropici* was co-inoculated with *Chryseobacterium balustinum*, it enhanced the growth of French bean, in both salines and controlled situations (Estevez et al. 2009). Both the experiments reported a positive effect on microbiome health through the establishment and multiplication of beneficial microbes in the rhizosphere. These favorable reactions were seen due to the synergistic effect of the combination of microorganisms in the soil (Bellabarba et al. 2019). The simultaneous introduction of beneficial microbes has been also carried out by some researchers. For instance, five root-associated bacteria were introduced in soil and this was able to protect *Nicotiana attenuata* from a sudden-wilt disease. This enriched the microbiome composition by introducing them in the rhizosphere (Santhanam et al. 2015). However, the successful establishment and survival of inoculated microbes is still a major hurdle in the management of rhizospheric microbiome health. Despite the prevailing bottlenecks, these techniques give us promising opportunities to improve the health of the rhizospheric microbiome.

8.5.3 Recruitment of Beneficial Organisms

Rhizodeposition or the discharge of root leachates and volatile substances into the soil significantly alters the rhizosphere of plants (Jones et al. 2009). These leachates or exudates may comprise sugars, amino acids, organic acids, phenolics, secondary metabolites, or proteins (Badri and Vivanco 2009) and enhance the nutrient acquisition, help to mitigate mineral stress, and consequently favor beneficial microorganisms. Reportedly, the application of maize mucilage as a soil amendment improved the production of N_2O in soil (Mounier et al. 2004). In another instance, nitrate reduction and denitrification were also promoted by amendment with artificial root exudates (ARE) that simulated maize exudates (Henry et al. 2008). Micallef et al. (2009) elaborately studied eight *Arabidopsis thaliana* accessions using RISA and 16S-TRFLP analyses and found that every accession harbored a definite rhizobacterial community and secreted a distinct array of chemical compounds. Some root exudates have a far larger ability to influence the composition of microbiota than other substances (Shi et al. 2011). Phenolic compounds showed a significant positive link with bacterial operational taxonomic units (OTUs) when

leachates of 18- to 21-day-old *Arabidopsis* plants were added to fallow soil, while bacterial OTUs showed negative relationships with amino acids, carbohydrates, and alcohols (Badri et al. 2013). The symbiosis between plants and rhizobium or even mycorrhiza is influenced by the root exudates such as flavonoids or strigolactones that sustain the growth and activity of microbiota near the root zones of plants (Schiltz et al. 2015; Perez-Jaramillo et al. 2016; Nelson 2017). Definite association of useful bacteria, i.e., plant growth-promoting rhizobacteria (PGPR), may also have an impact on root exudates. For instance, Rudrappa et al. (2008) reported that the infection of *Pseudomonas syringae* pv. *tomato* in *Arabidopsis* leaves elevated the secretion of malic acid from the roots. *Pseudomonas fluorescens* WCS365, *Paenibacillus polymyxa* SQR- 21, *Bacillus amyloliquefaciens* SQR9, *B. subtilis* N11, and *Bacillus subtilis* FB17 have been shown to exhibit a positive chemotactic response toward L-malic acid and citric acid exuded by roots of different crops such as tomato, cucumber, banana, and watermelon (de Weert et al. 2002; Ling et al. 2011; Zhang et al. 2014). Interestingly, the establishment of *B. subtilis* strain FB17 on *Arabidopsis* was also induced by foliage treatment with microbe-associated molecular patterns (MAMPs) that enhanced the activity of the root malic acid transporter (ALMT1) (Lakshmanan et al. 2012). Several additional substances found in root exudates may encourage helpful bacteria to colonize the roots. The root colonization of *Pseudomonas putida* KT2440 is encouraged by the chemoattractant 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) released by maize roots (Neal et al. 2012). Finally, chemicals from plants may also influence the expression of genes in bacteria that produce antifungal substances. In *P. fluorescens* CHAO, the phlA and pltA genes, for example, control the synthesis of the antifungal compounds 2,4-diacetylphloroglucinol (DAPG) and pyoluteorin (PLT). However, a number of chemical substances originating from plants, as well as various plant phenolics and pectin, can influence how it expresses (de Werra et al. 2011). In a seemingly intrinsic defense response, barley plants infected with *Pythium ultimum* secreted phenolic and organic acids that promoted the expression of the phlA factor of *P. fluorescens* CHAO (Jousset et al. 2011). Similar mechanisms operate against insect pests as well. Roots of *teosinte* and the European maize lines secreted a volatile sesquiterpene compound, (E)-β-caryophyllene, that could attract an insect-infecting nematode counter to an insect infestation (Rasmann et al. 2005; Kollner et al. 2008). These findings show the ability of plants to engage and stimulate the beneficial rhizomicrobiome members through the production of specific compounds in their root exudates. On the other hand, these exudates can have a detrimental effect on communities underground. In a study evaluating how a weed, *Centaurea maculosa*, affects the composition of mycorrhizal plants (arbuscular, AMF), it was discovered that the amount and variety of AMF plants decreased in comparison to samples of indigenous grasslands (Mummey and Rillig 2006). It is fair to assume that plant domestication will significantly alter root exudation profiles, which will have an impact on the composition and functionality of the rhizosphere microbiome, even though the evidence currently available is insufficient to draw strong conclusions.

8.5.4 Genetic Manipulation of Plants

In the rhizosphere, plants are the primary determinants of microbial populations. They have devised a number of functions and tactics for modifying the rhizosphere in order to minimize environmental pressure (Table 8.3). Plant genetic engineering is a novel field of study, thus there are still certain obstacles to be addressed before a

Table 8.3 Pros and cons of different strategies used in health management of rhizosphere microbiome

Strategies	Pros	Cons
Cultural practices	Widely used in agriculture Adds to organic carbon and nutrients (mainly N and P) in soil Improves soil physical and chemical properties Increases microbiome diversity in soil rhizosphere	Not well understood
Microbial inoculation and recruitment of beneficial organisms	Easy to introduce in soil Promotes plant growth and development by providing nutrients either by nitrogen fixation or phosphorus solubilization Management of abiotic stress in plant Reduces biotic stress in plant through antibiotics and siderophores production, hyperparasitism or induced resistance (SAR/ISR) Production of plant growth promoting hormones Enriches soil fertility and rhizospheric microbiome diversity	Establishment and survival of inoculates are difficult Applicable for only culturable microorganisms
Genetic manipulation	Manipulated plant induces beneficial functions of microbiome such as production of siderophores, antibiotics against biotic stress factors Improves resistance against abiotic conditions May be applied for bioremediation of soil contaminants Blocks communication among the plant and their harmful microbial community, thus increases plant disease resistance Establishes a direct interaction between the plant and microbiome	The compound produced by modified plants might get inactive and degraded or their rate of release might be too slow to act as a determining factor in the rhizosphere Communication blockage might occur in between plant-associated beneficial microbial community Establishment and survival might be difficult for modified organism

successful outcome can be reached. Phenotypes in transgenic plants can be undetectable, mild, or even completely absent. The next section discusses other approaches that involve genetically engineering plants to create substances that either affect the growth of specific bacterial populations in rhizosphere or change their biochemical performance.

The opine model: Several research have produced transgenic plants that leak xenotropic substances into the rhizosphere. In a certain habitat, xenotropic chemicals are those that do not normally occur there. It is suggested that low molecular weight derivatives of amino acids that are present in tumors caused by *Agrobacterium tumefaciens* are the substances that these research are focusing on (Dessaux et al. 1998). Plants with one to three biosynthetic genes are capable of producing and releasing them into the rhizosphere (Savka et al. 1996).

In a study, bacteria from *Lotus corniculatus* plants that were genetically modified to produce opines were compared to those from plants that were nearly isogenic to the wild type (Oger et al. 1997). The population densities of sporulating bacteria, thermotolerant bacteria, agrobacteria, pseudomonads, and bacteria in the rhizospheres of the transgenic and control plants were the same. However, opiate-producing plants' rhizospheres had two to three orders of magnitude more bacterial populations that could break down opines than did plants of the wild type. Additionally, the quantity of opinions produced by the transgenic plants was correlated with the size of this community (Oger et al. 2004). A few modifications were also found. The fraction of pseudomonads that could break down opium was larger in the rhizosphere of opium-producing plants than in the rhizosphere of wild-type plants, even though the total density of all pseudomonads was unaffected by opium production. However, this change in the makeup of the population was dependent on the kind of opiates generated (Oger et al. 1997). The population density of opine-degrading microorganisms gradually decreased but remained higher than in control tests with only wild-type plants when opine-producing plants were eliminated from the soil and replaced (Oger et al. 2000). Three different plant species—*Lotus corniculatus*, *L. japonicas*, and *Solanum nigrum*—as well as two different soil types—a clay-rich soil and a sandy-loam soil—showed evidence of the opine-induced bias, indicating that it was not unique to any one soil or plant system (Mansouri et al. 2002). These findings support three main conclusions. First, they demonstrated that plant exudates directly affected the rhizosphere microflora's composition. Second, they showed how the rhizosphere's "bias" can persist past the time when opinions are produced, which is a noteworthy property for ecological engineering. In addition to root exudation that is occurring at the time of the inquiry, they also note as a precaution that previous root exudation has an effect on the structure and function of the microbial population of rhizosphere. More research has been done to evaluate whether the opine technique may be used to increase the multiplication of a single bacterial strain. An epiphytic strain of *Pseudomonas syringae* that can degrade mannityl opines showed a double- to triple-fold increase in growth when compared to control plants (Wilson and Lindow 1995). Similar investigations found that the growth of a *Pseudomonas* sp. strain in the rhizosphere that was designed to use specific opines (of opium-producing plants) was slightly

improved in roots (Savka and Farrand 1997). The *Agrobacterium* opine system is just one way that can be used to choose specific plant–bacteria interactions. A chemical called rhizopine, which is present in the nitrogen-fixing nodules of legumes, is synthesized (3-*O*-methyl-scyllo-inosamine) and degraded (by plants) in other research (Murphy et al. 1987). *Sinorhizobium meliloti* was used to identify the genes involved in rhizopine production and degradation (Saint et al. 1993; Murphy et al. 1993). None of the transgenic plants produced any rhizopine despite *Arabidopsis* expressing all three of the rhizopine biosynthetic genes (McSpadden-Gardener and de Bruijn 1998). The engineering of plant–microbe interactions based on rhizopine metabolism appears to be more challenging than anticipated. However, the tactic is effective because rhizopine-degrading bacteria prefer a rhizopine-rich environment (McSpadden-Gardener and de Bruijn 1998). This is especially important since rhizopine may encourage the growth of good bacteria that fix nitrogen. Despite some promising advances, it remains difficult for humans to develop the rhizosphere consistently and predictably. A significant scientific barrier to development is a complete understanding of the intricate chemical and biological interactions that take place in this zone. Indeed, it is exceedingly unlikely that most nations will conduct trials of genetically modified species in the near future, especially ones that may benefit the environment. It is essential that scientists continue their work in order to provide safe, sustainable, and environmentally sound alternatives to a future population that will accept them.

8.6 Conclusion

The rhizospheric microbiome mainly represents the root-associated microbiome that inhabits the rhizosphere of plants and includes both beneficial and pathogenic microorganisms that can be found either in plants or humans. These microbial communities are diverse in nature consist of thousands of bacterial and fungal taxa and are also got affected by multiple factors such as abiotic stress, host genotypes, and microbe–microbe interactions. These microorganisms have a great impact on the physiology and development of plant, and hence on human health as well (Mendes et al. 2013; Foo et al. 2017). Recent advances in molecular techniques have made it possible to precisely manipulate the genes that influence microbiome functions. The parallel advancement in biotechnology and microbiology ensures promising progress for the future. This would open the door for the creation of more environmentally friendly substitutes including organic fertilizers, fresh biocontrol agents, and potential genetically modified products. In addition to these benefits, these techniques would increase crops' resistance to abiotic stresses (such as heat, drought, and salinity), which are expected to increase the frequency under the prevailing scenario of continued climate change.

8.7 Future Prospects

Our current understanding of the rhizomicrobiome has demonstrated the fact that trivial of the underlying diversity is known to us (Quiza et al. 2015). Tremendous potential of the rhizomicrobiome members to increase worldwide crop production under climate resilient agriculture still lays unexplored (Barea 2015; Nehra and Choudhary 2015). On the application side, bio-inoculants and PGPR-based formulations may be put to use for commercial agriculture as alternatives to the synthetic agrochemicals. The plants that are native to highly saline coastal environments or geothermal soils harbor unique endophytic microbes in their vicinity (Rodriguez and Redman 2008). It may be possible to find and isolate stress-relieving bacteria in the microbiomes of plants that are prospering in such harsh settings. Therefore, methods such as next-generation sequencing of 16S rRNA can prove the prevalence of particular microorganisms and their function in sustainable agriculture while also providing a greater representation of the diversity of microorganisms.

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Detection and Management of Basal Stem Rot of Oil Palm: Classical to Modern Approaches

9

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Abstract

Oil palm (*Elaeis guineensis* Jacq.), also referred as 'Golden palm', is the most efficient oil-yielding perennial crop in the world. Unfortunately, basal stem rot (BSR) disease poses a major menace to the palm oil industry and hence to farmers' livelihoods. *Ganoderma*, the causal agent, has been known for almost a century and is still a growing economic concern without proper remedy. A crucial factor in managing the BSR disease is the lack of well-grounded diagnostic method(s) for early and accurate diagnosis. Rapid and early on-field detection is very essential for proactive management of BSR. Practice of curative methods in infected trees and their economic feasibility is a matter of great concern as the disease is asymptomatic till its advanced stages of infection. Integrated BSR disease management should employ all successful cultural practices control, chemical control and biological agents.

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Keywords

Oil palm · Basal stem rot · *Ganoderma* · Detection · Management

9.1 Introduction

Oil palm (*Elaeis guineensis* Jacq.), also referred as 'Golden palm', is world's most efficient oil-yielding perennial crop, extensively cultivated in South-East Asia. It is presumed to be originated in Africa and classified under order Arecales and family Arecaceae. Palm oil (75.45 Mt) surpasses soybean oil (60.27 Mt) and rapeseed oil (28 Mt) in terms of global vegetable oil production ranking first among the oil-yielding crops (Shahbandeh 2021). The unparalleled yield advantage of the oil palm to that of other oilseed crops in terms of seasonal long harvest and high productivity (4–6 tonnes of vegetable oil per hectare) led to expansion of oil palm cultivation in the last two decades. Oil palm is highly productive in humid tropical regions receiving high light intensity, thus extensively cultivated in Indonesia, Malaysia, Thailand, Nigeria and Colombia. Recent estimates state that oil palm occupies 19.04 million hectares of global agricultural land (0.36%) (Kushairi et al. 2018). There are two types of oils, such as palm oil and palm kernel oil, that can be extracted from mesocarp and kernel of fruits, respectively. Palm oil has worldwide demand for consumption and accounts for 15% of oil requirements of the local food industry. In addition, palm oil is exploited for biofuels, lubricants, cosmetics and other products. Palm oil is widely recognized as the healthiest oil since it is rich in phenolic antioxidants, carotene and free of trans-fatty acids and cholesterol. Cultivation of oil palm in India is also fast expanding to the tune of 3.5 lakh ha with a production of 16.33 lakh tonnes of fresh fruit bunches and 2.70 lakh metric tonnes of crude palm oil (Oil World 2020). India is one of the largest consumers of vegetable oil, and 133.5 lakh tonnes of edible oil worth Rs. 80,000 crore imported in 2020–21, with palm oil accounts for 55% of the total vegetable oil imports. India may witness a substantial increase in oil palm area and production in future in order to achieve self-sufficiency in palm oil.

The rapid expansion of oil palm cultivation in both forest and arable land is posing severe disease and pest outbreaks, threatening its commercial cultivation. Aderungboye (1977) described 32 different types of diseases and disorders in palm cultivation, emphasizing nine major diseases that greatly hamper production of palm oil. Basal stem rot (BSR), Fusarium wilt, spear rot-bud rot and sudden wither (Corley and Tinker 2003) are becoming devastating fungal diseases in the recent past. Several other diseases such as Armillaria trunk rot (*Armillaria mellea*), blast (*Pythium splendens*), Corticium leaf rot (*Corticium solani*), Marasmius bunch rot (*Marasmius palmivora*), red ring (*Rhadinaphelenchus cocophilus*) (Aderungboye 1977) and heart rot (*Phytophthora palmivora*) (Elliott and Uchida 2004) are also reported in oil palm. Of all the diseases reported in oil palm, basal stem rot disease

caused by wood rotting basidiomycete, *Ganoderma* spp., is becoming the most annihilating disease in major oil palm cultivating areas of the world (Flood et al. 2005; Chong 2010).

9.2 Basal Stem Rot of Oil Palm: Phytopathological Aspects

9.2.1 Geographical Distribution and Economic Loss

BSR is categorized as most devastating disease of oil palm cultivation in the recent years (Corley and Tinker 2003; Susanto et al. 2005). It was first described by Wakefield in 1915 from Republic of Congo of West Africa. When incidence of oil palm basal stem rot was initially reported by Thompson in 1931, the disease was of negligible economic importance. Later, with the rapid extension of oil palm cultivation from 1960, the young plantations in South-East Asian countries witnessed the real and destructive impact of BSR (Turner 1981). The disease has now been reported in every oil palm-producing regions of the world, with the most severe cases occurring in Indonesia and Malaysia, the world's largest producers and exporters of palm oil. Infection with basal stem rot reduces the number and weight of fresh fruit bunch, along with the stem weight of oil palm bunches. In extreme cases, the disease has a potential to kill more than 80% of crop stands during its normal economic life and necessitates early replanting (Razak et al. 2004). In spite of the fact that it has been known for nearly a century, it continues to be a major economic problem, with annual losses ranging from RM 225 million to RM 1.5 billion (up to US$ 500 million) (Arif et al. 2011; Ommelna et al. 2012). *Ganoderma* infection has caused 80% and 50% yield losses in Malaysia and Indonesia, respectively, during the last 40 years (Chong 2010; Chong et al. 2012a; Idris et al. 2010b; Susanto et al. 2005), and it is predicted that BSR has the potential to wipe out 860,610 hectares of oil palm farms in Malaysia by 2040 (Olaniyi and Szulczyk 2020).

9.2.2 Causal Organism

Ganoderma is a basidiomycetes fungi, grouped in the sub-phylum Hymenomycetes, order Polyporales and family Ganodermataceae (Cannon and Kirk 2007). Karsten (1881) established *G. lucidum* as the single species under genus *Ganoderma*. According to Seo and Kirk (2000), currently 322 species names of *Ganoderma* are included in species Fungorum, although the number of true species may be limited to 60–80 (Moncalvo 2000). Among them, a handful of important species are *G. applanatum*, *G. australe*, *G. boninense*, *G. cupreum*, *G. lobatum*, *G. lucidum*, *G. oerstedii*, *G. platense*, *G. resinaceum*, *G. sinense*, *G. tornatum*, *G. tsugae* and *G. weberianum* (in alphabetical order) (Roberts 2004).

Although basal stem rot is known for its high disease severity and wide occurrence in oil palm, there is no general consensus on *Ganoderma* species

associated with the disease. Turner (1981) identified potential association of 15 species of *Ganoderma* such as *G. boninense*, *G. applanatum*, *G. chalceum*, *G. miniatocinctum*, *G. pseudoferreu*, *G. lucidum* and *G. tornatum* with BSR, and he also believed that the sole cause of the disease could not be attributed to a single species in any given area. *G. boninense* is the major species that is highly pathogenic to oil palm (Ho and Nawawi 1986; Khairudin 1990; Moncalvo 2000) in South-East Asian countries. *G. boninense* has been reported to be more aggressive, causing yield reductions of 20–40% and/or losses of 46–67%, if infected oil palm aged 15 years followed by *G. zonatum* (moderately aggressive) and *G. miniatocinctum* (least aggressive). However, *G. tornatum* is reported to be non-pathogenic species associated with BSR (Singh 1991; Hisham 1993; Idris et al. 2001). In spite of that, *G. boninense* as the BSR real causative agent in different oil palm growing region is yet to be confirmed. In India, *G. lucidum* and *G. applanatum* are known to cause basal stem rot disease in oil palm as well as coconut (Mandal et al. 2003).

9.2.3 Host Range

BSR disease incited by *Ganoderma* spp. has got a wide host range, infecting mainly palms, forest, avenue and fruit trees belonging to 19 families, 36 genera and 48 species (Naidu et al. 1966). *Ganoderma* exhibits saprophytic and parasitic life on logs (Singh et al. 2007) and plays a significant ecological role. It acts a as good decomposer in the breaking down or delignification of hard wood as well as soft wood causing white rot. Apart from oil palm, many *Ganoderma* species have long been found to cause stem and root rot diseases in many commercial perennial crops such as coconut (*Cocos nucifera*), tea (*Camellia sinensis*) and betel nut (*Areca catechu*) (Miller et al. 2000).

9.2.4 Morphology

Ganoderma fructification produces bracket like large and woody basidiocarps. Their fruiting bodies are generally double walled, generating truncate spores (with round base and shorter tip) having yellow to brown ornamented inner layers (Adaskaveg and Gilbertson 1988). Basidiocarps are made up of hymenium (tissue that produces spores) and pileus (cap-like structure) (Seo and Kirk 2000). *G. boninense* is morphologically distinguished from other species by light coloured thin pileus with elongated basidiospores that are uniformly brown coloured. The colonies of *G. boninense* grows into undulating whitish mycelial growth on top and having dark pigmentation on the reverse side of growth media plate.

9.2.5 Taxonomy

Many attempts have been made in the past in differentiating Ganodermataceae *taxonomically* based on host specificity, geographical distribution and phenotypic characteristics such as colour and consistency of basidiocarp, shape of margin of pileus, stipitate or sessile fruiting body and size and shape of basidiospores (Adaskaveg and Gilbertson 1986; Bazzalo and Wright 1982; Pegler and Young 1973; Steyaert 1972, 1980). The colour (deep red, light yellow to white) and size of pileus and hymenium vary between different species. However, the size of the pore remains almost similar in all species of *Ganoderma*. Later, the cultural, morphological and physiological characters of mycelial state of *Ganoderma* was used for taxonomic delimitation at species level (Miller et al. 2000). Variation in morphological features under different growth conditions resulted in ambiguity in species identification of *Ganoderma* (Ryvarden 1991). This can also be interpreted from a study conducted by Mandal et al. (2009) on colony morphology and sporulation stating the morphological plasticity in different isolates of *Ganoderma* in India. As a result, molecular identification using ribosomal DNA (rDNA) region (Moncalvo 2000; Smith and Sivasithamparam 2000), intergenic spacer (IGS1) region, cultural and mating features, isozyme-based studies and cladistic methods (Seo and Kirk 2000) are exploited nowadays. However, only a limited number of taxa have been identified in this way so far.

9.2.6 Symptoms

The delayed expression of symptoms is one of the biggest obstacles in diagnosing and managing BSR disease. The initial symptoms are observed generally after 60–70% of damage to vascular tissue of the plant and consequently mortality is quick in young palms (Rees et al. 2007). The infection by *G. boninense* results in lignin degradation of xylem vessels of plants, which is expressed as water stressed wilt conditions in the palms. The earliest symptoms include multiple unfolded fronds that become chlorotic on one side with subsequent necrosis of tips in young plants. Adult palms also produce similar symptoms giving sickened yellow canopy and skirting of lower leaves (Turner 1981). Gradually, all necrotic leaves shed off, leaving twigs die back resembling stag-horn-like appearance to the palm. Under severe xylem decay on lower parts of stem, stem bleeding symptoms can also be observed. A cross-sectional view of the diseased trunk appears as rotten tissue with irregular zones and cavities representing active growth of the white mycelium. Infected roots become friable and gives desiccated appearance internally. The cortical tissue discolours to brown and peel off easily, whereas the stele turns black in colour (Singh 1991). *Ganoderma* takes at least 2–3 years to kill mature palms, whereas the young palms are killed within a short span of 6–24 months. Fructification of basidiomata at the stem base or base of leaf, or infected root during rainy seasons is a critical sign in the in situ diagnosis of the disease (Paterson 2007). In advanced stages, infected trees fall over due to high winds, leaving bole tissue within the ground or some dead palm remains erected with hollow trunks (Fig. 9.1).

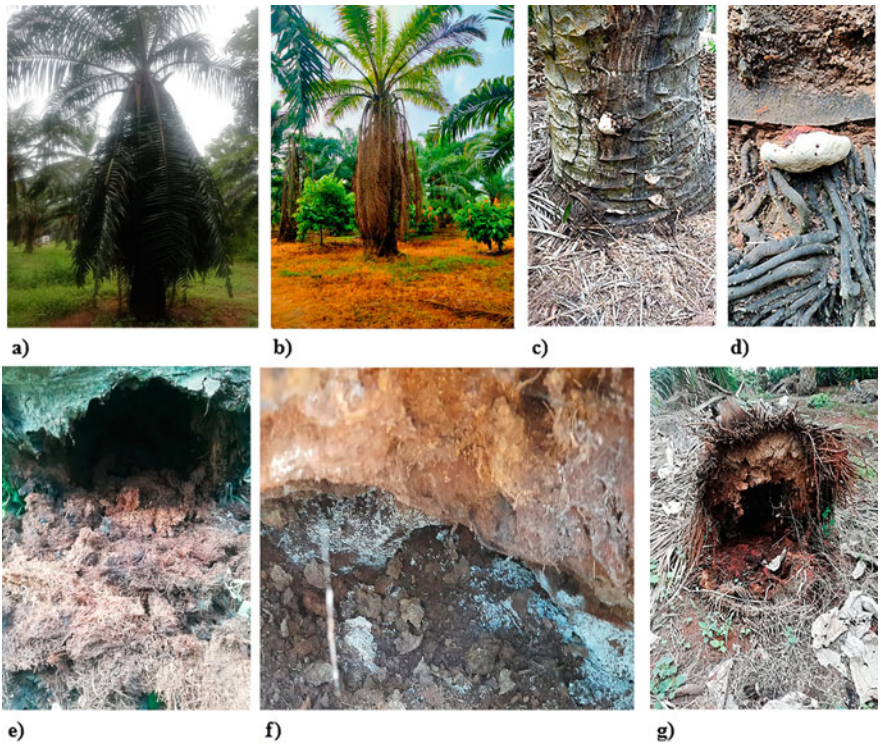

Fig. 9.1 Symptoms of basal stem rot: (**a**) snapping of old fronds at the petiole and drooping, (**b**) skirting and severe desiccation of lower leaves, (**c**) stem bleeding, (**d**) formation of basidiocarp, (**e**) internal disintegration of basal portion, (**f**) mycelial mat formation and (**g**) collapse of palm

9.2.7 Epidemiology and Favourable Conditions

Generally, wide occurrence of BSR had been reported in poorly managed and older plantations. However, in the recent past, the infection has been observed in palms regardless of plant growth stage, making it a major economic concern for oil palm growers. It is noteworthy that younger palms incited by more aggressive isolates of *Ganoderma* species when compared to older palms (Nur-Rashyeda et al. 2021). *Ganoderma* is a soil-borne pathogen and directly depends on various soil factors. Sandy soils or sandy loam soils of the coastal tracts and peat soil favour the disease development and spread. Soils having poor drainage facilities and prolonged water stagnation in rainy seasons also aggravate the disease (Latiffah and Ho 2005). Now, it is known to occur in all oil palm-cultivating soil types (Idris 1999; Khairudin and Tey 2008). Soil pH level is another important factor in determining the microbial activity and disease severity (Chong et al. 2017). High pH (Parthiban et al. 2016) as well as very low pH (Chong et al. 2017) do not favour growth of the fungus. An optimum pH range of 3.7–5.0 along with a temperature range of 27–30°C favour the fungal growth (Nawawi and Ho 1990). Interestingly, *Ganoderma* has the potential to

manipulate the pH levels of the surrounding host tissue in accordance with its favourable range (Vylkova 2017). Thus, this ability of *Ganoderma* to survive and adapt in varying pH poses a serious imbalance in the soil micro-ecosystem and paves a way for other soil-borne infections. When coconut was a previous crop, early infection of *Ganoderma* was observed in 12–24 months old palm plantings (Singh 1991), and subsequently, disease progressed to 40–50% by the time they reached the age of 15 years. Similar observations were recorded for high incidence of BSR in oil palm when rubber (Ariffin et al. 1989) and pineapple were grown as the previous plantations (Ariffin et al. 1989; Rao 1990). Later, Khairudin (1993) proved the direct relationship among nature of previous crop, age of palm and BSR disease severity is inappropriate, and suggested that high disease inoculum that comes into contact with palm roots and subsequent congenial factors for disease development are more critical. Organic debris with high inoculum load left behind by previous natural forest ecosystem, infected stumps of previous trees, poor maintenance of the plantation, non-adoption of the recommended cultural operation, type of planting and poor management of irrigation and drainage are other possible reasons for severity of BSR disease.

9.2.8 Survival and Spread

Rees et al. (2009) suggested that *Ganoderma* infection cycle comprises initial biotrophic and subsequent necrotrophic phases. This is followed by the formation of melanized mycelium that results in the degradation of lignin and white rot symptoms by *G. boninense* (Adaskaveg et al. 1990). The formation of blacklines in the infected tissue transforms *Ganoderma* hyphae into thick-walled, swollen structures, which might play an important role in the perpetuation of inoculum in soil (Ariffin et al. 1989). The inoculum left by coconut (Abdullah 2000) and rubber (Flood et al. 2005) plantations, both of which contain *Ganoderma* as an endophyte, is the major primary source of inoculum for the BSR in oil palm.

The spread of BSR disease to the healthy palms occurs in two possible ways. The fungus, without a doubt, is a soil-borne pathogen but the air-borne basidiospores and secondary mycelium are speculated to be involved in its spread in the existing planation. However, there are no conclusive evidences on mode of initiation of the disease and spread of the disease in the plantations. The infection from leftover inoculum/tissue or diseased roots to healthy roots by contact is presumed to be main mode of spread of BSR disease in oil palm (Turner 1965; Flood et al. 2000). The infection from roots slowly spreads to trunk and is known to infect all kinds of tissues there onwards in advanced stages.

The idea of basidiospore's role in disease spread was put forth by Miller et al. (1995) and Ariffin et al. (1996), who reported the existence of different vegetative compatible groups and basidiomata within the same area of oil palm plantations, indicating different sources of primary inoculum. Basidiospores that can germinate and grow in non-living tissues may be the main sources for the disease dispersal (Pilotti et al. 2003; Sanderson 2005). It is recorded that 14,000 spores/min can be

spread from 10 cm^2 of the fruiting body (Rees et al. 2012). The significance of basidiospores is overlooked when symptoms appear late after a long period of incubation. However, the different isolates and long infection process are the outcome of formation of dikaryotic strains from monokaryotic ones, which usually takes long time (Bridge and Utomo 2005). Basidiospores germinated by monokaryotic mycelium can colonize palm (Hasan and Flood 2003; Rees et al. 2007), but a dikaryotic heterokaryon, formed after anastomosis with a compatible mating type, is essential for potential infection and disease production. The possible little role of wind, rain and insect such as *Oryctes* beetle (Turner 1981) and larvae of the *Sufetula* spp. in dissemination of basidiospores (Genty et al. 1976) was also speculated.

9.2.9 Artificial Inoculation Methods

Due to the asymptomatic phase and slow progression of BSR in mature palms, screening cultivars resistant to BSR is an arduous task. Rubber wood blocks (RWB) method has been conventionally used as a standard method for artificial inoculation and proving Koch's postulates throughout the world. This method was also successfully deployed in roots of the seedlings (Sariah et al. 1994; Breton et al. 2006) as well as in germinated seeds (Rees et al. 2007). Alternatively, Chong et al. (2012b) achieved successful root infection with spraying of *Ganoderma* mycelial suspension onto seedling roots. However, artificial inoculation with basidiospores was not successful in initiating the disease in oil palm (Turner 1981; Ho and Nawawi 1986; Hasan et al. 2005; Cooper et al. 2011; Idris 2013). However, Lim et al. (1992) proved that spore contact through wounded tissue of fronds could cause infection. Despite its extensive usage, the RWB method is time-consuming and labour-intensive, requiring at least 6 months from preparation to disease evaluation (Chong et al. 2012a, b). Due to the long incubation period and difficulty in sterilizing RWB, contamination rate of other fungi is also quite high. Alternatively, Purnamasari et al. (2018) developed a rapid inoculation method using a root immersion technique for routinely infecting oil palm seedlings that can be used to develop resistant oil palm cultivars to *G. boninense*. In addition, Angel et al. (2021) made first report of a non-invasive tissue culture method in plantlets using a sawdust substrate for the establishment of *Ganoderma* infections.

9.3 Basal Stem Rot of Oil Palm: Detection and Diagnostic Tools

It is vital to monitor plant health and to detect infections at early stages in order to avoid disease spread and to approach appropriate management strategies. Oil palms must be assessed for disease severity and then categorized in terms of resistant and susceptible cultivars to apply pesticides cost-effectively. Early detection of BSR can extend the economic life of oil palms, although this is constrained by a number of variables. The delayed detection of BSR in oil palm is due to the fact that it is

frequently misdiagnosed as *G. zonatum*, which is moderately pathogenic to oil palm, and *G. boninense* isolates have a high intraspecific diversity.

9.3.1 Manual Methods/Field Based

9.3.1.1 Based on Visual Symptoms

In the earlier days, the only way to diagnose disease was to look for symptomatic indicators in the field (Lelong et al. 2010). BSR is identified by the presence of unopened spear leaves of oil palm and basidiocarp at near soil level on the tree trunk or primary roots (Aswad et al. 2011). The most common symptom is mild to severe wilting of all leaves except the spear leaf. Other symptoms include general deterioration, reduced growth, and off-colour foliage. Symptoms of the unhealthy plant appear only after the plant becomes at least 7–16 years old (Abdullah et al. 2013).

9.3.2 Lab-Based Methods

9.3.2.1 Cultural Methods

***Ganoderma*-selective medium (GSM)**: *Ganoderma*-selective medium (GSM) developed by Ariffin and Idris (1993) could isolate the pathogen selectively from any portion of diseased tissue collected from the field, with or without surface sterilization, to assist various studies on *Ganoderma* in oil palm. Infected oil palm samples are obtained by drilling into a diseased stem at a height of about 5–10 cm above the soil surface to culture the samples on semi-selective media (Utomo and Niepold 2000).

9.3.3 Biochemical Methods

9.3.3.1 Ethylenediaminetetraacetic acid (EDTA)

It is a colorimetric technique using ethylenediaminetetraacetic acid (EDTA) to identify *Ganoderma* in oil palm (Natarajan et al. 1986; Ariffin et al. 1995; Utomo and Niepold 2000). The use of lab-based methods is limited due to their time-consuming and labour-intensive nature. These techniques may provide non-specific and inaccurate results and the necessity to bring all samples to laboratory makes them extremely unsuitable for large-scale field monitoring.

9.3.3.2 Isozyme Analysis

Isozyme analysis, such as pectinase zymograms, is used to identify palm-associated fungal isolates by producing band patterns (Bridge et al. 2000). By using polyacrylamide gel electrophoresis (PAGE) and cellulose acetate gel electrophoresis (CAGE), the isozymes of five Australian *Ganoderma* species were investigated. Pectic isozymes were found to be sufficient in distinguishing three laccate Australian

species, *G. weberianum*, *Ganoderma* sp. and *G. cupreum*, but not the non-laccate *G. australe* or *G. incrassatum* (Smith and Sivasithamparam 2000).

9.3.3.3 Ergosterol Analysis

Ergosterol is a fungus-specific, primary sterol present in cell membrane of fungus, which has been found in *Ganoderma* species as well (Axelsson et al. 1995; Paterson 2006). It is absent in plants and other microbes; hence, it could be used as an effective biomarker for determining the amount of fungal biomass present (Choon et al. 2012). According to Chong et al. (2009), ergosterol is linked with the proliferation of *G. boninense* and disease severity in oil palm. The separation and characterization techniques of ergosterol from *G. boninense* mycelium are now commonly available. Thin liquid chromatography (TLC) and ultra performance liquid chromatography (UPLC) have been used to discover the ergosterol structure, which was then verified using gas chromatography combined with mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) analyses (Choon et al. 2012). The major limitation with this technique is that it cannot discriminate between the ergosterols produced by target *Ganoderma* or any other fungi present.

9.3.3.4 Altered Proteins

Infection of oil palms with *G. boninense* has been shown to alter the gene expression and protein concentrations, which can serve as an important biochemical marker in the detection process. Root proteins from both healthy and *G. boninense*-infected oil palm seedlings were examined using two-dimensional gel electrophoresis. After evaluation, proteins show a significant change in abundance under *G. boninense* infection and 21 proteins with changed abundance were discovered, which might be used as disease biomarkers (Al-Obaidi et al. 2014).

9.3.3.5 Metabolic Profiling

Plant metabolomics is a significant tool for system biology research. It has been utilized to determine the whole profile of measurable metabolites in a biological system. Plant metabolites play an essential role in host–pathogen interactions (Hu et al. 2019). Rozali et al. (2017) used a metabolomics approach to investigate the *G. boninense*-infected oil palm leaf using two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF-MS). They discovered that mannose, xylose, glucopyranose, myoinositol and hexadecanoic acid were higher in partially tolerant oil palm, whereas cadaverine and turanose were found to be more abundant in susceptible oil palm (OPLS-DA), demonstrating the differential pattern of metabolites under infected and healthy oil palms.

9.3.4 Molecular Methods

9.3.4.1 Nucleic Acid-Based Detection

Nucleic acid-based detection techniques are based on the sequence of DNA; hence, it is important to get sufficient sequencing data to use them.

1. **Molecular markers**: Molecular markers can be successfully used in biodiversity screening, phylogenetic analysis, evolutionary research (Meyer et al. 2010), geographical distribution and host–pathogen associations (Hong and Jung 2004). The following are some molecular markers used for the detection of *Ganoderma*:
   (a) Manganese-superoxide dismutase (antioxidant defence mechanism of the cell).
   (b) 18S rDNA (the small ribosomal subunit RNA: 16S in prokaryotes and 18S in eukaryotes). Analysis of nuclear 18S rDNA can be exploited to give molecular evidences for *Ganoderma*'s long-distance spread over the southern hemisphere (Moncalvo and Buchanan 2008). Furthermore, this technique has previously been used to demonstrate the diversity of wood-decaying fungi in India (Singh et al. 2013).
   (c) (Mitochondrial small subunit) mt SSU rDNA: It is recognized to be a locus that led to the division of *Ganoderma* into six monophyletic groups, indicating that complicated situations such as geographical region and pathogen–host connections, as well as phylogenetic linkages, must be examined (Hong and Jung 2004).
2. **PCR**: Polymerase chain reaction (PCR) techniques were used to amplify and detect certain DNA sequences of *G. boninense* in order to identify *Ganoderma* species (Moncalvo et al. 1995; Idris et al. 2003). To discover and identify the pathogen, researchers have used internal transcribed spacer (ITS) regions, polymerase chain reaction (PCR) amplification of ribosomal DNA, repetitive DNA polymorphism analysis and oligonucleotide hybridization to amplified ribosomal DNA (rDNA) spacers. The PCR detection approach can be used as a realistic screen for *Ganoderma* detection and identification. Two primers, PER44-123 and LR1 primer, were used to produce a 580 bp product solely for *Ganoderma* isolates and not for any other fungi (Idris et al. 2003). Similarly, Idris et al. (2010a) used multiplex polymerase chain reaction to distinguish *Ganoderma* isolates, although further tuning is required to produce convincing results.
3. **RFLP**: Using the restriction fragment length polymorphism (RFLP) approach on both highly conserved and variable ITS or rDNA sequences, it allows genetic variation investigations to be facilitated at the species level (Nusaibah et al. 2011a, b). Because ITS1 sequences have more divergent than ITS2 sequences (Moncalvo and Buchanan 2008), an experiment based on ITS1 sequences is advised. Restriction fragment length polymorphism has the benefits of combining highly conserved sequences in the ITS, 5.8S-ITS4 rDNA regions with variable sequences in the ITS regions at the species level, where the ITS has a high interspecific variability but a very low intra-specific variability (Moritz et al. 2000).
4. **RAPD**: Random amplified polymorphic DNA (RAPD) can be modified for examining various *Ganoderma* spp. isolates. The use of RAPD-PCR and ITS sequence data yielded diverse results, with RAPD being shown to be better method for lower taxonomic level systematics that cannot be resolved using ITS sequence data. RAPD analysis indicated differences across *G. boninense* isolates even if they showed a greater degree of similarities (Zakaria et al. 2009).

Hence, RAPD can be effectively used in differentiating various *G. boninense* isolates with identical ITS sequences (Hseu et al. 1996), although it cannot provide accurate identification.

5. **AFLP**: A PCR-RFLP technique was created based on several sequential changes between pathogenic and non-pathogenic *Ganoderma* spp. Compared to RAPD and RFLP, AFLP (amplified fragment length polymorphism) is more accurate and less prone to contamination (Utomo et al. 2005).
6. **LAMP**: A nucleic acid-based gene amplification approach, known as loop-mediated isothermal amplification (LAMP), could be used to detect BSR in oil palms in the field or at remote places. It amplifies DNA under isothermal conditions with high specificity, rapidity and efficiency. The technology eliminates the necessity for the reaction to be carried out in a thermal cycler (Tomlinson and Boonham 2008). A battery-operated device has recently been developed to identify phytoplasma infections in coconut farms by combining a quick DNA extraction step of less than 2 min with the LAMP test (Dickinson 2015).
7. **DNA microarray**: DNA microarray is a detection method that makes use of the selectivity of DNA binding to complimentary sequence nucleic acids. The application of DNA oligonucleotide arrays for the sensitive and selective detection of *G. boninense* was discussed, along with the use of polymer pen lithography for the production of DNA oligonucleotide arrays. This technique can yield a clearly detectable result in the presence of the target DNA when utilized in a sandwich assay format using DNA-conjugated gold nanoparticles (Rani and Devaraj 2019).
8. **DNA biosensors**: Advances achieved in the field of molecular techniques have allowed for the introduction of a number of novel tools for the identification of BSR disease of oil palm. For the detection of *G. boninense*, an electrochemical-based DNA biosensor was devised and calibrated (Dutse et al. 2012, 2013). An interdigitated electrode (IDE)-based electrochemical biosensor for the early detection of *G. boninense* DNA was proposed by Thivina et al. (2021). The performance of IDE in combination with gold nanoparticles has been demonstrated with hybridization times ranging from 30 min to 2 h.
9. **Lateral flow assay**: LFMs (lateral flow microarrays) enable quick nucleic acid identification based on hybridization and utilization of a colorimetric signal that is clearly visible (Carter and Cary 2007). These arrays are made of a tiny lateral flow chromatography nitrocellulose membrane, hybridize rapidly with detection limits similar to microarrays, and can help laboratories save money by reducing the usage of expensive laboratory equipment. The system depends on the availability of robust and reliable host and pathogen biomarkers found using transcriptomic methods (Martinelli et al. 2015).

Although nucleic acid-based detection and molecular techniques offer an efficient and reliable tool for detection of *G. boninense*, they do suffer from few limitations such as being complex, expensive and time-consuming since these techniques necessitate the collection of samples for laboratory testing (Ishaq et al. 2014).

9.3.4.2 Protein-Based Detection Methods

1. **Immunoassay**: Immunoassays are based on the antigen–antibody interactions and were used for the detection of *Ganoderma* in culture media (Reddy and Ananthanarayanan 1984). To improve the accuracy of standard BSR disease detection methods, enzyme-linked immunosorbent assays (ELISA) and dot immuno-binding assays (DIBA) were developed (Rajendran et al. 2009). Antibodies, both monoclonal and polyclonal, have been utilized to detect pathogenic *Ganoderma* spp. The only problem is that other species of *Ganoderma* and saprophytic fungi often present on diseased oil palm roots and trunks, such as *Penicillium*, *Aspergillus* and *Trichoderma*, display cross-reactivity in the assays (Shamala et al. 2006; Idris and Rafidah 2008), but when compared to the culture-based approach GSM, ELISA-PAb demonstrates an 18% improvement in detection.

9.3.5 Remote-Based Methods

9.3.5.1 VOC Profiling

VOCs (volatile organic compounds) are biomolecules having low molecular with a high vapour pressure and low boiling point. Plants emit a variety of VOCs into their immediate environment that are important for growth, defence, communication and survival (Baldwin et al. 2006). Headspace solid-phase microextraction (HS-SPME) method paired with gas chromatography mass spectrometry (GC-MS) could detect VOCs generated from oil palm wood tissue infected by *G. boninense*. The approach was capable of sampling VOCs with good repeatability and a well-balanced VOC profile across chemical classes (Cheah et al. 2019).

1. **E-nose**: A platform for VOC profiling is the electronic nose (e-nose). These systems employ a variety of specialized metal oxide sensors, each of which is selective for specific VOC classes. Individual sensors provide an impedance response when volatiles are introduced into the e-nose, which is measured and presented concurrently (Gardner and Bartlett 1994; Laothawornkitkul et al. 2008). Artificial neural network (ANN) is another accurate method for distinguishing healthy oil palms from diseased ones. By using the ANN classification algorithm and multivariate statistical analysis approaches, hand-held e-nose sensors are created, which are capable of categorizing the samples into two groups, namely infected and non-infected based on the odour (Abdullah et al. 2014).

9.3.5.2 Tomography

Mohd Shu'ud and his colleagues sought to locate *Ganoderma* in oil palm stems using tomography scans (Shu'ud et al. 2007). Tomography involves several quantity measurements of ray transmission over the object cross section (Wang 2015). Four types of tomography used for detection are described below.

1. *Electrical Capacitance Volume Tomography* (ECVT) is a technology that uses the considerable difference in permittivity values between air, soil and water to quantify soil water content. The principle of ECVT is to rebuild a 3D image using a signal from a capacitance sensor, with any changes in phase causing non-linearity in the electric field distribution. The research was executed to see the potential of electrical impedance tomography technology in early detection of BSR-infected oil palm trees. It has the advantage of identifying unhealthy and asymptomatic palms, hence reducing inoculum in the fields. As a result, it is a valuable tool for detecting basal stem rot early and implementing a disease management strategy (Arango et al. 2016).
2. *GammaScorpion* is a portable computed tomography (CT) technology that employs gamma rays and a small amount of sealed radioactive source. Without cutting the tree, it can detect BSR non-invasively and also accurately estimate the extent and location of BSR damage. Radiation detectors capture gamma-ray transmission data from a variety of angles inside the image plane, which are then utilized to reconstruct meaningful cross-sectional images (Abdullah et al. 2013).
3. *X-ray computed tomography* (CT) is a minimally invasive structural imaging technique that permits three-dimensional (3-D) reconstruction of scanned structures (Khosrokhani et al. 2016). CT is now widely used in animal sciences, mostly for cancer research, bone architecture studies, angiogenesis and small animal in vivo imaging (Hamidon and Mukhlisin 2014).
4. *Sonic tomography*: Sonic tomography image is an internal construction of a solid object generated by recording the speed differences of sonar wave transmissions and it is used to detect the presence of lesions inside the stem (Khosrokhani et al. 2016).

9.3.5.3 Microfocus X-Ray Fluorescence (μXRF)

Microfocus X-ray fluorescence (XRF) sends a micro-sized X-ray beam to a specified target for element mapping and analysis as a dispersive energy source. It is based on the fact that *Ganoderma*-infected palms had a lower rate of inorganic elements than healthy palms (Khosrokhani et al. 2016). When compared to an electronic microscope, it does not necessitate sample degradation or coating. X-ray fluorescence has been proposed as a useful sensor for detecting plant diseases (Yokhin and Tisdale 1993).

9.3.5.4 Electrical Resistance

Differential electrical resistance has been used to determine plant vigour using electrical resistance (ER) (Paysen et al. 1992). Two devices, called Shigometer and Resistograph, can measure ER and are used to diagnose faults in wood (Johnstone et al. 2010; Aziz et al. 2019).

9.3.5.5 Hyperspectral Imaging

Hyperspectral imaging sensors (HRS) have a large number of continuous spectral bands to record spectral responses of materials over a long period of time. It represents plant cell structure condition, chlorophyll pigment status, plant structural water content and other useful information. High reflectance in the near infrared and low reflectivity in the visible regions of the electromagnetic spectrum indicate healthy vegetation covering. Visible–near infrared (VIS-NIR) hyperspectral imaging was also used to detect *G. boninense* infections in palm trees that were 5 months old and had no BSR symptoms. The uninoculated and inoculated seedlings were classified with 100% accuracy using this approach (Azmi et al. 2020).

9.3.5.6 Multispectral Imaging

Sensors that capture reflected or emitted energy from a given area or item in numerous discrete bands of the electromagnetic spectrum are known as multispectral remote sensing sensors (Jensen 2006). The reflectance of BSR-infected oil palms was reduced in the NIR and greater in the RGB electromagnetic areas (Santoso et al. 2011). For detection of BSR in oil palm plantations, ground-based (Bejo et al. 2015) and spaceborne multispectral sensors (Santoso et al. 2011) could be used.

9.3.5.7 Terrestrial Laser Scanning

Terrestrial laser scanning (TLS) is a relatively recent technique that has a wide range of applications. Precision agriculture has also used it to diagnose a variety of biophysical and structural plant factors. One of the TLS uses would be the calculation of the leaf area index (LAI) (Zheng et al. 2012).

Ground-based LiDAR (Light Detection and Ranging) is an active remote sensing imaging approach for plant phenotyping that employs laser light. Using point clouds data from the TLS, a study presented a unique BSR classification technique for oil palm canopy analysis. To get a full 3D image, the TLS scanner was installed at a height of 1 m, and according to statistical research, the best single measure for early detection of BSR disease was frond number with an average accuracy of 86.67% (Husin et al. 2020).

9.3.5.8 RGB Cameras

A visible camera sensor is an imager that captures visible light (400–700 nm) and transforms it to an electrical signal before organizing it to output images and video streams. Visible cameras use light wavelengths between 400 and 700 nm, which are the same wavelengths as the human eye sees. Using visible aerial photographs (RGB-aerial photographs), a study was conducted with the goal to determine the degree of severity of *G. boninense* infection in oil palm. The resulting images could distinguish the infection severity on each individual palm with an average accuracy value of 83% (Wiratmoko et al. 2020) (Table 9.1).

Table 9.1 Summary of different types of detection and diagnosis methods for *Ganoderma*

Method type	Particulars		References
Manual method	Visual symptoms		Lelong et al. (2010); Aswad et al. (2011); Abdullah et al. (2013)
Lab-based methods	Cultural methods	Ganoderma selective medium (GSM)	Utomo and Niepold (2000)
		Ethylenediaminetetraacetic acid (EDTA)	Ariffin et al. (1995); Utomo and Niepold (2000)
Biochemical methods	Isozyme analysis		Smith and Sivasithamparam (2000)
	Ergosterol analysis		Chong et al. (2009); Choon et al. (2012)
	Altered proteins		Al Obaidi et al. (2014)
	Metabolic profiling		Rozali et al. (2017); Hu et al. (2019)
Molecular methods	Nucleic acid-based detection	Molecular markers	Hong and Jung (2004); Meyer et al. (2010)
		PCR	Idris et al. (2003); Wong et al. (2012)
		RFLP	Moritz et al. (2000); Nusaibah et al. (2011a, b)
		RAPD	Zakaria et al. (2009)
		AFLP	Utomo et al. (2005)
		LAMP	Dickinson (2015)
		DNA microarray	Rani and Devaraj (2019)
		DNA biosensors	Dutse et al. (2013); Thivina et al. (2021)
		Lateral flow assay	Martinelli et al. (2015); Ishaq et al. (2014)
	Protein-based detection	Immunoassay	Shamala et al. (2006); Idris and Rafidah (2008); Rajendran et al. (2009)
Remote-based methods	VOC profiling	E-nose	Gardner and Bartlett (1994); Laothawornkitkul et al. (2008); Abdullah et al. (2014); Tan et al. (2021)
	Tomography	Electrical Capacitance Volume Tomography (ECVT)	Arango et al. (2016)
		Gamma-ray computed tomography	Abdullah et al. (2013)
		X-ray computed tomography (CT)	Khosrokhani et al. (2016)
		Sonic tomography	Khosrokhani et al. (2016)
	Microfocus X-ray fluorescence (μXRF)		Yokhin and Tisdale (1993); Khosrokhani et al. (2016)

(continued)

Table 9.1 (continued)

Method type	Particulars		References
	Electrical resistance		Johnstone et al. (2010); Aziz et al. (2019)
	Hyperspectral imaging		Azmi et al. (2020)
	Multispectral imaging		Jensen (2006); Santoso et al. (2011); Bejo et al. (2015)
	Terrestrial laser scanning	Ground-based LiDAR	Zheng et al. (2012); Husin et al. (2020)
	RGB cameras		Wiratmoko et al. (2020)

9.4 Basal Stem Rot of Oil Palm: Integrated Disease Management Strategies

Management of BSR under field conditions is a challenging task. Although there are different management strategies, none of them give satisfactory results for managing *Ganoderma*. Slow progressing nature of BSR leads to difficulty in detection at early stages of infection. Therefore, basal stem rot disease is often detected at advanced stages and by then the infected trees may not be able to respond to any treatment given (Sapak et al. 2008). Moreover, inefficient performance of existing management strategies is attributed to the systemic infection, soil-borne nature, production of resting structures, melanized mycelium, basidiospores and pseudo-sclerotia and the ability to penetrate deeply inside palm (Bivi et al. 2010). Unfortunately, the resistant sources for combating the disease are also limited (Chong et al. 2012a, b). The curative methods are not economically feasible to save the infected trees; hence, the current management practices are aiming at reducing the incidence of BSR and delaying the progression of *G. boninense* (Azadeh et al. 2010).

9.4.1 Cultural Practices

Even if it is not possible to manage a field without pathogens (Sanderson et al. 2000), yet, a good management system by maintaining healthy stands and further prevention of various pathogen can reduce the hostile effects of a disease. Cultural practices are effective and economical for managing *G. boninense* in oil palm. These measures normally constitute eradication and reduction of the pathogen inoculums to prevent further disease spread (Khairudin 1990; Susanto et al. 2005).

9.4.1.1 Preventing the Entry of Pathogens

The entry of pathogens can be prevented easily by carefully accomplishing the harvesting process, preventing wounds in trees and improving treatment. Regular practice of paint or dressing should be followed to treat large wounds in oil palm trees.

9.4.1.2 Clean Clearing/Sanitation

Clean clearing is the most important recommendation for reducing the incidence and spread of *Ganoderma* in both existing and replanted oil palm plantations (Turner 1965; Singh 1990; Flood et al. 2000). The main goal is to clear the old-aged trees before they reach extreme susceptibility and thereby eradicate all possible inoculum that remain within an infected palm area. It is commonly implemented in two situations, from where it is apparent and at the replanting stage. Gurmit (1991) had studied that this technique gave lower disease incidence of 14% in comparison to other replanting techniques. Different practices such as ploughing, harrowing, trenching and burning are employed in this method to lower BSR incidence (Flood et al. 2000; Rees et al. 2009; Hushiarian et al. 2013). In disease prone areas of oil palm, it is often recommended that before planting of new seedlings, one round of harrowing and two rounds of ploughing should be done to finely chop the leftover roots (Flood et al. 2000). Idris et al. (2004a) described that large hole of 2 m × 2 m × 1 m of depth can be dug out for sanitation operation. Infected materials are removed, cut into pieces and left for decaying. It is generally practiced at the time of replantation. Researches show that trials up to 14–15 years sanitation, if done properly, reduced the BSR incidence or if done poorly, the inoculum helped to increase the incidence (Chung 2011). In addition to this, Khairudin (1990) showed that at different levels of BSR points in which seedlings were bait, 93% of seedlings grown around diseased stumps left in the field with 0.3 m distance, which became infected within a period of 18 months. However, open burning is prohibited in many palm oil growing countries, including Malaysia, under Air Regulation Act of 1978, which deals with the issues of air pollution. These regulatory frameworks, however, suffer from weak execution. Although it is exorbitantly expensive, this approach is practiced in many palm producing countries.

9.4.1.3 Windrows

It is a technique in which excised root tissue and fallen palm trunks are laid beside the old rows. Diseased palms are often pulverized, chipped and stacked to enhance the process of natural decomposition. This method demands less efforts than clean clearing and has been found capable in reducing losses in the successive oil palm plantation. Hashim (1991) conducted a comparative study and find out clean clearing as the most efficient way in lowering BSR incidence. Reduction in disease incidence from 27.3% in the preceding stand to 14% in the replanted stand after next 15 years was observed in plantations, followed by windrowed treatment (27.3–17.6%). This is due to the efficiency of windrowed materials to cope up with the problems of potential source of inoculum (Flood et al. 2000).

9.4.1.4 Soil Modification Practices

It is an economic practice, which is followed in almost all oil palm producing countries. It commonly involves collection of healthy soil from the adjacent areas and creation of a heap of about 75 cm height to prevent the toppling of infected palm trunk by wind. Ho and Khairuddin (1997) and George et al. (2000) in their studies found comparative economic advantage of soil mounding in controlling BSR

disease. However, this method could only extend the economic life of affected palm. It could not even stop the spread of *Ganoderma*.

9.4.1.5 Surgery

In this method, excision of infected tissue is done with the help of a black-hoe blade (Singh 1991) or hand-held chisel in order to eradicate the primary source of inoculum, i.e., basidiocarp (Turner 1981). Fungicides and paints are the commonly used protectants that prevent further decay of the infected plants. Hasan and Turner (1994) showed through their study that surgery enhanced the survival and yields in case of palms. However, it is less successful due to delay in detection or an extended underground lesion that includes infectious root masses. Furthermore, surgery requires more rigorous efforts and repetition, as the revival of the infection is possible if lesions are not removed completely. Studies by Panchal and Bridge (2005) showed that if fresh cut is sealed, it would prevent the spores from coming in contact with the wound region. In addition, Ho and Khairuddin (1997) reported that surgery followed by soil mounding could decrease the loss of palm from 34% to 2% in 2 years. Surgery would extend the lifespan of the infected palms up to 2–3 years (Priwiratama et al. 2020).

9.4.1.6 Isolation Trenches

It is a common practice that is used to prevent contact between palms by digging trenches (Hasan and Turner 1998; Chung 2011) and has been found to be a more successful technique in delaying BSR occurrence for about 14 years. Trenches are created in accordance with the size and age of the trees. Generally, the diseased palm is isolated with 0.5 m wide and 1 m deep trench (Lim and Udin 2010). It is found to be a better method than clean clearing and windrowing. Sometimes drenching of chemicals in trenches is also practiced for enhancing effectiveness. However, if it is not maintained properly, or the depth of trench is not enough, it will not prevent the spread of infectious roots.

9.4.1.7 Fallowing

It is a process in which the land is left fallow for a certain period in order to reduce the disease incidence in the subsequent plantation crop. Studies were conducted by Virdiana et al. (2010) to assess the optimum time period for fallowing and the effect of other potential crop to create a balance in the environment.

9.4.1.8 Planting Legume Cover Crops (LCC)

Legumes are widely grown as cover crops in oil palm plantation areas as they have potential to fix the atmospheric nitrogen and their decomposition usually adds nitrogen for the palm. It also helps to control soil erosion and weeds (Chung 2011).

9.4.2 Nutritional Management

Nutritional status of plants plays a crucial role in disease resistance. Considering the fact, optimum nutrient uptake by the plants is essential to avoid nutritional deficiency. Mineral fertilizers have a major impact on overall plant health, and in many circumstances, they are the foremost line of defence activators against plant pest and diseases. It can also activate the disease resistance through induced defence responses including the production of different types of metabolites, toxins and lignification (Engelhard 1989). Supplementation of soil with nutrients is found to influence the susceptibility of plants towards various fungal diseases (Veresoglou et al. 2013). A balanced mineral nutrients application in the form of fertilizers can improve the plants' disease resistance in most of the cases (Usherwood 1980). In this regard, manipulation of nutrient uptake is a key approach, as all essential plant nutrients have influence on the plant health and their susceptibility to diseases (Agrios 2005). Therefore, apart from fungicide treatments, enhanced nutritional programmes (ENPs)—by employing mineral nutrients and plant hormones that are applied at seedling stage—make plants resistant to BSR disease after transplanting them in the field.

9.4.2.1 Major Nutrients

Experimental studies using macro- and microelements such as nitrogen (N), phosphorus (P) and potassium (K) have resulted in positive changes to disease status and productivity of plant, but the actual role of fertilizers in controlling BSR disease is still uncertain (Singh 1990; Chung 2011). Lately, Hasmah Mohidin (personal communication) observed that seedlings raised on peat soil in nursery showed better vegetative growth and reduction in BSR incidence when applied with a combination of primary macronutrients such as N, P_2O_5 and high K_2O at 17.37 g, 17.37 g and 41.34 g per plant, respectively. In addition to this, activities of defence-related enzymes, including chitinase, β-1,3-glucanase, PAL and POX, were found to be enhanced in the oil palm roots, thus confirming the role of macronutrients in inducing resistance against *G. boninense*. In addition, potassium modifies plant metabolism and thus limits the invasion of pathogen by inducing thicker outer wall formation in epidermal cells (Dordas 2008).

9.4.2.2 Micronutrients

Micronutrients are less considered in BSR management strategies even if their involvement in plant defence activation is well known. Micronutrients such as boron (Stangoulis and Graham 2007), copper (Evans et al. 2007) and manganese (Thompson and Huber 2007) are shown to assist in controlling many plant diseases and they are closely associated with phenol synthesis in plants and have major impact on plant susceptibility to diseases (Graham 1983).

Earlier reports revealed that application of calcium nitrate was adopted to suppress the symptoms of BSR on oil palm (Sariah and Zakaria 2000). In addition, it was noticed that supplementing soil with calcium nitrate could enhance the population of *Trichoderma harzianum* and other antagonistic fungal population. These

discoveries are in agreement with the findings of Nur Sabrina et al. (2012). Boron (B), copper (Cu) and manganese (Mn) were shown to reduce the disease incidence and severity in seedlings of oil palm inoculated with *G. boninense* (Bivi et al. 2014). Under glass house conditions, oil palm seedlings exhibited increased resistance against *Ganoderma* when applied with calcium (Ca) and copper (Cu) in combination. In studies conducted by Tengoua et al. (2014), double combination treatments, namely B + Mn and Cu + Mn, alleviated the disease severity in oil palm seedlings under nursery condition with reduction of 16% and 24%, respectively.

9.4.2.3 Beneficial Elements

Studies conducted by Najihah et al. (2015) revealed that the use of calcium silicate, potassium silicate, sodium silicate, silicon oxide and sodium meta-silicate reduced the severity of BSR in oil palm seedlings. Endodermal deposition of Si enhanced the cellular features by forming a mechanical barrier, hence restricting movement of pathogen into the stems. Nursery study using beneficial nutrient proved that supplementation of 1200 mg/L of SiO_2 contributed to highest BSR reduction of 53%, with less number of primary roots and bulb tissue lesions infected with the fungus. Salicylic acid (SA) is a crucial plant hormone (Raskin 1992) that is well known for activating host defence responses during pathogen infection and abiotic stress (Gautam and Singh 2009; Pieterse et al. 2009) and is an essential factor in the systemic acquired resistance (Nie 2006). Bivi et al. (2014) demonstrated that application of a combination of calcium chloride, copper-EDTA and salicylic acid (SA) has reduced the disease symptoms in BSR-infected palms. EDTA has potential to inhibit ligninolytic enzymes produced by *G. boninense* (Siddiqui et al. 2019). Calcium/copper/SA supplementation on a continuous basis can be a key approach to improve resistance in oil palm (Bivi et al. 2016). An apparent increase in lignin content was observed, which explained how resistance was induced. A new fertilizer technology, GanoCare®, that is formulated by combining powdered empty fruit bunches (EFB) and beneficial elements, was found to be effective in preventing BSR infection in oil palm (Rebitanim et al. 2020).

9.4.2.4 Soil Amendments

Soil amendments application is one of the strategies for managing *Ganoderma* in palms. Applying decomposed green manure or farmyard manure at 50 kg/palm/year in combination with 5 kg of neem cake can check the disease spread in the field (Prakasam et al. 1997). In a trial conducted, it is shown that phosphobacteria (200 g peat inoculum + 10 kg of farmyard manure) could cause significant reduction in BSR disease in coconut (Bhaskaran et al. 1994). In another study, Bhaskaran (2000) showed that supplementation of phosphobacteria (200 g in 10 kg of farm yard manure/tree/year) evidently lessened severity of the disease in coconut compared to treatments with *Azospirillum* or the VAM fungus, *Gigaspora calospora*.

9.4.3 Management of *Ganoderma* Using Chemical Fungicides

The use of chemicals tends to be a well-founded strategy for the oil palm plantations. Chemical method of management is shown to be efficient only if applied judicially. The use of fungicides requires careful consideration, and field level evaluation of the results is necessary. This method combined with soil mounding shown to be effective and the benefit-costs need reassessment.

A number of fungicides have detrimental effect on the growth of *Ganoderma*; systemic fungicides, especially those in the triazole group, were noted to be highly effective as they could penetrate and spread to different parts of the plant (Khairudin 1990; Gurmit 1991). Azoxystrobin (EC50 of 0.53 g/mL), carbendazim (EC50 of 0.026 g/mL), hexaconazole (EC50 of 0.026 g/mL) and pyraclostrobin (EC50 of 0.25 g/mL) are among the fungicides that have exhibited inhibition of *G. boninense* (in vitro) with low EC50 values (Idris et al. 2010a, b; Said et al. 2019). It was recorded that 74.4% of the hexaconazole-treated oil palms could stand alive with the production of fruit bunches up to 5 more years. In contrast, none of the untreated palms could survive the disease (Idris et al. 2010b).

9.4.3.1 Delivery Systems

Control of BSR by chemical method can be achieved only if properly applied. Application of chemical fungicides for control of soil-borne pathogens constitutes soil drenching, pressurized trunk injection or combination of both. Similarly, trunk injection method was also assessed in the field by use of systemic fungicides with the help of a pressure injector (Idris et al. 2010a). According to a study conducted by the Malaysian Palm Oil Board (MPOB), it was demonstrated that the application of hexaconazole onto infected standing palms using a trunk injector restricted the spread of *Ganoderma* infection within the palm trunk (Idris et al. 2004b; Mohammed et al. 2014). It was also recorded that cyproconazole was capable to hold up 97% of the standing palms infected with *Ganoderma*. In another field study, trunk injection treatment with carboxin–quintozene mixture was found efficient by extending the lives of about 91% of the palms by 69 months after treatment (George et al. 1996). These findings manifest promising results by confirming the restriction of the disease progression in affected palms and thus prolonging the productive life of palms.

Eradication of wood-decay fungi of tree crops using chemicals, which are normally used for soil fumigation, has shown success in field studies. Dazomet (a soil fumigant), which releases methyl isothiocyanate, was shown to move within the stem tissues of palm and thus could subsequently limit the growth of *G. boninense*. Furthermore, prophylactic spraying with dazomet was successful in eradicating the pathogen inoculum in the infected stumps, hence limiting the spread of the fungus within plantations (Idris and Maizatul 2012).

Nevertheless, the development of alternative means to manage diseases is the need of current scenario as there is an increasing concern about the ecological issues and high cost of pesticides. However, employing chemical control measures delay

the spread of the disease. Additionally, inhibition of defence mechanisms in plants could be affected by the fungicidal action (Oostendorp et al. 2001).

9.4.3.2 Chitosan-Based Nano Fungicides

Recently, chitosan-based nano-fungicides have emerged as a remarkable breakthrough in enhancing the efficacy of fungicides. Such nano-delivery systems put forward controlled release characters with high potency and efficacy in delivering the fungicides at the target, compared to their counterparts (Duhan et al. 2017). It also aims to enhance uptake and reduce volatilization as well as toxicity level, thus keeping down their adverse effect on the environment (Worrall et al. 2018). In addition, chitosan is non-toxic and compatible with other bio-agents and also known for its potential to check the spread of pathogens and enhance the plant defence responses (Maluin and Hussein 2020). Chitosan-based encapsulated formulations of hexaconazole and/or dazomet can be encapsulated into the chitosan nanoparticles and it would act as an efficient antifungal agent for the control of *Ganoderma*. This new nano-formulation comprising chitosan (carrier) and hexaconazole (active ingredient) can be a better option for the management of BSR, and the results suggested the ability of these nanoparticles to persist in the crop longer than the conventional formulations. As reported in previous works, the release period of chitosan–hexaconazole nanoparticles was six times more than that of their counterpart (Maluin et al. 2019). The findings also indicated the movement of the nanoparticles in the internal parts of the stem and leaf, rather than being mobilized to the fruit. It was noticed that the crude palm oil and crude palm kernel oil were devoid of residue. Additionally, increased accumulation of the active ingredient in stem and leaf after treatment with the hexaconazole nanoparticles is ideal for enhanced bioavailability of the product for the prevention of *G. boninense*. Thus, the chitosan–hexaconazole nanoparticles offer a better platform for the effective control of BSR disease as the disease can be managed over a long period without any residue in the palm oil matrices. This is the ideal property for furnishing nano-based fungicides for basal stem rot disease management in oil palm (Maluin et al. 2019).

9.4.4 Biological Control of *Ganoderma* in Oil Palm

As the conventional control measures such as chemical, cultural and mechanical practices are found to be unsatisfactory in field conditions (Susanto et al. 2005), there is a need to switch on to alternate strategies for managing the disease to extend the productive life of the palms in the field, and it is mainly focused on biocontrol agents (BCAs). Biological control is generally the prime choice of prevention and control in the integrated disease management approach. In this era of sustainable agriculture, biological control with the use of natural enemies is a promising green tool as compared to synthetics. The biological control and growth promotion activities of these microbes could offer sustaining economic benefits for the palm oil industry. Development and exploitation of biocontrol agents mainly focus on four pivotal points: (1) biocontrol properties of the microbial agents, (2) evaluation of microbe–

plant interactions, (3) assessment of ecological and beneficial effects of the agent in the rhizosphere and (4) formulation and proper delivery of the microbial agents (Herrmann and Lesueur 2013; Miransari 2013). Studies conducted in plantation seedlings with microbial antagonists have shown remarkable results in managing BSR, but large-scale plantation-based evaluation is needed to validate the success of using BCA for the long-term control of *Ganoderma* in field condition.

A list of BCAs studied for the management of *Ganoderma* in oil palm is shown in Table 9.2. Growth promotional activities of these microbes, including enhanced root and plant development, induced resistance and solubilization of inorganic nutrients, would assist in the control of the disease (Susanto et al. 2005).

9.4.4.1 Fungal and Bacterial Antagonists

Ascomycetes

Trichoderma spp. are the commonly utilized bio-agents to combat a wide range of plant pathogenic organism, especially soil-borne pathogens. This beneficial free-living fungus has antagonistic effects on many phytopathogens and can inhibit growth and survival of pathogens by employing multiple mechanisms including mycoparasitism, antibiosis, hydrolytic enzyme production, competition and plant resistance induction (Nusaibah and Musa 2019).

Biocontrol of *G. boninense* using *Trichoderma* spp. has recorded high effectiveness and potency in controlling the pathogen in both green house and field conditions (Ilias 2000; Sariah et al. 2005; Susanto et al. 2005). Similarly, it is found to induce plant defence activation in oil palm by enhancing the production of fungal cell wall-degrading enzymes such as chitinases and glucanases (Naher et al. 2011). These two enzymes have synergistic effect on each other and adversely affect the hyphal growth of filamentous fungi (Latgé 2007). Similar defence responses have previously reported in *Ganoderma* spp. infected tissues (Siswanto and Darmono 1998). Investigations on control of *G. boninense* using *T. harzianum* in green house showed that the *T. harzianum*-treated oil palm seedlings had reduced disease incidence compared to the control (Naher et al. 2012; Izzati and Abdullah 2008; Susanto et al. 2005). *T. harzianum* solely or mixed with dried palm oil mill effluent, calcium nitrate and mycorrhizal preparation were tried out in a nursery and have showed a notable effect on the seedlings (Sariah and Zakaria 2000). Furthermore, disease suppression was reported in seedlings of oil palm treated with *T. harzianum* isolate FA1132 conidial suspension (Izzati and Abdullah 2008). This isolate showed notable antagonistic activity against *G. boninense* in trials conducted in plant house. It was also suggested that *T. harzianum* was a more efficient biocontrol agent against *G. boninense* than other species such as *T. longibrachiatum* and *T. virens* (Ilias 2000).

In addition to *Trichoderma*, many other ascomycetous fungus have been found to be parasitic on *Ganoderma* spp. From oil palm, numerous mycoparasitic ascomycetous fungi were isolated, which are capable of sporulating asexually and/or sexually on *G. boninense* (Goh et al. 2015). Out of these, *Scytalidium parasiticum* was shown to be a necrotrophic parasite on *G. boninense* and could be a possible biocontrol agent against this basidiomycetous pathogenic fungi. In the in vitro studies,

Table 9.2 Potential biocontrol agents for the control of BSR in oil palm

Bio-agent	Effects	Reference
1. *Fungus*		
Hendersonia isolate (GanoEF1)	Reduced incidence of BSR disease in nursery seedlings after 6 months of treatment	Nurrashyeda et al. (2018)
Scytalidium parasiticum	Suppressed fruiting body regeneration of *G. boninense* Reduced disease incidence and severity in nursery trials Improved seedling growth	Goh et al. (2016)
Trichoderma sp.	Growth inhibition of *G. boninense* in laboratory trials and plant growth promoting activities Delay of infection at early stages Enhance the defence response in oil palm by inducing the fungal cell wall-degrading enzymes production, including chitinases and glucanases	Hasan and Turner (1998); Ilias (2000); Sariah et al. (2005); Susanto et al. (2005) Naher et al. (2011)
Trichoderma sp. + dried palm oil mill effluent, calcium nitrate and mycorrhiza	Notable effect on disease suppression in oil palm seedlings	Sariah and Zakaria (2000)
Soil mounding + *T. harzianum*	Extended life of infected palm by 3 years in field trials	Priwiratama and Susanto (2014)
Hymenomycetes (*Pycnoporus sanguineus*, *Trametes lactinea* and *Grammothele fuligo*)	Growth inhibition of *G. boninense* under in vitro screening	Naidu et al. (2018)
Talaromyces apiculatus and *Clonostachys rosea*	Disease reduction by 4.9–60% in treated seedlings Plant growth promoting traits	Goh et al. (2020)
Scytalidium parasiticum	Reduced fruiting body regeneration and inhibited the mycelial survival of *G. boninense*. In nursery trials, reduced disease severity of *Ganoderma*	Goh et al. (2016)
2. *Bacteria*		
Burkholderia sp.	Decreased disease incidence in seed-treated plants up to 3 months in nursery	Buana et al. (2014)
Burkholderia cepacia, *Pseudomonas aeruginosa* and *Serratia marcescens*	Inhibition of *G. boninense* in nursery trials	Sapak et al. (2008); Azadeh et al. (2010)
Bacillus sp. and *Enterobacter* sp.	Reduced incidence of disease in seedlings Growth promotional activity	Suryanto et al. (2012)
3. *Actinomycetes*		
Streptomyces spp. and *Nocardiopsis* sp.	Reduced disease incidence and severity by 81.6% in seedlings Reduced severity of foliar symptoms in nursery trials	Tan et al. 2002; Ting et al. 2014; Sujarit et al. (2020)

S. parasiticum remarkably reduced fruiting body regeneration and inhibited the mycelial survival of *G. boninense*. In addition, nursery trials suggested that *S. parasiticum* was non-pathogenic on seedlings of oil palm and it could also limit infection by *Ganoderma* and thus the disease severity (Goh et al. 2016).

Basidiomycetes

Attempts to control stump infection using basidiomycetes have been made in forest trees (Roy et al. 2003). No such investigation has been done in BSR-affected oil palm. Non-pathogenic hymenomycetes, naturally found on oil palm trunk, were tested for their antagonistic activity against *Ganoderma*. Out of 25 fungi isolated, 8 appeared to be antagonists against the pathogen. Three potential antagonists including *Grammothele fuligo*, *Pycnoporus sanguineus* and *Trametes lactinea* restricted the mycelial growth of *G. boninense* with higher PIRG (percentage inhibition of radial mycelial growth) in dual culture (Naidu et al. 2018). Nonetheless, further studies are required to validate their potential use for the management of infection of *G. boninense* in field.

Actinomycetes

The actinomycetes isolated from mangrove area, including *Streptomyces* and *Micromonospora* sp., were antagonistic to *G. boninense* in oil palm, and *Streptomyces* genus has high inhibitory effect on *G. boninense* in vitro by hyphal lysis and antibiosis (Tan et al. 2002). Non-pathogenic actinomycetes such as *Nocardiopsis* sp. and *Streptomyces* spp. isolated from empty fruit bunches of oil palm were also identified as antagonists against *G. boninense* (Ting et al. 2014). Anti-*Ganoderma* activity of three *Streptomyces* species such as *S. palmae*, *S. sioyaensis* and *S. noursei* was proved in vitro, and *S. palmae* CMU-AB204^{T} isolate was found as effective inoculant, which reduced the severity of foliar symptoms and showed lowest percentage disease severity. Additionally, the treated seedlings marked highest plant vigour in terms of biomass and stem diameter (Sujarit et al. 2020). Thus, *S. palmae* could be an assuring biocontrol candidate to protect the palm trees from BSR.

9.4.4.2 Fungal and Bacterial Endophytes

Nowadays, antagonistic endophytes are drawing attention as a promising bio-agent for plant disease control, resulting in replacement of harmful chemicals (Kobayashi and Palumbo 2000). Endophytes live asymptomatically within plants, bringing with them additional benefits such as improved crop development and health, as well as the ability to generate plant resistance through the secretion of secondary compounds and antibiotics (Zhao et al. 2015). As they colonize and move within the plant, they are suitable for the holistic control of diseases like BSR and are usually unaffected by environmental changes. Apart from *Trichoderma* spp., microbes such as *Gliocladium viridae*, *Bacillus* spp. (Susanto et al. 2005), *Burkholderia cepacia* and *Pseudomonas aeruginosa* (Sapak et al. 2008) were also studied as a promising biocontrol agent for BSR management. Shamala (2013) made the first record of endophytic *Trichoderma* isolated from oil palm with biocontrol activity towards

G. boninense. In an attempt to evaluate their competency for managing BSR disease, four endophytic fungi—a Dothidiomycetes species, *Lasiodiplodia venezuelensis*, *T. longibrachiatum*, and *T. harzianum*—were investigated. It was hypothesised that these fungi could stimulate the production of pathogenesis-related protein in the palm (Esyanti et al. 2017).

Some of the gram-negative and gram-positive endophytic bacteria can be a potential biocontrol agent against *G. boninense* pathogen of oil palm. Bacteria such as *Bacillus* spp., *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Serratia marcescens* have been recorded as possible bio-agents to control BSR disease (Zaiton et al. 2006; Bivi et al. 2010). Chitinolytic bacteria including *Bacillus* sp. and *Enterobacter* sp. could cause hyphal abnormalities in *Ganoderma* in vitro and are capable of minimizing the disease incidence in nursery seedlings. Endophytic *Bacillus subtilis* was isolated from oil palm, and it was revealed that these antagonistic isolates would limit the growth of *Ganoderma* with an inhibition of 8.13–49.38% (Nasahi et al. 2016). The effectiveness of induction of resistance by *B. subtilis* has not been evaluated well in oil palm. Other species including *B. cepacia* and *B. amyloliquefaciens* were also shown to restrict the mycelial growth of *G. boninense* in vitro (Azadeh et al. 2010; Azizah et al. 2015). *P. aeruginosa* has been reported to enhance the growth of plants by producing various growth promoting hormones such as auxin and cytokinin and also other volatile compounds including ethylene, acetonin and 2,3-butanediol (Lambrecht et al. 2000; Persello-Cartieaux et al. 2003; Ryu et al. 2003). *P. aeruginosa* was found to improve the root mass and seedling growth and was effective in controlling *G. boninense* in comparison to *B. cepacia* (Zaiton et al. 2008). Furthermore, Ramli et al. (2016) reported the effectiveness of *P. aeruginosa* in reducing the disease incidence and foliar symptom severity in treated oil palm seedlings, compared to *P. fluorescence* and *B. cepacia*.

9.4.4.3 Arbuscular Mycorrhizal Fungi (AMF)

In recent times, the usage of endophytic arbuscular mycorrhizal fungi has accelerated in the field of agriculture in an attempt to enhance yield and plant health with further advantage of restricted use of pesticides and fertilizers (Barea et al. 2002; Gianinazzi et al. 2010). AMF are symbiotic fungi of mycorrhizal origin which carry out essential ecological functions such as augment plant nutrients uptake, enhancement of plant tolerance to environmental stress and improvement of soil structure (Smith and Read 1997). AMF are found to be associated with oil palm roots and may hinder *G. boninense* (Sundram et al. 2015). Azizah (2003) recorded that oil palm seedlings treated with mycorrhiza could combat the infection by *Ganoderma*. Evaluations in nursery trials proved the efficacy of the arbuscular mycorrhizal fungi in suppression of the *Ganoderma* incidence (Priwiratama and Susanto 2014). Application of the mycorrhizal fungus, *Glomus intraradices*, restricted the disease progression of BSR, and a combination of this fungus with endophytic bacteria further improved the biocontrol potency (Sundram et al. 2015). Moreover, treatment with the mycorrhizal fungi was effective in prolonging the incubation period of the pathogen, and mycorrhizal fungal inoculants could significantly enhance the growth of the seedlings of oil palm artificially inoculated with *Ganoderma*, in terms of dry

and fresh weight of seedling and leaf number (Widiastuti 2011). Thus, the use of AMF can be a promising approach for the management of BSR disease, but there is a necessity for further large-scale trials and review of their field efficacy.

9.4.4.4 Delivery Mechanism

A possible approach to deliver the biocontrol agents like endophytes is the seed enrichment. The use of microbes such as *Trichoderma* spp. and AMF is regarded as a standard operational procedure (SOP) in the production of seedlings of oil palm especially in *Ganoderma* endemic area. The dose of these microbial agents to be applied can vary based on the developmental stage of the plant. Thus, seed coating of these biocontrol agents can be a solution for the efficient transportation and delivery in the field. Even though seed coating and enrichment is a general approach in horticultural seeds, this method has not been practiced much in oil palm due to the susceptibility of the seeds to mechanical damage. The delivery of the consortium of AMF, *T. asperellum* and *E. sacchari*, followed by Carboxymethyl cellulose (CMC) coating, is likely to improve the seedling vigour of oil pam in pre-nursery stage (Jawak et al. 2018).

9.4.4.5 Challenges in Field Level Testing of Biocontrol Agents

- The use of biocontrol agent (BCA) in field conditions often faces difficulty because of susceptibility of microbes to combative environmental conditions. Various obstacles such as alteration in the rhizosphere, inability to colonize in different soil conditions, interaction with non-target organisms, genetic diversity of the pathogen, the presence of other microbes and vulnerability to climate change lead to poor performance of BCAs in field (Meyer and Roberts 2002). The use of bio-agents for field applications can be effortful due to (a) difficulty in handling and transport, (b) poor storage and (c) intricate application requisite (Vidhyasekaran et al. 1997). In addition, some fungal bio-agents produce mycotoxins that are harmful to the environment and also contaminate the economical product (palm oil). Thus, only few BCAs could be commercialized due to the instability of many of the microbes in field.
- Although the potency of BCAs for the management of BSR has proven, most of the studies are nursery-based trial without field assessments, which is a time-taking process. The effectiveness of different biocontrol agents has been investigated in nursery (Soepena et al. 2000; Izzati and Abdullah 2008; Sapak et al. 2008; Sundram et al. 2008; Suryanto et al. 2012), but a regular field evaluation needs at least 3–5 years monitoring to obtain relevant results. Hence, establishment of a shorter time scale-based effective system is required for the appraisal of biocontrol agents at the field level. Flood et al. (2000) exploited a bait seedling trial to assess the implication of inoculum intensity of *G. boninense*, in which the bait seedlings were planted adjacently to the differing inoculum intensities of the pathogen for determining the significance of removal of infected tissues while replanting. This technique comes up with benefits such as possibility of field assessment and shorter observation time.

9.4.4.6 The Concept of Biocontrol Consortium

Most of the investigations on biocontrol agents for the plant health management are focused on the application of a solitary BCA against a single pathogen. Yet, the use of a single BCA may not be efficient in all types of soils, as optimum conditions for growth and multiplication of each microbe vary. Combining multiple microbial agents has benefits over a sole biocontrol agent in controlling diseases (Lemanceau et al. 1993; Pierson and Weller 1994; Crump 1998). Thus, researchers have been trying to improve the efficacy of biocontrol by exploiting multiple biocontrol agents (Multi-BCAs). Moreover, it is obvious that the naturally happening biocontrol is the result of action of a mixed population of antagonists rather than by an individual organism. Hence, introduction of Multi-BCAs will help in expanding their mode of action for the management of pathogens with stable broad spectrum activity (Mishra et al. 2011).

Based on initial studies, a combination of *T. asperellum* and *P. aeruginosa* was selected and assessed for the control of *G. boninenese* in terms of antagonistic activity, enzymatic action and also plant growth promoting properties. Both could inhibit the mycelial growth of *G. boninense* with Percentage of inhibition radial growth (PIRG value of more than 50%. In addition, both showed positive results to IAA production and phosphate solubilization, whereas only *T. asperellum* exhibited siderophore production properties (Muniroh et al. 2019). Studies have also reported that endophytic bacteria could assist the mutualistic interaction of AMF with the host plant and encourage the defence responses against plant pathogens (Garbaye 1994; Pivato et al. 2009). This combination would offer notable benefits including enhanced chitinase production and growth promotion of plants. Even though AMF could assure protection against pathogens (Smith and Read 1997; Gianinazzi et al. 2010), picking the suitable endophytic bacteria was crucial for assessing the biocontrol potential of the consortium against *G. boninense*. Two such potential endophytic bacteria, *Burkholderia cepacia* UPMB3 and *Pseudomonas aeruginosa* UPMP3, were isolated and evaluated by Sapak et al. (2008) and found effective suppression of *G. boninense* both in vitro (Sundram et al. 2011) and in vivo studies (Sapak et al. 2008). In the previous study, it was observed that the same strains of endophytic bacteria could also increase the hyphal growth and spore germination of *Glomus clarum* BR152B and *Glomus intraradices* UT126 (Sundram et al. 2011). It was also recorded that the endophytic bacterial strains had similar activities like mycorrhizal helper bacteria (Garbaye 1994).

Other possibilities were also explored by researchers, and apart from the common endophytic microbes, two ascomycetous fungi were studied for their compatibility and potential use in the control of BSR. Plant growth promoting activity and biocontrol traits of *Clonostachys rosea* AAB0114 and *Talaromyces apiculatus* AT0115 consortium against BSR disease were evaluated in nursery. Inoculation of the consortium as well as the individual fungus brought about significant increase in both bole girth and leaf area of the seedlings after 5 months of treatment. Additionally, the treated seedlings showed considerable reduction in disease incidence compared to the control treatment. Co-inoculation of the two fungi came up with

notable disease control efficiency, indicating its potential use as a biocontrol strategy against *G. boninense* (Goh et al. 2020).

9.5 Breeding for Genetic Resistance

The use of resistant planting materials would be a better option for the long-term control of BSR disease in plantations. The crucial factor in the breeding programmes for the disease resistance is the source of resistance. Sources of susceptibility and genetic resistance against *Ganoderma* have been recognized in field experiments, suggesting the reflection of genetic resistance as a component of Integrated disease management (IDM) of BSR (Idris et al. 2006; Chung 2011). Oil palms with varying genetic origins have been shown to be tolerant to *G. boninense* (Durand-Gasselin et al. 2005; Idris et al. 2004b). Franqueville et al. (2001) reported sources of genetic resistance in field studies in North Sumatra and it was further confirmed by Durand-Gasselin et al. (2005). Oil palms of Deli origin (both Indonesia and Malaysia) were appeared to be more susceptible compared to those originated from Africa (Durand-Gasselin et al. 2005), revealing the presence of possible genetic resistance. These findings point out that the enhancement of resistance of planting materials using available genetic sources could be a promising disease management strategy in BSR risk area. Breeding and selection of palms with greater lignin deposition (Casler et al. 2002) or modifying the lignin structure may be another key approach for the improvement of resistance in palms (Rees et al. 2009).

9.5.1 Genetic Engineering

Exploitation of genetic engineering tools could be a surpassing choice for the improvement of planting materials owing to their cost and time effectiveness (Sambanthamurthi et al. 2009). Enhanced expression of chitinases and glucanases to combat *Ganoderma* can be realized with the help of genetic engineering approach. In previous studies, two genes, rice chitinase (RCH10) and alfalfa glucanase (AGLU1), were employed to genetically engineer oil palm in view of resistance against *G. boninense*. Rashdan and Abdullah (2000) were able to successfully transform the oil palm through *Agrobacterium*-mediated transfer of chitinase gene against *Ganoderma*.

9.5.2 Application of Omic Technologies

9.5.2.1 Transcriptomics

The global-based gene expression analysis in oil palm in response to pathogen could be achieved through transcriptomic databases. This information available on the differential gene upregulation and expression upon oil palm–pathogen interaction is a great source for designing markers for the selection of resistance in oil palm. In

contrast, the down-regulated genes during the interaction can be utilized for identification of markers linked with susceptibility, and therefore it further helps in selecting the susceptible seedlings. The use of the molecular markers at least avoids the deployment of susceptible materials to the field. Moreover, these susceptible genes would be a potential target for the gene editing approaches.

Transcriptomic analysis conducted by Tee et al. (2013) indicated involvement of defence-associated genes, encoding phenylalanine ammonia lyase (PAL), isoflavone reductase (IFR) and cinnamate 4-hydroxylase (C4H). Chong et al. (2012b) identified the accumulation of three antifungal compounds including caffeic acid, 4-hydroxybenzoic acid and phenolic acids such as syringic acid (SA) during oil palm–*Ganoderma* interaction. In another study carried out by Wulandari et al. (2018), it was shown that the EMLP1 gene was up-regulated amid the *G. boninense* infection. Faizah et al. (2019) identified 16 susceptibility response-related genes from the oil palm roots.

9.5.2.2 Proteomics

Comparative proteomic analysis during *Ganoderma* and oil palm interaction has revealed the variation in expression of defence-related proteins, including adenosine triphosphate (ATP) synthase, cysteine synthase, caffeic acid *O*-methyltransferase (COMT), caffeoyl-CoA *O*-methyltransferase (CCoAOMT), malate dehydrogenase, enolase and fructokinase, upon the progression of BSR disease (Al-Obaidi et al. 2014). Likewise, proteins associated with oxidative burst such as ascorbate peroxidase (APx), 2-Cys peroxiredoxin (Prx), APx catalase and superoxide dismutase (SOD) were also identified (Daim et al. 2015) and characterized (Caverzan et al. 2012).

9.5.2.3 Metabolomics

A wide range of metabolites can be assessed by employing metabolomics approach for phenotyping and diagnostic analysis in plants (Fernie and Schauer 2009). There have been several reports on metabolite diversity of oil palm roots with a potential role in disease resistance, including the pre-formed phytoanticipins and inducible phytoalexins (Diabaté et al. 2009). Dzulkafli et al. (2019) detected and identified chelidonic acid in the leaves of oil palm upon artificial inoculation with *G. boninense*. Syringic acid (a phenolic acid) accumulation in *Ganoderma*-infected oil palm roots indicates the possible anti-fungal activity of these compounds against the pathogen (Chong et al. 2012a, b). Furthermore, the presence of sterols and tocopherols was detected in infected oil palm roots by using gas chromatography-mass spectrometry (GC-MS) (Nusaibah et al. 2011b). In previous studies, it has been reported the resistance against vascular wilt pathogen (*Fusarium oxysporum*) in the presence of phenolic acids, and thus it suggests their role in indication of disease infection (Diabaté et al. 2009).

9.6 Conclusion

Basal stem rot caused by wood rotting basidiomycete, *Ganoderma* spp., is considered to be the deadliest disease of oil palm. For proper monitoring and early identification of disease in oil palm, more automated, objective and sensitive approaches such as artificial intelligence, multispectral imaging and sensor-based techniques may be used. Management strategies for controlling BSR in oil palm should integrate the existing and advanced technical knowledge. Different models of integrated disease management should be evaluated under large scale across plant species and ecosystems to come out with an effective package of practice. Marker-assisted early disease resistance phenotyping, histological characterization, pathogen genome and diversity analysis, and host–pathogen interaction investigations should all be included in disease resistance breeding in oil palm, in addition to traditional breeding methodologies.

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Talaromyces flavus: An Important Rhizospheric Inhabitant

10

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Abstract

The *Talaromyces*' story started about 100 years ago, with isolation and description of the first strain, albeit under another name. It continued with taxonomic studies, secondary metabolites identification, and the study of their effect on biological models. Subsequently, it continued with attempts to apply this strain in agriculture for biocontrol of phytopathogenic microorganisms and culminated with the study of *T. flavus* genetic equipment. Biological studies with metabolites such as vermiculine, vermistatin, dehydrolatenusin, or purpactins have broadened our horizons in immunology, cancer treatment, or metabolic diseases. This chapter describes some of the important metabolites produced and the role of *T. flavus* as biocontrol agent in sustainable agriculture system.

Keywords

Talaromyces flavus · Biological control · Phytopathogen · Secondary metabolites · Glucanase · Chitinase

10.1 Introduction

Talaromyces flavus is the most common species of the genus *Talaromyces*, which has been studied and applied as a biocontrol agent, a producer of secondary metabolites or enzymes. *T. flavus* is an extremely variable species found in soils and on organic materials that undergo slow decomposition. The species is widespread in its distribution but it is most commonly reported from the warmer regions

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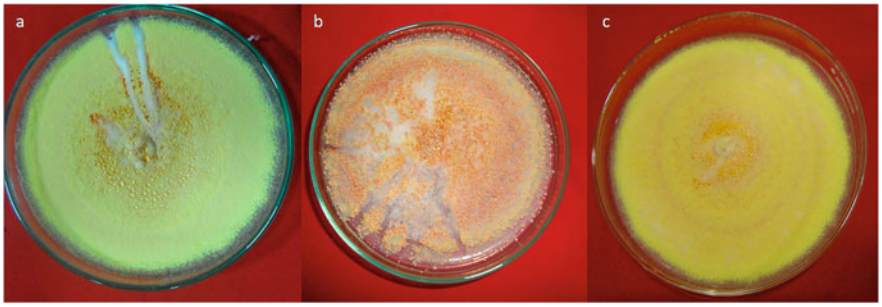

Fig. 10.1 *Talaromyces flavus* isolates showing different colony characteristics: (**a**) *Tf1*, (**b**) *Tf2*, and (**c**) *Tf3*

of the world. It also occurs in foods, probably as a contaminant (Pitt and Hocking 2009). Dangeard (1907) provided a detailed report of *Penicillium vermiculatum*, which Benjamin (1955) relocated to the genus Talaromyces, and Orr et al. (1963) demonstrated the identity of this strain with *Gymnoascus flavus* described by Klocker (1902). *Arachniotus indicus* (Chattopadhyay and Das Gupta 1959) and *P. liani* were also declared identical with *T. vermiculatus* (Ghosh et al. 1961). Stolk and Samson (1972) redefined the genus *Talaromyces* and distinguished two new varieties of *T. flavus*, namely *T. flavus* var. *flavus* (isolates varies in color and colony characteristics; Fig. 10.1) and *T. flavus* var. *macrosporus*.

10.2 Classification

Species classified in the phylum Ascomycota, class Eurotiomycetes, order Eurotiales, family Trichocomaceae, and genus *Talaromyces* are as follows (MycoBank 2010):

- *Talaromyces flavus* (Klocker) Stolk and Samson (1972)
- Basionym: *Gymnoascus flavus* Klocker (1902)
- Synonyms: *Arachniotus indicus* Chattopadhyay and Das Gupta (1959)
- *Talaromyces flavus* var. *flavus* (Klocker) Stolk and Samson (1972)
- Synonyms: *Penicillium liani* Kamyschko (1962), *T. vermiculatus* (Dangeard) Benjamin (1955), *G. flavus* Klocker (basionym), *A. indicus* Chattopadhyay and Das Gupta (1959), *A. indicus* var. *major* Chattopadhyay and Das Gupta (1959)

Status conidialis (anamorphs):

- *P. vermiculatum* Dangeard (1907)
- *P. liani* Kamyschko (1962), *Penicillium vermiculatum* Dangeard (1907)
- Synonym: *Eupenicillium vermiculatum* (Dang.) Ram and Ram (1972)

Teleomorphs:

- *P. dangeardii* Pitt (1979)
- *T. flavus* var. *flavus* (Klocker) Stolk and Samson (1972)

10.3 Secondary Metabolites

The organic soluble metabolites of this fungus include D-glucono-1,4-lacton, 5-hydroxymethylfurfural, 4,6-dihydroxy-5-methylphthalimide, methyl-4-carboxy-5-hydroxyphethalal dehydrate, hexaketide, 7-hydroxy-2,5-dimethylchromone, 3-hydroxymethyl-6,8-dimethoxycoumarin, altenusin, desmethyldehydroaltenusin, talaroflavone, deoxytalaroflavone, 2-methylsorbic acid, sorbic acid, bromomethylsorbic acid, and bromosorbic acid (Ayer and Racok 1990a). Some of the above-mentioned metabolites (2-methylsorbic acid, sorbic acid, bromomethylsorbic acid, and bromosorbic acid) play a fundamental role in the biogeochemical cycling of phosphorus (P) in natural and agricultural ecosystems. Some of the metabolites produced by the *T. flavus* are reported in Table 10.1.

10.4 Enzymes

Esterase/amidase from *P. vermiculatum* stereoselectively hydrolyzed only 2R,3S enantiomer from the synthetically prepared monoalkylesters or dialkylamides of racemic phosphomycin (Demain et al. 1972). Amine oxidase (EC 1.4.3.4) catalyzes the oxidative deamination of amines by the formation of aldehyde, hydrogen peroxide, and ammonia. *T. flavus* var. *flavus* isolated from a soil sample collected from a rice field produced amine oxidase stable up to 40 °C with the optimum pH in a range of 7.5–8.5. This enzyme was intended to be used in biochemical analysis, e.g., determination of the freshness of meat by assaying tyramine or other amines (Matsumoto and Takada 1984). α-Amylase and glucoamylase *P. vermiculatum* precultured on amylose gel and cultured on a medium containing corn starch secreted crude α-amylase (EC 3.2.1.1), which was further purified on a cross-linked starch (Augustín et al. 1983). Thermostable amylases are used for starch hydrolysis in the liquefaction step of industrial starch syrup production. *T. flavus* secreted a thermophilic glucoamylase (EC 3.2.1.3), which exhibited peak activity at 50 °C and a pH of 4.0–4.8 (Hang and Woodams 1993). Chitinase: *T. flavus* grown in the presence of chitin produced two chitinases (EC 3.2.1.14). The isolated enzymes with a molecular mass of 41 and 32 kDa decomposed the cell wall of *Verticillium dahliae*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* (Duo-Chuan et al. 2005) at the optimum pH between 4.0 and 5.0, respectively and 40 °C. *P. vermiculatum* produced dextranase (EC 3.2.1.11) at the optimum temperature between 50 and 55 °C and pH in a range of 5.0–5.5. It hydrolyzed dextran to isomaltose, isomaltotriose, and glucose (Sun et al. 1988). The main application field of dextranases is sugarcane processing. The use of dextranases has also been extended to dental care as a

Table 10.1 List of important metabolites produced by *T. flavus*

S. no.	Secondary metabolite	Description	Reference	Function
1	Coumarins	3-Hydroxymethyl-6,8-dimethoxycoumarin (III)	Ayer and Racok (1990a)	Endophytic fungus *Pestalotiopsis* sp.
		Talacoumarins A (**1**) and B (**2**)	He et al. (2007)	
2	Chromones	2,5-Dimethyl-7-hydroxychromone (IV)	Ayer and Racok (1990a)	Until now, chromone IV has only been isolated from higher plants, e.g., *Bupleurum longicaule* wall and *Lycopus europaeus* L.
3	Short-chain organic acids	(–)-*Trans*-2,3-epoxysuccinic acid (V)	Sakaguchi et al. (1939), Martin and Foster (1955)	Antibiotic activity
		Fosfonochlorin (VI)	Hendlin et al. (1969)	
		2-Methylsorbic acid (VII)	Proksa et al. (1992a)	
	Phthalide derivatives	4,6-Dihydroxy-5-methylphthalide (IX) 2-Formyl-5-hydroxyterephthalic acid	Ayer and Racok (1990a)	Antioxidant activity
		Rubralide C (XV)	Kimura et al. (2007)	Antioxidant activity
		Funiculosic acid (XI)	Qureshi et al. (1980)	Antioxidant activity
4	Vermistatin and derivatives	Vermistatin (XVI, F)	Fuska et al. (1979a)	Vermistatin (XVI) is a cytotoxic agent without any marked antibiotic effect. This compound inhibited the utilization of precursors of nucleic acid and protein synthesis in Ehrlich ascites

(continued)

Table 10.1 (continued)

S. no.	Secondary metabolite	Description	Reference	Function
				carcinoma cells and suppressed proliferation of P388 cells in vitro (Fuska et al. 1979a)
5	Altenusin, dehydroaltenusin, and derivatives	Altenusin (XXIX)	Ayer and Racok (1990b)	Anticancerous
		Dehydroaltenusin (XXX), desmethyldehydroaltenusin (XXXI) together with the structurally related talaroflavone (XXXII) and deoxytalaroflavone (XXXIII)	Ayer and Racok (1990b)	Anticancerous
	(–)-Mitorubrin and related compounds	(–)-Mitorubrin (XXXV) and (–)-mitorubrinol (XXXVI)	Proksa et al. (1994, 1997)	Suggested for the treatment of trypanosomiasis, Chagas' disease, malaria, or coccidiosis (Hayashi et al. 1996)
6	Purpactin, penicillide, and related compounds	Vermixocins A (XL) and B (XLI)	Proksa et al. (1992b)	Acts as an acyl-CoA:cholesterol acyltransferase inhibitor
7	Vermiculine and derivatives	Vermiculine (XLVI)	Fuska et al. (1972)	Antimicrobial
		Vermiculinol (XLVII) and vermiculidiol (XLVIII)	Massias et al. (1989)	Antimicrobial
	Vermicillin	–	Fuska et al. (1979a, b)	Affected the synthesis of RNA in leukemia P388, EAC, NK/Ly, and L 1210 cells and suppressed the proliferation of P388 cells
8	TAN-2177A and B	Esterified oligopeptides TAN-2177A and B (LII) and (LIII)	Tozawa et al. (1996)	Specific inhibitors of squalene synthase may inhibit cholesterol biosynthesis

(continued)

Table 10.1 (continued)

S. no.	Secondary metabolite	Description	Reference	Function
9	Saccharides and polysaccharides	D-Glucono-1,4-lactone	Ayer and Racok (1990a)	Fungicidal activity
		Talaron	Mizuno et al. (1974)	

toothpaste additive, since dextran has been shown to be involved in dental plaque formation (Galvez-Mariscal and Lopez-Munguia 1991). *T. flavus* var. *flavus* cultured aerobically at 28 °C for 48 h on a medium containing fructan-afforded fructanase (EC 3.2.1.80), which has been applied in the production of fructose from Jerusalem artichokes (Ishibashi et al. 1974). *T. flavus* secreted α-D-galactosidase in the presence of 6-deoxyglucose. The crude enzyme was composed of three isoenzymes. The most important isoenzyme, αGal-1, showed a different regioselectivity than the other two isoenzymes. Purified αGal-1 catalyzed the transglycosylation of *tert*-butanol and split off D-galactose from raffinose and stachyose. This enzyme, inhibited by α-D-galactopyranosylazide, D-xylose, melibiose, or lactose (Simerská et al. 2007), catalyzed the reaction of 4-nitrophenyl-α-D-galactopyranoside (LXX) and its 6-acetyl derivative LXXI to 4-nitrophenyl-α-Dgalactopyranosyl-(1→3)-6-*O*-acetyl-α-D-galactopyranoside (LXXII) (Simerská et al. 2003). A mixture of biosides, which composed of 86.5, 3.5, and 8.0% of LXXIII, α(1→2), and α(1→6) regioisomers, respectively, was prepared after incubation of LXX with α-D-galactosidase from *T. flavus* (Weignerová et al. 2001). *P. vermiculatum*, grown in a culture enriched with α-1,3-glucan, secreted a remarkable amount of α-1,3-glucanase (EC 3.2.1.84). The production of this enzyme was stimulated by the addition of surfactants Tween 80 or Tergitol NPX to the culture medium (Reese et al. 1972). The antifungal activity of this enzyme was studied in relation to its role in mycoparasitic processes, especially in degradation of the host cell wall (Sanz et al. 2004). Glucose oxidase (EC 1.1.3.4) catalyzes the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide using molecular oxygen as the electron acceptor. *T. flavus* produces glucose oxidase, which is involved in the biocontrol of fungal plant pathogens. Pectinolytic enzymes are important for several industrial applications such as improving juice yields and clarity. Other areas of application include the paper and pulp industry, waste management, animal feed preparation, or the textile industry. *T. flavus*, precultured for 24 h on a solid substrate culture like passion fruit peel and then transferred into a new medium supplemented with 0.5–0.8% citrus pectin, secreted pectinesterase (EC 3.1.1.11) and polygalacturonase (EC 3.2.1.15) into the medium. High levels of pectinases cultivated in solid state fermentation using citrus pulp pellets were produced by *T. flavus* isolated from Brazilian soil (Siessere and Said 1989). Phytase, an enzyme that breaks down the indigestible phytic acid/phytates (inositol penta-, tetra-, and triphosphate) found in grains and oil seeds, was produced by *T. flavus* cultured in a medium containing up

to 6% of saccharides and 2% of nitrogen-containing compounds (Jiang et al. 2007). Proteinase K (EC 3.4.21.64), a serine protease, is used in molecular biology because it rapidly inactivates nuclease, which might otherwise degrade the DNA or RNA during purification and maintains its activity in the presence of chemicals that denature proteins. Proteinase K was secreted by *T. flavus* in media containing goat hairs. The highest enzyme production was observed at pH 6.5 after a 9-day incubation and the isolated raw enzyme was separated into two fractions characterized by a molecular mass of 31.5 and 36.75 kDa, respectively (Mohawed and Badran 1995). The purified product was tested as an antifungal agent against brown spot disease caused by *Botrytis fabae* on a bean of *Vicia faba* (Haggag et al. 2006). α-L-Rhamnosidase *T. flavus* produced an extracellular α-L-rhamnosidase when incubated with inducers such as L-rhamnose, rutin, or naringin but not hesperidin (Monti et al. 2004). Fungal α-L-rhamnosidases have applications mainly in the food industry (Yadav et al. 2010). The enhancement of wine aroma by enzymatic hydrolysis of terpenylglycosides was also studied (Spagna et al. 2000). Cell wall-degrading enzymes, such as β-1,3-, β-1,4-, and β-1,6-glucanases, cellulase, and chitinase, are involved in the antagonistic activity of biocontrol agents against phytopathogenic fungi (Madi et al. 1997; Inglis and Kawchuk 2002). Some of the enzymes produced by *Talaromyces flavus* are listed in Table 10.2.

10.5 *Talaromyces flavus* in Biological Pest Control

T. flavus suppresses Verticillium wilt of tomato (Dutta 1981), aubergine (Fahima and Henis 1995; Marois et al. 1982), potato (Fravel et al. 1986), or cotton (Nakova 2003) and parasitizes *Sclerotium rolfsii* (Madi et al. 1992, 1997), *Sclerotinia sclerotiorum* (McLaren et al. 1986), *Rhizoctonia solani* (Boosalis 1956), and *Gaeumannomyces graminis* var. *tritici* (Mohammadi and Ghanbari 2015).

Dethoup et al. (2007) characterized 122 isolates of *Talaromyces flavus* from 45 soil samples in 38 provinces of Thailand. *Talaromyces flavus* isolates were found in both non-agricultural and agricultural soil in Chiang Mai and Mae Hong Son provinces following heat and alcohol treatments. Twenty isolates of *T. flavus* were selected for antagonistic tests against 15 species of plant pathogenic fungi in vitro and in the greenhouse. All the selected isolates of *T. flavus* inhibited the mycelial growth of *Phytophthora palmivora*, *P. parasitica*, *Peronophythora litchii*, *Colletotrichum capsici*, *C. gloeosporioides*, *Pestalotiopsis guepinii*, *Phyllosticta* sp., *Curvularia lunata*, *Helminthosporium maydis*, *H. oryzae*, and *Fusarium oxysporum*. However, none of the isolates controlled *Pythium aphanidermatum*, *Lasiodiplodia theobromae*, *Rhizoctonia solani*, and *Sclerotium rolfsii* in vitro. However, in the greenhouse experiment, 20 isolates of *T. flavus* controlled stem rot of mung bean, caused by *S. rolfsii*, 7 and 14 days inoculation, and 6 isolates gave control up to 30 days inoculation. Bashyal (2018) reported that *T. flavus* is effective against *Fusarium fujikuroi* responsible for bakanae disease in rice (Fig. 10.2).

Cell wall-degrading enzymes, such as β-1,3-, β-1,4-, and β-1,6-glucanases, cellulase, and chitinase, are involved in the antagonistic activity of biocontrol agents

Table 10.2 List of the important enzymes produced by *Talaromyces flavus*

S. no.	Enzyme	Functions
1	Acetyl hexosaminidase	Fungal β-*N*-acetyl hexosaminidase (EC 3.2.1.52) catalyzes the hydrolysis and transfers β-GlcNAc and β-GalNAc (*N*-acetyl galactosamine
2	Amine oxidase	Amine oxidase (EC 1.4.3.4) catalyzes the oxidative deamination of amines by the formation of aldehyde, hydrogen peroxide, and ammonia
3	α-Amylase, glucoamylase	Thermostable amylases are used for starch hydrolysis in the liquefaction step of industrial starch syrup production
4	Chitinase	Decomposed the cell wall of *Verticillium dahliae*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani*
5	Dextranase	It hydrolyzed dextran to isomaltose, isomaltotriose, and glucose (Sun et al. 1988). The main application field of dextranases is sugarcane processing
6	Fructanase	It has been applied in the production of fructose from Jerusalem artichokes
7	Galactosidase	Purified αGal-1 catalyzed the transglycosylation of *tert*-butanol and split off D-galactose from raffinose and stachyose
8	α-1,3-Glucanase	The antifungal activity of this enzyme was studied in relation to its role in mycoparasitic processes, especially in degradation of the host cell wall
9	Glucose oxidase	Glucose oxidase (EC 1.1.3.4) catalyzes the oxidation of β-D-glucose to gluconic acid and hydrogen peroxide using molecular oxygen as the electron acceptor. *T. flavus* produces glucose oxidase, which is involved in the biocontrol of fungal plant pathogens
10	β-Glucosidase	The enzyme showed the capacity to resolve diastereoisomeric mixtures of alkyl β-D-glucopyranosides
11	Pectinase	Pectinolytic enzymes are important for several industrial applications such as improving juice yields and clarity
12	Phytase	Phytase, an enzyme that breaks down the indigestible phytic acid/phytates (inositol penta-, tetra-, and triphosphate) found in grains and oil seeds, was produced by *T. flavus* cultured in a medium containing up to 6% of saccharides and 2% of nitrogen-containing compounds
13	Proteinase K	Proteinase K, a serine protease (EC 3.4.21.64), is used in molecular biology because it rapidly inactivates nuclease, which might otherwise degrade the DNA or RNA during purification and maintains its activity in the presence of chemicals that denature proteins
14	α-L-Rhamnosidase	Fungal α-L-rhamnosidases have applications mainly in the food industry (Yadav et al. 2010)

against phytopathogenic fungi (Madi et al. 1997; Inglis and Kawchuk 2002). In addition, *T. flavus* antagonizes *Verticillium dahliae* by parasitism and antibiosis (Fahima et al. 1992; Marois et al. 1984). Microsclerotia of *V. dahliae* were suppressed by a culture filtrate of *T. flavus* and this effect was attributed to the action of glucose oxidase (Fravel and Roberts 1991; Kim et al. 1988). In a recent study, the

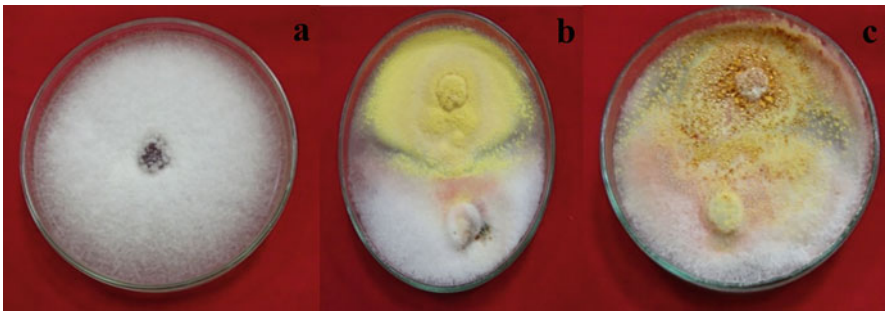

Fig. 10.2 Dual culture assay of *Fusarium fujikuroi* with different isolates of *Talaromyces flavus*: (**a**) control, (**b**) *Fusarium fujikuroi* + Tf_1, and (**c**) *Fusarium fujikuroi* + Tf_2

effect of seed treatments of *Talaromyces flavus* on sugar beet seedling damping-off disease under greenhouse conditions reported that the isolates reduced the percentage of damping-off disease from 40 to 7.5% compared to infected control (Naraghi et al. 2012). Furthermore, in another field study, a significant decrease in the incidence of sugar beet seedling damping-off disease was observed along with increase in yield in treatments containing antagonistic fungi *T. flavus* and *T. harzianum* compared to the control (Naraghi et al. 2014; Lamichhane et al. 2017). Further, improved growth was reported on cotton and potato when they were treated with biocontrol agent *Talaromyces flavus* (Naraghi et al. 2012). Bashyal et al. (2020) reported increased drought tolerance in *T. flavus*-treated rice seedlings.

Chattopadhyay and Das Gupta (1959) isolated *T. flavus* from paddy rhizosphere and reported phosphate-solubilizing activity of *T. flavus* under in vitro conditions that positively influenced the growth of the rice, *Cicer arientinum*, and *Vigna radiata* under greenhouse conditions. He further reported that *T. flavus* have the capacity to convert insoluble phosphorus to soluble form.

Haggag et al. (2006) purified proteases from the culture filtrate of *T. harzianum* and *T. flavus* and tested for their antifungal activity against brown spot disease caused by *Botrytis fabae* on faba bean. *T. flavus* exhibited high levels of extracellular protease activity compared with *T. harzianum*. Germination and growth rate, extracellular polygalacturonase (*PGase*), and carboxymethyl cellulase (*CMCase*) activities of *Botrytis fabae* were inhibited by the purified protease at a concentration of 40–120 U/ml. Growth and extracellular production of *B. fabae* were completely inhibited by the protease enzyme of *T. flavus* at a concentration of 80 U/ml, while protease from *T. harzianum* was effective at 120 U/ml. Proteases were effective in reducing brown spot disease severity and pathogen sporulation on faba bean leaves inoculated with *B. fabae*.

Glucose oxidase, secreted by *T. flavus*, retards hyphal growth and kills microsclerotia of *V. dahliae* in vitro, probably by generating toxic peroxide (Kim et al. 1988; Stosz et al. 1996), but only if a sufficient amount of glucose is available (Murray et al. 1997). Partial disintegration of melanin was observed near hyphae of *T. flavus* colonizing sclerotia of *S. sclerotiorum* (McLaren et al. 1989). The

inhibition of germination and melanin formation in sublethally heated microsclerotia of *V. dahliae* and additive suppression by sublethal heating and *T. flavus* treatment was studied (Tjamos and Fravel 1995). A glucose oxidase gene was located and isolated, and mutants of *T. flavus* with both high and low production ability of this enzyme were constructed. A high level of glucose oxidase in tobacco and cotton as a result of the expression of its gene from *T. flavus* was associated with phytotoxic effects such as reduced root growth, slow germination on culture medium, or reduced lateral root formation (Murray et al. 1999).

To increase the effectiveness of *T. flavus* isolates obtained from greenhouse cucumbers and field-grown tomatoes, five chemical stabilizers were evaluated. Based on the results of previous studies, the most effective substrate for the growth, sporulation, and stability of *T. flavus* isolates related to the above-mentioned plants was a mixture of rice bran and peat moss. Different chemical stabilizers were mixed with the above-mentioned substrate containing spore suspensions of various *T. flavus* isolates. Completely randomized experiment was conducted under greenhouse conditions with seven treatments and three replications. The results of this study indicated that treatments containing sodium nitrate and D-cycloserine were more effective than those containing other stabilizers (Bahramiyam et al. 2016).

Fravel et al. (1986) evaluated the use of pyrophyllite clay (Pyrax), milled chitin, maize cobs, fish meal, neem cake, groundnut hulls, soya fiber, and wheat bran to make alginate prill with or without ascospores of *T. flavus*. The formulations were compared for their ability to induce *T. flavus* to control Verticillium wilt of aubergine in the greenhouse in field soil and to increase populations of *T. flavus* in three field soils (two loamy sands, one silty clay). Survival of *T. flavus* in prill at 5 °C or ambient temperature (22–24 °C), as well as the carbon (C) and nitrogen (N) contents of the prill, was also determined. Two formulations (maize cobs and pyrophyllite) consistently enhanced biocontrol activity.

10.6 Conclusion

Talaromyces flavus is a significant soil-inhabiting fungus that produces important metabolites and enzymes that have established their effects on biological models. The application of its enzymes has enabled stereoselective preparation of many useful saccharides. *T. flavus* is a very variable species and not all of its secrets have as yet been uncovered. At the same time, this microbe was utilized as biological control agent against many important diseases. However, genomic studies are lacking on this fungal species. Further, emphasis should be given on genes involved in secondary metabolite production, whole genome studies of this microbe, and tripartite interaction to understand this fungal species further.

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11 Harnessing Beneficial Rhizospheric Microorganisms for Biotic Stress Management in Medicinal and Aromatic Plants

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Abstract

Medicinal and aromatic plants (MAPs) are important source of life-saving drugs even after progress on synthetic substitutes. However, its yield, productivity, and quality are severely hampered by several biotic stresses such as fungi, bacteria, nematodes, and viruses. Agricultural stresses and associated therapeutic security issues need the optimization of reliability, efficient use of resources, and alleviation of the environmental impacts of herbal drug production. Several approaches may be used to manage these diseases in MAPs, e.g., synthetic/chemical, which, however, are incompetent and hazardous to the environment. Soil microbes in sustainable agriculture have provided new insights to green agro-economy and are being looked upon as an alternative tool for the management of MAPs diseases. They enhance plant health and disease eradication through various mechanisms such as induced plant defense, rhizosphere competence, and improvement of nutrition to the plant. In this chapter, we have focused on the diverse mechanisms of soil microbes in MAPs cultivation and biotic stress

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management by discussing present knowledge of the field, covering all aspects of biotic stress management, and further summarized essential mechanisms applied by microbes in mitigating biotic stresses.

Keywords

Biotic stress · Plant–microbe interactions · Medicinal and aromatic plants · Soil microorganisms

11.1 Introduction

Medicinal and aromatic plants (MAPs) are cultivated worldwide for the production of herbal medicine and are expected to be increased by more than 80% in developing countries like India till 2050 (Zahra et al. 2020). In India, the cultivation of MAPs is approximately 617,000 ha, generating 1,156,000 metric tons of biomass with a yield of around 1.9 t ha^{-1} (Gahukar 2018). At this time, many agricultural techniques are implemented on the global scale to protect MAPs as natural resources and to meet challenges related to the world's herbal medicine demands for future (Hamilton 2004; Bhat et al. 2018). In recent decades, the large-scale cultivation of MAPs such as *Bacopa monniera* (Brahmi), *Chlorophytum borivilianum* (Safed musli), *Coleus forskohlii* (Coleus), *Emblica officinalis* (Indian gooseberry), *Ocimum sanctum* (Holy basil), *Plantago ovata* (Isabgol), *Withania somnifera* (Ashwagandha), *Rauvolfia serpentina* (Sarpagandha), *Mentha* spp. (Peppermint), and *Pelargonium graveolens* (Geranium) depends upon use of chemical/synthetic pesticides against several phytopathogens. Due to the increasing environmental and health problems, utilization of these chemicals is being discouraged (Köhl et al. 2019). Therefore, search for suitable, ecologically compatible and eco-friendly approach-based strategies against phytopathogens are alternative options for better protection and production of MAPs. In recent decades, a wide range of soil microorganisms have been applied to provide benefits to MAPs production and are rising in demand for the development of bio-fertilizers/pesticides (Pii et al. 2015; Finkel et al. 2017).

Rhizospheric microorganisms play an essential role in increasing the agricultural productivity by inducing plant resistance, enhancing nutrient availability, synthesizing iron-chelating siderophores, maintaining the levels of phytohormones, producing volatile organic compounds, and degrading quorum-sensing signals in phytopathogens (Lugtenberg 2015). However, recent research findings continually demonstrated an intimate interaction of soil microbes with their host plants, which can manage phytopathogens as well as develop MAPs fitness (Dojima and Craker 2016; Gupta et al. 2019, 2020). A wide range of approaches has been and being developed to help sustainable eco-friendly techniques to enhance the potential of soil microbes as a low-cost technology. For optimization of soil microbes' effectiveness, maintaining the natural microbial communities is essential, which can be achieved through application of organic or inorganic amendments and utilization of beneficial microbes as plant growth promoters (Egamberdieva and da Silva 2015). Recently,

conventional and organic farmers recommended awareness in exploitation of product based on soil microbes, signifying that the possible application of beneficial microbes will boost up in the future (Ab Rahman et al. 2018; Gupta et al. 2020).

In recent years, the application of soil microbes in combinational mode with different properties is gaining interest (Sarma et al. 2015; Liu and Brettell 2019). Combining number of microbial activities is expected to provide better crop production and protection (Kumar et al. 2016a). The mixture of soil microbes promote variability of microbes and increases stress tolerance, which may linearly influence plant health, microbial colonization, and activity of one another. The fitness of MAPs under biotic stress can be accomplished by the direct/indirect interaction between host and soil microbes. This chapter is an overview of the effective utilization of soil microbes to manage the diseases cause by the phytopathogens in MAPs (Table 11.1). In view of several advantages of soil microbes over the synthetic chemicals such as environmental friendliness, targeted activities, enhanced stress tolerance, and better management of plant yield, microbes are being looked upon as better substitute for managing plant diseases. This chapter focuses on the current status of research and application of soil microbes to improve plant health and management of phytopathogens. Finally, a fundamental part of this chapter is devoted to discuss the future perspectives and opportunities for improving our understanding of the mechanism behind antagonistic properties of microbes to combat MAPs diseases caused by the different pathogens by enhancing the ability of stress alleviation. In addition, the development of a microbial community in a mixture will enrich beneficial microbial activities, leading to the enhancement of protection and MAPs yield.

11.2 Mechanisms Employed by Rhizospheric Microorganisms

Rhizospheric microbes exhibit several mechanisms for alleviating biotic stress either directly by antagonism of phytopathogens or indirectly by stimulating a host defense response in MAPs. Most of beneficial microbes are involved in antagonistic mechanism resulting from physical attachment and/or provide a high range of selectivity for the phytopathogen, whereas indirect antagonism results from activities, which do not directly involve sensing or targeting pathogens. Most of the soil microbes are involved in competition and bioactive secondary metabolites production that directly affect a pathogen. Soil microbes also suppress phytopathogens by diverse modes of action, which include secretion of antibiotics, rhizospheric colonization, siderophores production, detoxification and degradation of virulence factors, and induction of systemic resistance. By combining all these microbes-mediated mechanisms, strong resistance against various plant diseases-causing agents is obtained. Activation of MAPs defense mechanisms by rhizospheric microbes is the indirect form of biocontrol. Figure 11.1 indicates detailed mechanisms employed by antagonistic rhizospheric microbes.

Table 11.1 Management of medicinal and aromatic plants (MAPs) diseases using various rhizospheric microorganisms

MAPs	Pathogens	Diseases	Microorganisms	References
Allium sativum (garlic)	*Sclerotium cepivorum*	White rot	*Trichoderma harzianum*	Miranda et al. (2006)
Asparagus officinalis	*Phytophthora megasperma*	Root rot	*Pseudomonas aureofaciens*	Carruthers et al. (1995), Godfrey et al. (2000)
Azadirachta indica (neem)	*Meloidogyne incognita* and *Fusarium oxysporum*	Root knot, *Fusarium* wilt	*T. harzianum*, *T. piluliferum*, *Asergillus niger*, and *Penicillium*	
Bacopa monnieri (brahmi)	*M. incognita*	Root knot disease	*B. megaterium*, *Glomus intraradices*, *T. harzianum*, *Chitiniphilus* sp., and *Streptomyces* sp.	Gupta et al. (2015, 2017c)
Cassia angustifolia	*M. incognita*	Root knot	*T. viride*, *P. fluorescens*, *G. fasciculatum*, and *G. mosesae*	Ramakrishnan and Senthilkumar (2009)
Catharanthus roseus (madagascar periwinkle or rosy periwinkle)	*Pythium ultimum*	Damping-off	*T. virens* and *Rhizoctonia* spp.	Burns and Benson (2000)
Chlorophytum borivilianum (safed musli)	*S. rolfsii*, *Colletotrichum dematium*, *Phoma* sp., and *Rhizoctonia bataticola*	Foliar and root diseases	*T. viride* and *P. fluorescens*	Sharma et al. (2010)
Coleus barbatus syn.	*Fusarium oxysporum*	Root-rot/wilt	*T. viride* and *P. fluorescens*	Singh et al. (2011)
Coleus forskohlii (patharchur)	*F. chlamydosporum* and *Ralstonia solanacearum*	Root diseases	*P. monteilii* and *G. fasciculatum*	Singh et al. (2013a)
	M. incognita	Root knot	Fluorescent *Pseudomonas*	Lakshmanan et al. (2013)
Curcuma longa (turmeric)	*P. aphanidermatum*	Rhizome rot	*P. chlororaphis* and *B. subtilis*	Kavitha et al. (2012)
Jatropha curcas	*R. bataticola*	Root rot	*P. fluorescens*	Kumar et al. (2011b)
Justicia gendarussa	*Puccinia thwaitesi*	Leaf rust	*T. harzianum*	Ragi et al. (2013)

(continued)

Table 11.1 (continued)

MAPs	Pathogens	Diseases	Microorganisms	References
Matricaria recutita (chamomile)	*M. incognita*	Root knot disease	*B. megaterium*, *T. harzianum*, and *Glomus intraradices*	Gupta et al. (2017a)
Mentha (mint)	*M. incognita*	Root knot	*T. harzianum* strain Thu, *B. megaterium*, *G. aggregatum*, and *P. fluorescens*	Pandey (2005)
	R. solani	Stem and stolon rot	*T. viride*, *P. fluorescens*, and *B. subtilis*	Kamalakannan et al. (2003)
	Linaria vulgaris	Yellow toadflax	*Eteobalea serratella*	Volenberg et al. (1999)
Ocimum basilicum (basil)	*F. oxysporum* f. sp. *basilici*	Vascular wilt/rot	*B. flexus*, *B. subtilis*, *B. megaterium*, and *B. aryabhattai*	Singh et al. (2013b)
	M. incognita	Root knot	*B. subtilis*	Gupta and Pandey (2015)
	Peronospora belbahrii	Downy mildew	*P. fluorescens*	Gilardi et al. (2013)
Papaver somniferum (opium)	*Peronospora* sp.	Downy mildew	*P. putida*	Barnawal et al. (2017)
Pelargonium graveolens (geranium)	*Botrytis cinerea*	Blight disease	*T. hamatum*	Olson and Benson (2007)
	Pythium ultimum	Root rot and damping-off	*Actinoplanes* spp.	Filonow (1999)
Piper nigrum (black pepper)	*Phytophthora capsici*	Foot rot and Nursery wilt	*Pseudomonas* spp. and *T. harzianum*	Paul and Sarma (2006), Anith et al. (2003)
Rauwolfia serpentine (sarpagandha)	*Alternaria alternatai*	Leaf spot	*T. viride* and *Beauveria bassiana*	Thakur and Harsh (2016)
	C. gloeosporioides	Anthracnose disease	*T. harzianum*	Ghosh and Chakraborty (2012)
Stevia rebaudiana (stevia)	*Alternaria alternata*	Leaf spot disease	*P. Monteilii*, *Cronobacter dublinensis*, and *Bacillus*	Sen et al. (2012)
Withania somnifera (ashwagandha)	*A. dianthicola* and *Alternaria alternata*	Leaf blight disease	*P. aeruginosa*	Maiti et al. (2012)
	M. incognita	Root knot disease	*C. cellulans*, *F. johnsoniae*, *Chitiniphilus* sp., and *Streptomyces* sp.	Gupta et al. (2016a)

(continued)

Table 11.1 (continued)

MAPs	Pathogens	Diseases	Microorganisms	References
Zingiber officinale (ginger)	*F. oxysporum* and *F. solani*	Rhizome rot	*Streptomyces* sp., *P. fluorescens*, and *B. subtilis*	Shanmugam et al. (2013), Manasa et al. (2013)

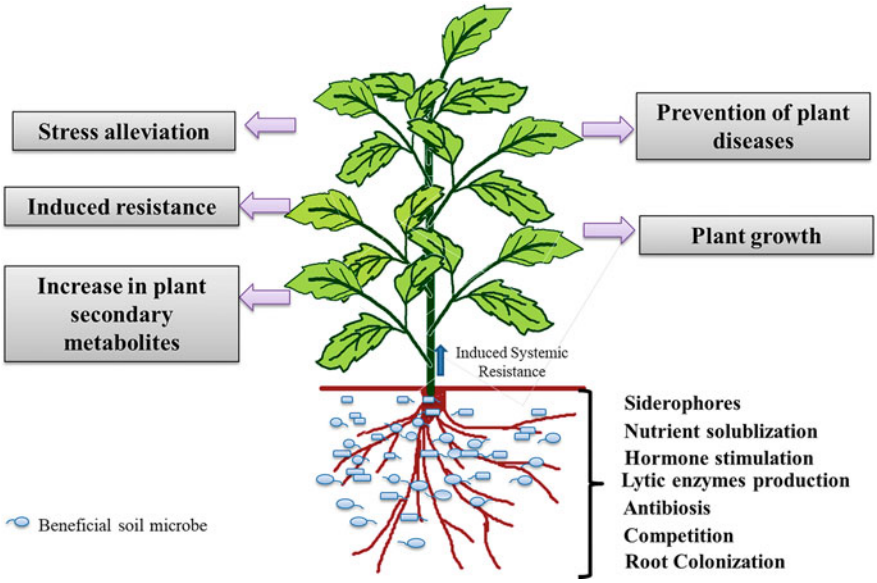

Fig. 11.1 Different mechanisms employed by rhizosphere microorganisms for enhancement of plant health and disease management

11.2.1 Soil Nutrient Solubilization

Microbes are recognized to reside in the rhizospheric region of plant and play a vital role in plant growth and health. Plant–microbe–soil interaction has gained much significance in recent years. A number of microbial strains possess a functional association and constitute a holistic system with the host plants. They easily colonize soil near plant rhizosphere to endorse host plant development (Mohamad et al. 2019). Soil microbes belonging to the genera *Arthrobacter*, *Azospirillium*, *Flavobacterium*, *Azotobacter*, *Erwinia*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Mycobacterium*, and *Serratia* spp. are known to fix atmospheric nitrogen through symbiotic and nonsymbiotic associative nitrogen fixing processes. The application of biofertilizer in a mixture of *A. chroococcum*, *A. liboferum*, and *B. megaterium* with chemical fertilizers in

medicinal plant fennel (*Foeniculum vulgare* Mill.) significantly enhanced the overall plant growth and essential oil content (Mahfouz and Sharaf-Eldin 2007). Awasthi et al. (2011) demonstrated that *Glomus mosseae* and *B. subtilis* Daz26 enhanced bioactive content (artemisinin) in *Artemisia annua* L. In addition, Arora et al. (2016) demonstrated that dual symbiosis between *A. chroococcum* and *Piriformospora indica* boosts the artemisinin content in *A. annua* L. A well-known genus *Rhizobium* sp. and fix atmospheric N_2 in leguminous root nodules were the first biofertilizers recognized and have been commercially used as bioinoculants. Another mode of action implicated by phosphorus-solubilizing soil microbes is to enhance nutrient availability to host plants (Khan et al. 2009). Phosphorus-solubilizing bacteria can help in increasing the availability of soluble phosphates in the soil and thus can enhance plant health by escalating the efficiency of biological nitrogen fixation and the availability of minor essential elements through plant growth-promoting activities (Menezes-Blackburn et al. 2018). In addition, potassium-solubilizing bacteria can also provide beneficial effects on plant development through suppressing phytopathogens, improving soil nutrients and soil structure (Parmar and Sindhu 2013). The MAPs were found to be a reservoir of large number of phosphate and potassium-solubilizing bacteria that have been shown to play an effective role in nutrient and plant growth promotion. For example, certain phosphate-solubilizing bacteria enhance plant growth, nutrient uptake, and secondary metabolites (stevioside and rebaudioside-A) of *Stevia rebaudiana* Bertoni (Rahi et al. 2010; Vafadar et al. 2014). The role of soil microbes as biofertilizers and biocontrol agents would diminish requirement of synthetic fertilizers, decline adverse environmental effects, and enhance soil fertility. Therefore, in the improvement and implementations of sustainable agriculture techniques, bioinoculants have an enormous importance in reducing environmental pollution (Vassilev et al. 2015).

11.2.2 Competition for Available Resources

In spite of the ability of rhizospheric microbes for disease management, utilization of microbes has been hindered by inconsistent performance under various natural conditions owing to their poor colonization (Ghoul and Mitri 2016). The root exudates of the plant contain numerous nutrients such as various organic acids, sugars, amino acids, enzymes, vitamins, siderophores, phenolics, and flavonoids (Dakora and Phillips 2002). The rhizospheric competence plays a major role in microbial activities and is associated with their ability to use carbon source and the root exudates composition. Microbial diversity in the rhizosphere is probably related to species of MAPs due to dissimilarity in root exudates (Marschner et al. 2004). *Fusarium* and *Pythium* infect through appressoria and infection pegs and thus directly germinate on the host plant surfaces. An effective fatty acid catabolism has been recognized as a fundamental mechanism of *Enterobacter cloacae* to control *P. ultimum* infection (Kageyama and Nelson 2003). In addition, chemotactic response of microbes toward chemical attractants present in root exudates (organic acids, sugars, amino acids, inorganic ions, purines, and vitamins) governs the arrival

of beneficial microbes to the root surface (Levy et al. 2018). Plant root exudates serve as essential nutrients for microbes present in soil, and thus provide ecological niche advantages to microbes that have adequate metabolic machinery to detoxify pathogens (Wallenstein 2017). Microbial competence highly depends on the quantity and composition of chemo-attractants and antimicrobials secreted by plant roots. As an example, strain-specific chemotaxis of *Azospirillum* sp. is induced by various chemoattractant exudates from plant root (Alexandre 2015). Zhang et al. (2014) demonstrated that microbes may be uniquely equipped to sense specific chemo-attractants, e.g., *B. subtilis* showed chemotactic response toward organic acids such as citric acid and fumaric acid, whereas *B. amyloliquefaciens* showed only chemotactic response toward citric acid.

11.2.3 Rhizosphere Colonization

Rhizospheric microbes multiply near the host plant root system and survive for many weeks in the presence of the native microbial community, thereby resulting in effective root colonization that directly endows with a selective adaptation to host plants toward specific ecological habitats (Bloemberg and Lugtenberg 2001). These soil microbes applied in initial high cell numbers, following infection of the pest or pathogen, provide the host plant with an additional competitive advantage (Nautiyal et al. 2002). Therefore, rhizosphere competence is related to microflora that displays enhanced growth in response to developing plant root systems and is also considered as essential for diseases management (Bach et al. 2016). The microbial density is constantly higher in the rhizosphere in comparison to the non-rhizospheric soil (Foster and Bowen 2012). Many researchers observed root colonization capability of soil microbes and they found a strong correlation between microbial multiplication and the efficiency of disease management against various plant enemies (Gupta et al. 2017b, c, d; Köhl et al. 2019). Once rhizospheric microbes establish themselves, the mechanism of competition develops for nutrients, space, antibiosis, and lytic enzymes production (Compant et al. 2005). Therefore, the ability to curtail plant pathogens by application of microbes relies mainly on their capability to better colonize the root (Verbon and Liberman 2016) and their rhizosphere population density (Martinez-Viveros et al. 2010). Singh et al. (2012) demonstrated that the significant threshold population density is required for sufficient suppression of root-rot (*F. chlamydosporum*) and wilt (*F. chlamydosporum* and *R. solanacearum*) of the medicinal plant *C. forskohlii* by *T. viride* and *P. fluorescens*, *B. subtilis*, and *A. chroococcum*, respectively. The effect of biotic factors along with host genotype and microbial genotype also enhances microbial root colonization (Schweitzer et al. 2008). For instance, Gupta et al. (2015) reported that plant growth promotion and diseases management were more pronounced with two *B. megaterium* (1.0×10^8 CFU ml^{-1}) and *T. harzanium* (1.2×10^6 CFU ml^{-1}) isolates than the chemical nematicide carbofuran in *B. monnieri*.

11.2.4 Iron Chelating Siderophores

Iron (Fe) is an essential factor for cellular growth and metabolism of all the microorganisms in rhizosphere and rhizoplane where its paucity creates a furious competition (Trapet et al. 2016). Low molecular weight compounds, siderophores, are produced by plants and microorganisms that act as a strong soluble high-affinity iron chelating agent (Dimkpa 2016; Saha et al. 2016). Siderophore-producing soil microbes can prevent the multiplication of phytopathogens by sequestering Fe^{3+} in the rhizosphere (Kumar et al. 2015). Various studies have isolated siderophore-producing microbes from different MAPs such as *Ocimum*, *Catharanthus*, *Withania*, and *Geranium*, belonging to the *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Streptomyces*, *Serratia*, *Enterobacter*, and *Rhizobium* (Hayat et al. 2010; Mishra et al. 2010; Tiwari et al. 2010). Siderophore-producing *Alcaligenes feacalis* promoted growth and seed germination in *Chlorophytum borivillianum* and *W. somnifera* (Sayyed et al. 2007). This microbial strain also possesses antifungal activity against *Aspergillus niger*, *A. flavus*, *F. oxysporum*, and *A. alternata* (Sayyed and Chincholkar 2009). Murugappan et al. (2013) isolated siderophores-producing *B. pumilus* from surface-sterilized tissues of the medicinal plant *Ocimum sanctum*. Siderophore-mediated iron sequestration by *B. pumilus* in host plant may confer a competitive benefit for pathogen suppression. Some of earlier reports have demonstrated the importance of microbial siderophores in management of plant diseases (Saha et al. 2016).

11.2.5 Antibiosis

Antibiosis is a widely found mechanism of soil microbes and inhibits the pathogen by the metabolic products. Antibiotics that are antimicrobial in nature inhibit the growth of other microorganisms at very little concentrations. Antibiotics-producing beneficial microorganisms have been displayed to be particularly effective in curbing phytopathogens or diseases caused by them. A large number of antimicrobial compounds produced by microbes include volatiles, non-volatile polyketides, heterocyclic nitrogenous compounds (Ahanger and Dar 2014), phenylpyrrole antibiotics (pyrrolnitrin), and lipopeptides (Kenawy et al. 2019). Kumar et al. (2015) reported that microbes isolated from host plants' prevalent environment possessed better antagonistic activity. Beneficial microbes such as fluorescent *Pseudomonads* and *Bacillus* species frequently use this strategy for the suppression of phytopathogens (Shafi et al. 2017). The production of antibiotic is tightly linked to the overall metabolism of the microbial cell, which in turn is dictated by the presence of nutrients and other environmental factors. Several strains produce secondary antimicrobial metabolites, which enables the antagonists to suppress the plant diseases under various environmental conditions. The diacetyl phloroglucinol production by *P. fluorescens* was affected by the bacterial metabolites such as salicylates and pyoluteorin (Schnider-Keel et al. 2000). This ensures a degree of flexibility of the biocontrol under various abiotic and biotic conditions. Berg (2009)

demonstrated that the host plant genotype plays a vital role in the plant–microbe–pathogen interaction. Soil microbes enforce suppression of plant pathogens by the secretion of the above-mentioned extracellular inhibitory metabolites at a low concentration. There are several reports on the involvement of *Pseudomonas*, *Burkholderia*, *Bacillus*, and *Trichoderma* in improving plant growth by restricting the disease development (Compant et al. 2010). Similarly, Mishra et al. (2011) showed that the culture filtrate of *B. subtilis* isolate MA-2 was completely inhibited the growth of phytopathogens such as *Alternaria alternata* and *Curvularia andropogonis*, infecting medicinal and aromatic plants while the culture filtrate of *P. fluorescens* isolate MA-4 was comparatively less effective against *Fusarium moniliforme* and *Colletotrichum acutatum*. Additionally, different *Bacillus* species, which are able to produce multiple antibiotics in different ways, reduced growth of diverse pathogens, which likely enhanced disease suppression activity (Choudhary and Johri 2009). Gupta and Pandey (2015) reported that a subset of *Bacillus* showed better abilities to inhibit root knot disease infection in field-grown sweet basil.

11.2.6 Lytic Enzymes Secretion

Beneficial microorganisms are able to produce a wide range of extracellular hydrolytic enzymes attacking phytopathogens by excreting cell wall hydrolases and play essential role in disease management. Chitinase, protease, and β-1,3-glucanase attack on chitin, protein, and β-1,3-glucan, respectively, which are major constituents of cell walls of many fungal pathogens and nematode egg shell, resulting in its degradation that further inhibits the growth of pathogens (Howell 2003; Bird and Bird 2012). The β-1,3-glucanase produced by *Paenibacillus*, *P. cepacia*, and *Streptomyces* sp. was reported to destroy plant pathogen cell walls (El-Tarabily et al. 2000). Antagonistic properties were also established by generating transgenic, expressing the gene for endo-chitinase in plants from *T. harzianum* and *T. virens*, which not only increased the resistance against *Phoma tracheiphila* but *B. cinerea* as well (Shah et al. 2009). The application of *P. aeruginosa* WS-1-based bioformulation to *W. somnifera* grown under natural conditions significantly managed (80%) leaf blight disease caused by the fungus *A. dianthicola* with respect to untreated control (Maiti et al. 2012). The antifungal activity of this microbe has been shown to be associated with the production of siderophore, hydrocyanic acid, proteases, chitinases, and so on. Kumar et al. (2012) reported that lytic enzyme produced by *P. stutzeri* showed antagonistic activity against various phytopathogens.

11.2.7 Phytohormones Production

Rhizospheric microbes able to produce individual phytohormones such as cytokinins, gibberellins, auxins, and ethylene (ACC deaminase) have been studied by different researches over the decades (De-la-Peña and Loyola-Vargas 2014;

Bhandari and Garg 2019). The most studied plant growth regulator produced by microbes is indole acetic acid (IAA), which is synthesized in the presence of the precursor tryptophan (Trp). Soil microorganisms of various MAPs such as *Ocimum*, *Bacopa*, *Matricaria*, and *Pelargonium* have been reported to produce auxin as secondary metabolites (Mishra et al. 2010; Gupta et al. 2017a). Microbes belonging to the genus *Bacillus*, *Streptomyces*, *Azospirillum*, *Pseudomonas*, *Burkholderia*, *Rhizobium*, *Bradyrhizobium japonicum*, *A. faecalis*, *E. cloacae*, *S. marcescens*, *Mycobacterium* sp., and *Azotobacter* as well as *Trichoderma* have been shown to produce IAA (Duca et al. 2014; Ramanuj and Shelat 2018). IAA-producing microbes *P. putida* GR12-2 and *Azospirillum* have been found to significantly augment root system development and improve nutrient uptake of the host plant (Ahmad et al. 2005). Such effects demonstrated potential of these plant growth promoting microbes (PGPMs) as commercial bioinoculants and biofertilizers for agronomically important MAPs (Nelson 2004). In a study, Mishra et al. (2010) demonstrated that IAA-producing bacteria *B. subtilis* and *P. fluorescens* increased the yield of herb (*P. graveolens*) over the control by 9 and 27.6%, respectively. IAA and protease-producing *B. megaterium* and *T. harzianum* ThU significantly enhanced the plant growth and resistance against *M. incognita* in *B. monnieri* and *M. recutita* (Gupta et al. 2015, 2017a). Sergeeva et al. (2007) examined that the IAA-producing *Pantoea agglomerans* strains were able to promote the plant growth under genotobiotic conditions. Other hormone cytokinins produced by beneficial rhizospheric microorganisms may also affect growth and development of plant (Bowen and Rovira 1999). It has been recognized that the phytostimulator regulatory pathway leading to overall plant development are differently regulated by biofilm production (Drogue et al. 2012). Researchers have identified that microbes such as *Rhizobium*, *Azotobacter*, *Azospirillum*, *Arthrobacter*, *Bacillus*, and *Pseudomonas*, as well as certain *Streptomycetes*, produce cytokinin, which enhances plant growth and stress tolerance (Khalid et al. 2006; Maheshwari et al. 2015). Cytokinin production by soil microbes is an innovative alternative to ameliorate plant development, diseases suppression, and may be used as a sustainable approach to improve the production and quality of MAPs plants. In addition, gibberellic acid (GA)-producing soil microbes have been reported to induce mechanism in host plants, which are beneficial for their growth. Boiero et al. (2007) and Joo et al. (2009) also found that *Rhizobium*, *Azospirillum*, *Bacillus*, and *Burkholderia* strains produced auxin (IAA) and gibberellin (GA_7). Similarly, Mishra et al. (2010) demonstrated that the plant growth response to microbial colonization was owed to gibberellin production and deconjugation of gibberellin glycosides by bacteria. They highlighted that the inoculated *P. graveolens* seedlings showed a better and significant response to the applied GA-producing bacteria.

Another plant hormone, ethylene, is also essential for plant growth and development, and at elevated concentration, it can be dangerous as it stimulates defoliation and other cellular processes such as inhibition of root growth that may affect the crop performance (Bari and Jones 2009). Rhizospheric microbes were able to produce an enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase (Glick 2014), which would reduce ethylene production in the host plant root. Ethylene is essential to

break seed dormancy but, following germination, a continued elevated ethylene production may diminish root elongation (Vejan et al. 2016). ACC deaminase-producing microbes such as *Rhizobium*, *Achromobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, and *Pseudomonas* can break ACC from the ethylene biosynthesis pathway in the form of ammonia and α-ketobutyrate in the plant root system, which can be later utilized by bacteria for their growth (Brígido et al. 2015; Bach et al. 2016). Such microbes attached to the seed or root may ensure that the ethylene level does not increase when root growth is hindered. Further, microbes assist in reducing the ethylene accumulation and re-establish a healthy root system needed to manage with various biotic stresses. It is emphasized that microbes containing ACC deaminase gene is a useful tool to reduce diseases caused by plant pathogens that are responsive to jasmonic acid and ethylene-dependent defenses. ACC deaminase-rich bacteria were also capable of antagonizing the phytopathogens *R. solanacearum* and *Rhizoctonia solani* (Rasche et al. 2006). Karthikeyan et al. (2012) reported that ACC deaminase-containing bacteria improve *C. roseus* resistance through reduced ethylene content and activation of antioxidant defense systems. *B. monnieri* plants inoculated with the bacterium *B. pumilus* and *Exiguobacterium oxidotolerans* had better yield and secondary metabolites content of plants under stress conditions (Bharti et al. 2013). Changes based on morphology in plant species were also observed after application of microbes-containing ACC deaminase activity. Therefore, it is speculated that modification at genetic level of microbial strains expressing ACC deaminase gene can be of great help in managing various plant diseases in MAPs. In addition, ACC deaminase gene-containing microbes, apart from directly inhibiting pathogens, sustain plant resistance against various pathogen assaults.

11.2.8 Detoxification of Virulence Factors

The microbial detoxification mechanism involves production of specific types of protein that attaches reversibly or irreversibly with toxin secreted by plant pathogen, resulting in reduced virulence ability of pathogen toxin. For instance, certain biocontrol microbes are able to detoxify albicidin toxin secreted by *Xanthomonas albilineans* (Lee et al. 2013). Recently, Defoirdt (2018) reported that certain antagonistic microbes quench pathogen quorum-sensing capacity by degrading virulence signals, thus delaying expression of numerous pathogenic genes. Antagonistic activity of microbes also involves the synthesis of various allelochemicals and secondary metabolites that contribute disease management in host plants (Saraf et al. 2014). Recently, Sattiraju et al. (2019) highlighted that toxins produced by plant pathogens also display a better activity and can inhibit the growth of microbial competitors or detoxify antibiotics secreted by microbes as a self-protection property against antagonistic microbes.

11.3 Functions of Soil Microorganisms in Rhizospheric Microbial Community Shifting

MAPs rhizosphere harbors versatile and vibrant microbes owing to the presence of potent secondary metabolites that play an important role in drug development process. MAPs soil microbes provide an essential link between plant and rhizosphere environments and are highly influenced by roots of the plants that overall develop the plant health (van der Voort et al. 2016). Due to the plant roots exudates and other rhizo-chemicals, soil microbes are attracted toward the plant. The community and diversity of soil microbes depend on several factors including plant age and species, the type and structure of soil, climate changes, pesticides applications, and environmental stress (Nannipieri et al. 2017). The role of soil microbes in shifting of microbial communities of medicinal and aromatic plants such as *O. basilicum* and *B. monnieri* showed interesting results (Bharti et al. 2016; Gupta et al. 2019), a remarkable shifting in microbial communities along with enhancement in therapeutic properties.

The effect of the soil microbes also depends on the release of root exudates that provide a healthy and rich environment for microbial activity. Colonization of different soil microbes depends on the chemical and biological properties of the organic compounds that are released from plants. Microbial activities highly affect the root cells permeability and metabolism rate. The plant–soil interactions are an essential factor, which are dynamic in nature (Lambers et al. 2009; Schlatter et al. 2015). Among various soil microbes, bacterial populations are the most abundant due to their ability to compete for root colonization and they strongly influence the physiology of plants as well. In MAPs, bacteria are the active groups, which can eventually compete with the other pathogenic microbes for nutrients and space. Therefore, such soil microbial inoculants can be beneficial for plant development under biotic and abiotic stress conditions.

11.4 Induced Resistance

Plant immune system elicited by the application of various chemicals is called systemic acquired resistance (SAR). Beneficial microbes and its metabolites can be used to increase plant's resistance against pathogens by induced systemic resistance (ISR). Their elicitor molecules as well as the signal transduction pathways within the host plant system (Pieterse et al. 2014) can differentiate both pathways. Beneficial microbes elicit ISR, whereas SAR is stimulated by phytopathogens. Host inoculated with microbes provided systemic resistance against various phytopathogens to diminish disease caused by them (Jain et al. 2016; Kumar et al. 2016b; Gupta and Bar 2020). An array of defense and antioxidant enzymes such as catalase, chitinase, lipoxygenase, ascorbate peroxidase, β-1,3-glucanase, polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, glutathione peroxidase, glutathione reductase, and superoxide dismutase have been widely reported to be linked with ISR (Gill and Tuteja 2010). The inoculation with *B. subtilis*, *P. chlororaphis*,

and *P. fluorescens* enhanced defense-related enzymes such as polyphenol oxidase, peroxidase, chitinase, and β-1,3-glucanase in *Phyllanthus amarus* infected with *C. cassiicola* (Mathiyazhagan et al. 2004). Several earlier findings suggest that MAPs inoculated with soil microbes led to the strengthening of host cell walls, alteration in physiology, and metabolic process, and finally enhanced overall plant resistance against phytopathogens (Conrath et al. 2002). Gupta and Pandey (2015) reported that different *Bacillus* isolates were most effective in improving the growth of sweet basil under nematode infection by the accumulation of phenolics and flavonoids.

11.5 Soil Microbial Mixture Against Biotic Stress

In natural field conditions, microbial communities that reside in the plant rhizosphere are highly affected by different environmental factors. When different combinations of microbes are applied in the rhizosphere of plants, their properties are generally enhanced as compared to single inoculation (Kumar et al. 2016a). In field conditions, soil microbes live in communities when they are applied as microbial mixture, providing specific benefits to plants. However, it is important to use accurate selection method for microbes; otherwise, it may lead to the decrease in efficacy of other beneficial microbes in the soil. The development of microbial mixture based on previous knowledge of microbial compatibility is more successful and effective against the target purpose. Thus, to reduce the breakdown of microbes in the environmental conditions, assessment for microbial compatibility is essential. Identification of the microorganisms with different properties is essential and a combination of microbes having such properties is the urgency for sustainable agricultural practices. Therefore, treating plants with a mixture of microbes has advance potentiality, particularly in existing agriculture system to reduce the application of pesticides. In recent years, few microbial combinations were developed and applied in various MAPs under different environmental conditions (Dojima and Craker 2016). Soil microbes when applied in combinational mode can enhance reliability, consistency, and efficiency of the microbes under different conditions (Sarma et al. 2015). Recently, researchers have developed some valuable and triumphant combination of microbes against plant parasitic nematode such as *M. incognita*. They demonstrated that microbial consortia consisting of potential microbes enhanced the resistance in various MAPs such as *B. monnieri* and *Matricaria recutita* (Gupta et al. 2015, 2017a).

11.6 Correlation Between Soil Microorganisms and Enhancement of Plant Secondary Metabolites

Enhancement of MAPs secondary metabolites has been associated with the application of soil microbes (Gupta et al. 2018; Singh et al. 2019). The mechanism by which soil microbes are able to enhance the *in planta* content is well documented. Some

researchers highlighted that an enhancement could be owing to plant growth activities of microbes or a modulation of biosynthetic pathway of MAPs (Gupta et al. 2017d, 2020). Soil microbes produce several growth hormones and volatile compounds that can serve as signals to trigger induction of secondary metabolites of plants. Volatile compounds produced by soil bacterium increased the essential oil in basil and *Mentha* (Banchio et al. 2009; Santoro et al. 2011). Numerous findings indicate that soil microbes can enhance the significance of cultivated MAPs (*B. monnieri*, *O. basilicum*, *C. roseus*, *W. somniferra*, *P. graveolens*, and *A. annua*) while improving plant development via conferring stress resistance (Gupta et al. 2016a, b, c, 2018). The effect of beneficial rhizospheric microbes established the enhanced effect on biomass and curcumin content of leaves of *Curcuma longa* (Kumar et al. 2016c). Recently, it has been reported that application of specific microbes enhance upregulation of biosynthetic pathways in various MAPs, and inoculation of microbes can strongly intensify the production of desired compounds via upregulation of key genes of pathway (Kushwaha et al. 2019; Ray et al. 2019).

11.7 Problem Associated with the Application of Rhizospheric Microorganisms

Microbial communities exert their beneficial properties in greenhouse conditions but there are only very few numbers of microbes that are successfully functioning under commercial agricultural field. Identifying the reason behind this failure in organic field conditions might lead us to the identification of potent strains followed by development of microbial consortia with superior efficacies. Mixtures of strains with antagonistic properties are easy to apply, and therefore they have been frequently used in diverse trials but sometimes they are futile. They could not succeed owing to their inability to colonize the root surface. In addition, the regulation of the secondary metabolites production by microbes is a complex process (Bloemberg and Lugtenberg 2001). This may be owing to adverse environmental conditions that direct initial failure of settlement of microbes in the host root region. In most of the cases, effective results in greenhouse studies have not been successful in the natural field conditions. Thus, preliminary selection of strains based on screening process may not be sufficient for selection and efficacy of strains in the field conditions. In greenhouse, better performance of various strains does allow prediction of their efficacy in field conditions.

However, a large number of researchers are evaluating field trials for validating efficiency of microbes. Thus, this development is probably going to raise the market for utilization of microbes worldwide. Assessment of field performance of microbial application against synthetic chemicals is necessary to develop plant immunity. For instance, plant biomass was improved with reduced disease infection in *O. basilicum* following inoculation of seeds with effective bio-inoculants under field conditions (Singh et al. 2013b). Similarly, Gupta et al. (2015) demonstrated that the effective field performance of microbial treatments (combinations of *B. megaterium*,

G. intraradices, and *T. harzianum* ThU) was also reported in *B. monnieri* in comparison with carbofuran treatment. The seed germination rates were maximum compared to those achieved using chemical treatments. Improved emergence rates and reduced plant mortality were also found with some potent bio-inoculants. Gupta and Pandey (2015) have reported that the *O. basilicum* seed primed with a subset of *Bacillus* spp. increased plant growth and essential oil content. In addition to increased biomass production, disease reduction can also improve crop health. Therefore, field trials for confirmation of microbial efficiency with extensive validation at multiple conditions such as effect of different soil quality and climatic conditions are necessary to authenticate biological products based on beneficial soil microbes.

In last few years, beneficial microbes in a combinational mode have supported MAPs cultivation in various organic field conditions. The yield parameters and cost economics of *W. somnifera* and *Plantago ovata* were studied using dual inoculation of *A. chroococcum* and *P. putida* (Kumar et al. 2009, 2011a). On the other hand, results are not always reproducible when using microbial mixture due to the incompatibility of microbes with one another and independent signaling pathways operating in two different microbial species. Gupta et al. (2016a) showed that co-inoculation treatments involving beneficial microbes affected growth, essential oil content, and number of glandular trichome in *P. graveolens*, and the consequences of the co-inoculated treatments were maximum compared to single application of bio-inoculants. Few researchers demonstrated that microbes in mixture were unable to enhance desirable traits as compared to their treatments alone in the host plants (Schmidt et al. 2004; Felici et al. 2008). Therefore, extensive selection of compatible microbes for application of microbial mixture is required. A necessity for reliable and successful biotic stress management would require screening of the microbes with different properties, which can mutually enhance the crop health under stress conditions. The cooperation of microbes and lack of rhizosphere competition among them are important requirements for development of microbial consortia to establish themselves in the plant rhizosphere. Another essential criterion for developing microbial combination is to understand deeply about the interaction between the indigenous microbial community and host in the plant rhizosphere.

11.8 Conclusion and Future Perspectives

Now it is very much clear that the application of soil microbes has positive effects in improving the growth and yield of MAPs under biotic stresses. Soil microbes are a nonhazardous environmentally friendly, and cost-effective alternative to synthetic chemicals for managing phytopathogens. Application of synthetic chemicals can achieve the disease management by 60–90%, but undoubtedly diminish the therapeutic properties of the MAPs along with damage to our environment and soil health. The use of beneficial microbes that are eco-friendly can improve the MAPs growth and secondary metabolite production. Till date, approximately 1400 biopesticides

(based on *Trichoderma*, *Bacillus*, *Streptomyces*, and *Pseudomonas*) have been widely marketed (Abhilash et al. 2016), but still there is a need to optimize the worth of these products. Although understanding the mechanism of soil microbes as biopesticides is still an interesting field of research, it is the accurate time to select the potential microbe that can enhance the plant fitness even under pathogen stress. Application of potential strain of microorganisms in the organic field infected with the pathogens may exert some reliable outcomes.

In general, use of single microbe under varying environmental conditions is not very effective. Hence, to achieve these targets in various environmental conditions, microbes in consortia are suggested. When different microbes are applied in combination, more than one mechanism such as mycoparasitism, competition, antibiosis, metabolites, growth promoters, and induced resistance acts in synergistic manner to achieve enhanced plant fitness. The molecular and physiological impact of microbial consortia on plant health are relatively unraveled. Owing to the diverse features of MAPs, future research could also pave a novel platform for understanding this issue. Comprehensive research in this thrust area could be a major breakthrough for the enhancement of health of various economically important MAPs.

Moreover, it will be interesting to see whether these microbial combinations apart from managing the phytopathogens could also trigger secondary metabolites pathways in MAPs to achieve value-added benefits (Gupta et al. 2016b; Singh et al. 2016). Bioformulations based on mixture of beneficial microbes and their secondary metabolites are useful to manage the plant diseases and improve stress tolerance by synergistic effects applied by them. Research based on the study to explore the role of microbes that help in managing the plant diseases in agricultural sustainability is needed. There are still many barriers preventing large-scale application of microbes in organic cultivation. The competence and prolonged survival of soil microbes along with nutrient availability and rhizospheric competence under varying environmental conditions are essential concerns. In addition, it is also necessary to uncover how microorganisms change host plant metabolism, thus enabling to enhance plant immunity. Novel bioformulations with enormous activity of soil microbes will enhance level of disease resistance in MAPs in an environmentally friendly manner (Fig. 11.2). The formulation based on the beneficial microbes is cost-effective and eco-friendly and can be easily replicated by farmers. Large-scale awareness activities in agricultural areas may therefore play an important role in enlightening farmers and industrialists. In India, movable plant health center can also be equally helpful and informative. Thus, beneficial rhizospheric microbes will be a green solution for sustainable production of natural drugs from MAPs through management of phytodiseases.

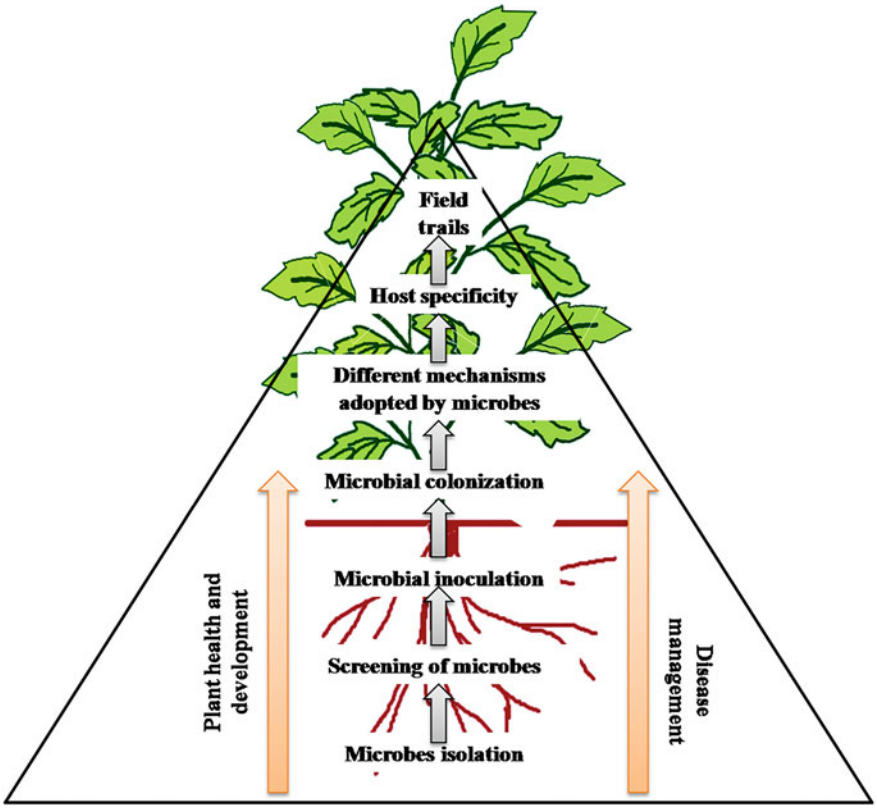

Fig. 11.2 A schematic representation of multifaceted approach to developed rhizosphere microorganisms as bio-fertilizers/pesticides for disease management

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Nematophagous Fungi: Biology, Ecology and Potential Application

12

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Abstract

Nematodes and fungi are soil inhabitants. Both are essential for maintaining the stability of food-web and facilitation of the nutrient cycle. Interaction between nematodes and fungi is possible in multiple ways. Here, we supply a platform for nematophagous (nematode destroying) fungi (NF), their mode of action, and their importance in agricultural ecosystems. They are potentially important for sustainable agriculture and play a major role in integrated pest management programs. Nematophagous fungi belong to a broad taxonomic group, such as Ascomycota, Oomycota, Basidiomycota, and distinct groups of fungi. Nematophagous fungi are broadly distributed in terrestrial and aquatic ecosystems that contain high densities of nematodes. Depending on the mechanism that affects nematode, NF can be divided into four types. Here, we described the classification, taxonomy, occurrence, distribution and ecology, types of nematophagous fungi, and potential mechanisms of NF in the control of plant-parasitic nematodes.

Keywords

Plant-parasitic nematodes · Nematophagous · Biological control · *Arthrobotrys oligospora* · Egg parasites · Second-stage juveniles

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12.1 Introduction

In this materialistic world, increasing food production for feeding the ever-growing population is the major world demand, while earning more profit from agriculture is the grower's demand, who faces an occasional setback due to the serious outbreak of the diseases and keeps looking for an effective method to save his crop from destructive pathogens. Among them, one of the major limiting factors is plant-parasitic nematodes (PPNs) that continuously affected agricultural production by and large. Further, the nature of crops, varieties, nematode species, the population of primary inocula, and environmental factors influence the losses to a great extent. It is estimated that 12.3% of global annual losses of major crops are due to phytoparasitic nematodes (Sasser 1989). Such type of losses become intolerable for poor and developing countries including India. Plant-parasitic nematodes cause 18–25% losses in vegetables, 20–25% in pulses, 18–23% in oilseed crops, and 15–18% in cereals crops (Indian Economy 2004).

Management of plant-parasitic nematodes is largely dependent on the use of toxic pesticides, the majority of which are soil fumigants. Farmers are using toxic pesticides intensively for the last few decades in order to reduce such a magnitude of losses to sustained crop production. However, the residual effects of these pesticides on nontarget soil flora and fauna are of great concern. Further, long-term residual effects are responsible for eroding biodiversity, increasing resistance and resurgence in the pathogen, and causing pollution that poses health hazards to humans, animals, and the environment. Present circumstances of environmental awareness evoke urgent need to search for and establish compatible alternatives to these hazardous agrochemicals. The persistence of pesticides in soils, deterrents to ecosystems, environmental contamination, detrimental impact on human health, deterrents to ecosystems, and the creation of resistant pathogenic strains are all consequences of heavy pesticide use. In order to reduce the use of pesticides, researchers have intensified resistance breeding programs along with transgenic plants to control the losses caused by these notorious pathogens. However, several constraints limit the scope of the resistance breeding program, i.e., unavailability of suitable donor parents having a high degree of resistance, detection of the source of resistance, and transferring desirable traits into a cultivar using a resistance breeding program is a great challenge. Biological control of plant-parasitic nematodes offers a promising alternative to pesticides, which had attained lots of attention over the years. Under these circumstances, using microbe-based strategies for the control of plant-parasitic nematodes has been reported to be an environmentally friendly, safe, and residue-free approach (Singh et al. 2012a, b, 2013, 2019a, b). Several biological control agents of microbial origin have been evaluated and used to control the plant-parasitic nematodes in many crops. Among them, *Trichoderma asperellum*, *T. harzianum*, *T. virens*, *Bacillus subtilis*, *B. licheniformis*, *Pseudomonas fluorescens*, *Purpureocillium lilacinus*, *Arthrobotrys oligospora*, *Pochonia chlamydosporia*, *Dactylaria* spp., *Monacrosporium* spp., *Drechslerella dactyloides*, *Syncephalastrum racemosum*, *Hirsutella* spp., and *Duddingtonia* spp. were noted worthy and are used to manage nematodes worldwide (Singh 2013; Singh et al.

2013, 2017, 2019b; Wang et al. 2014; Huang et al. 2014; Gupta et al. 2015a, b). Predacious fungi are an important part of the soil's biodiversity. Interactions between predatory fungi and parasitic nematodes are widespread and dynamic in the soil. Several reviews and research publications have demonstrated the ability of nematophagous fungi (NF or NPF) to suppress plant-parasitic nematodes; however, they are scattered. Nematophagous (nematode-eating) fungi are found in both terrestrial and aquatic habitats and are diverse in nature (Pramer 1964; Nordbring-Hertz et al. 2006). More than 200 fungal species, which can develop specific trapping devices, belong to the NF group such as adhesive knobs, constricting rings, and adhesive networks to capture nematodes juveniles, eggs, and adults and then use various strategies to extract nutrients from their nematode prey (Jansson and Lopez-Llorca 2001; Nordbring-Hertz et al. 2006; Yang et al. 2007; Schmidt et al. 2007). Most of the nematode-trapping fungi can function as both saprophytes and parasites (Pramer 1964; Nordbring-Hertz et al. 2006). Nematode-trapping fungi develop sophisticated hyphal structures, such as hyphal knobs, hyphal branches or rings, and hyphal nets, by adhesion or mechanical capture (Nordbring-Hertz et al. 2006; Singh 2007; Singh et al. 2012b). They have an essential role in maintaining nematode population density via natural settings. Many egg-parasitic and trap-forming fungi may exist in soil saprophytically while endoparasites are obligate parasites. Considering the importance of the problem and the potentiality shown by previous workers, the present study was undertaken with the objectives to give an overview of the biology, ecology, and potential application of nematophagous fungi to sustain crop production in changing climatic scenarios.

12.2 Historical Background

The word "predacious" comes from the Latin word *preada*, which means "to grab out all valuables and things of a victim after they have been killed." Predacious fungi are distributed in all types of soils. These fungi are more significant in decomposing plant waste, and the organic matter supplied to the soil increases the number of predacious fungi. *Arthrobotrys oligospora* was first described as a common inhabitant of organic plant debris by Fresenius (1852). Woronin (1870) reported that the conidia of *A. oligospora* germinated on the old manure and some of the hyphae-produced net-like bails, although he did not know the functions of such bails. Sorokin (1876) created the genus *Catenaria* with the type species *C. anguillulae*. He found *C. anguillulae* parasitizing eelworms in a vessel, which were eventually killed. He also described that round zoospores were liberated from the sporangia through a discharge tube. Zopf (1888) was the first to record the predacious behavior of *A. oligospora*. Further, his studies show that the cuticle of the captured nematode is penetrated, and the fungus grew within the nematode body and consumed it by its hyphae. Thus, Zopf established the predatory relationship of a fungus on nematodes. Drechsler (1937) established a base for studies on predacious fungi responsible for capturing and killing nematodes. He described that the predacious fungi produced different trapping devices for the predation of nematodes. Such capturing devices

include adhesive hyphae, adhesive branches, adhesive nets, and adhesive knots, whereas the nonadhesive organs include non-constricting rings and constricting rings. Some predaceous fungi produce sticky knobs that capture nematodes (Drechsler 1937; Barron 1977).

12.3 Occurrence and Distribution

Predacious fungi can be found as saprophytes in the soil or on decaying plant materials where they live saprophytically and/or feeding on plant-parasitic nematodes. The efficacy of capturing predacious fungi may be influenced by the nature of the soil and environmental conditions. The presence of nematodes in the soil and organic matter is necessary in maintaining the biodiversity of soil and increasing the population of predacious fungi. Most of the predatory species belong to either the Zoopagales or Moniliales, while endo-parasites are found in the lower fungi such as Chytridiales, Saprolegniales, Peronosporales, Lagenidiales, Mucorales, Entomophthorales, and in higher fungi Deutromycetes.

12.4 Classification

Fungi represent the fifth kingdom in the living organisms (Kendrick 2001). NPF are found in all lower and higher groups of fungi, such as Basidiomycetes, Ascomycetes, and Deuteromycetes in higher fungi and Chytridiomycetes, Oomycetes, and Zygomycetes in lower fungi. In distinct taxonomic groups of fungi, the habit of nematophagous fungi evolved separately. Barron (1992) reported that the habit of nematophagous fungi evolved from lignolytic (characterized by a unique ability to depolymerize and mineralize lignin) and cellulolytic fungi (hydrolyzing or having the capacity to hydrolyze cellulose) for adaptation to overcome nutrient competition in the soil.

In tandem with entomopathogenic species of *Verticillium*, which were relocated to the genus *Lecanicillium* based on both morphological and molecular features, egg-parasitic fungi previously placed within the genus *Verticillium* were recently shifted to the new genus *Pochonia* (Zare and Gams 2001; Zare et al. 2001). *Cordyceps* contains the teleomorphs of the *Pochonia* species. *P. chlamydosporia* and *P. rubescens* are the most well-known egg parasites, and other taxa reported to parasitize nematode eggs include *Paecilomyces lilacinus* and *Lecanicillium lecanii*. Based on the molecular evidence, Scholler et al. (1999) proposed the following classification: *Arthrobotrys* (adhesive three-dimensional networks), *Dactylellina* (stalked adhesive knobs or non-constricting rings), *Drechslerella* (constricting rings), and *Gamsylella* (non-constricting rings) (having adhesive branches and unstalked knobs).

The taxonomy and phylogeny of endoparasitic fungi are far less well-understood (Fig. 12.1). Some, such as the zoosporic *Catenaria anguillulae*, are classified as Chytridiomycetes, while others are classified as *Haptocillium* (previously

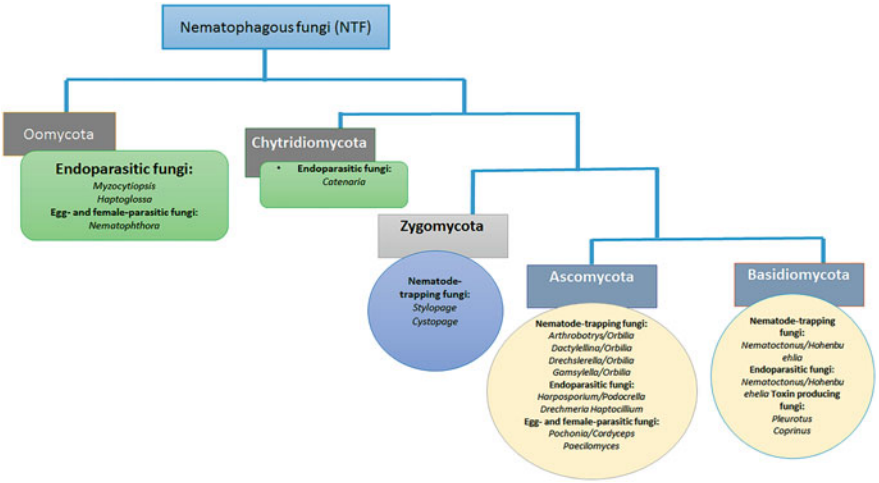

Fig. 12.1 Classification of nematophagous fungi

Verticillium), *Harposporium*, or *Drechmeria*. *Harposporium* spp. teleomorphs have lately been shifted to *Podocrella* from *Atricordyceps* (Chaverri et al. 2005). Pleurotus is a category of toxin-producing fungi that contains species such as the oyster mushroom *Pleurotus ostreatus*. Luo et al. (2004) reported that *Coprinus comatus* was recently discovered to have similar abilities, suggesting that nematophagy is more widespread among Basidiomycetes than previously thought.

12.5 Ecology

Nematophagous or nematode-trapping fungi (NF or NPF) are found in the soil. They are mostly found in the topsoil, meadows, leaf litter, mangroves, and some shallow aquatic areas. NF employ adhesive knobs, adhesive hyphal strands, and nets made of hyphal threads, hyphal loops, and non-constricting loops that capture nematodes. When the nematode is bridled, the NF hyphae enter the cuticle and eat the nematode's internal tissues (Zhang et al. 2014).

Arthrobotrys oligospora, a species of *Arthrobotrys*, is one of the most well-studied nematode-trapping fungi (Nordbring-Hertz et al. 2006). Strains of *A. oligospora* have been discovered in different soil conditions (Pfister and Liftik 2018; Money 1998). By creating intricate three-dimensional networks, *A. oligospora* enters the parasitic stage in the presence of nematodes to capture them. Nematode trapping triggers a chain of actions that include nematode adhesion, penetration, and immobilization (Nordbring-Hertz 2004; Nordbring-Hertz et al. 2006). The fungus' strong ability to capture nematodes makes it a promising candidate for controlling plant-parasitic worms. To catch nematodes mechanically, *A. oligospora* forms three-dimensional adhesive nets. The fungus actively seeks out its prey by creating

chemical signals or olfactory cues that are similar to those used by worms to find food and mate (Yu'e et al. 2005; Zhang et al. 2015; Hsueh et al. 2017). Some nematophagous fungi produce toxins that render nematodes immobile. The hypha of the shaggy ink cap (*Coprinus comatus*) attacks the nematode *Panagrellus redivivus* as a spiny ball structure, which immobilizes and breaks the nematode cuticle, following which the hypha pierces the skin and digests the contents (Luo et al. 2007). The spores of the most endoparasitic fungi are attracted to and concentrated in the mouth region of soil nematodes. The hyphae proliferate throughout the nematode after penetration of the cuticle and absorption of nematode tissues. Conidia are contacted by the nematode in other fungal species and are infected in a similar fashion. *Harposporium anguillulae* having sickle-shaped conidia are consumed by the nematodes and lodge themselves in the esophagus or gut, where they destroy the tissues (Aschner and Kohn 1958).

The hypha flattens itself against the egg in egg-parasitic species, and the presence of appressoria indicates that infection is about to occur or has already occurred. After piercing the egg and devouring the developing juvenile worm, the hypha produces conidiophores and moves on to nearby eggs (Money 1998).

12.6 Plant-Parasitic Nematodes have an Impact on Agriculture

Plant-parasitic nematodes are a serious constraint in agricultural crop production. These nematodes have been discovered in over 4100 different species (Decraemer and Hunt 2006). Crop loss is projected to cost between US$118 and 80 billion per year (Sasser and Freckman 1987; Nicol et al. 2011). The most economically important nematode species accounts for 15% of all identified nematode species. They directly target the plant roots of major crops, preventing nutrient uptake and water, resulting in decreased agronomic performance, overall yield, and quality of the crop. Surprisingly, just a small percentage of the nearly 4000 reported plant-parasitic nematodes cause major agricultural losses. In a survey, the principal genera of phytoparasitic nematodes identified to cause crop losses in the United States were *Meloidogyne*, *Heterodera*, *Hoplolaimus*, *Rotylenchulus*, *Xiphinema*, and *Pratylenchus* (Koenning et al. 1999).

12.7 Types of Nematophagous Fungi

Nematophagous fungi can be divided into four major groups (Fig. 12.2) depending on their mode of attacking nematodes (Jansson and Lopez-Llorca 2001):

1. Nematode-trapping fungi (previously sometimes called predacious or predatory fungi)
2. Endoparasitic fungi
3. Egg- and female-parasitic fungi
4. Toxin-producing fungi

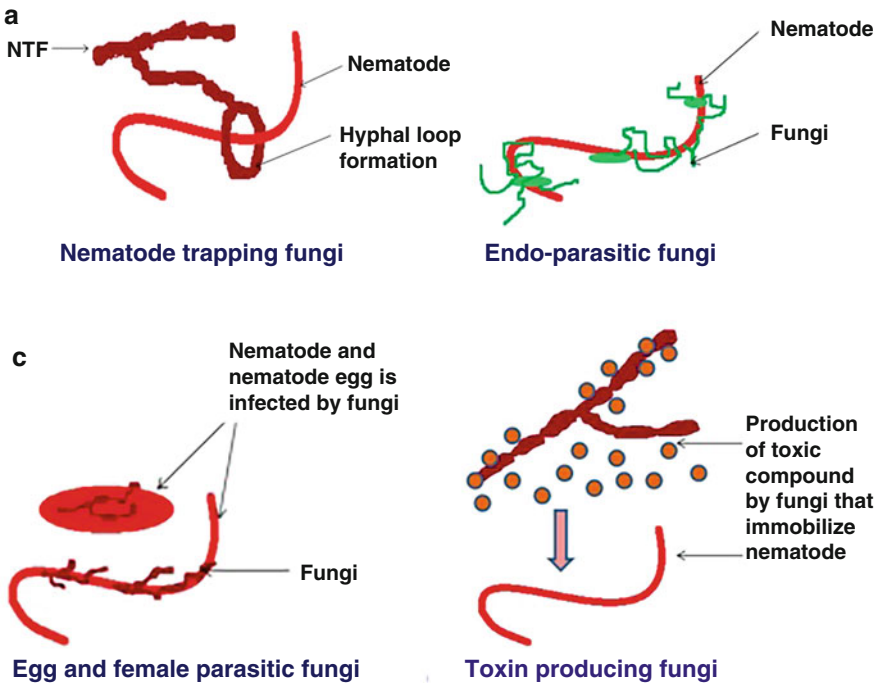

Fig. 12.2 Types of nematophagous fungi

The nematode-trapping fungi use hyphal trapping devices of various shapes and sizes to capture nematodes, such as sticky/adhesive three-dimensional nets, adhesive knobs, and nonadhesive constricting rings. Some "nematode-trappers" capture nematodes by an adhesive substance formed on their hyphae without any visible traps, e.g., *Stylopage* spp. Endoparasitic fungi use their conidia or zoospores to infect the nematodes. The propagules of fungi adhere to the cuticle of the nematode, and then spore contents are injected into them or spores are swallowed by the host. Most of them are obligate parasites and the entire vegetative stages of their life live inside the infected nematodes. The egg- and female-parasitic fungi are facultative parasites. They infect nematode females and their eggs, using appressoria or zoospores. Being facultative parasites, they grow on nematodes and parasitize the sedentary stages such as eggs. The toxin-producing fungi produce toxic compounds that can immobilize nematodes, prior to penetration by hyphae through the cuticle of the nematode. Parasitism of nematodes results in complete prey or egg digestion in all four nematophagous fungal groups, an action that provides the fungus with nutrients and energy for continuous growth.

12.8 Mechanism and Mode of Action of Nematophagous Fungi to Control Plant-Parasitic Nematode

Biological control of phytonematodes is described as a reduction in populations of nematodes caused by actions of living organisms other than those found naturally in the host plant, or by introduction of antagonist organisms into the environment (Kim 2015). More than 200 taxonomically distinct fungi have demonstrated the ability to kill live nematodes in all the stages of development such as juveniles, adults, and eggs (Nordbring-Hertz et al. 2006). Two types of barriers to fungus invasion are created by the morphology of nematodes. The eggshell is the first barrier, made up of three layers in root-knot and cyst nematodes: the outer vitelline (mostly proteins), the inner lipoprotein layer, and the chitin layer, and the cuticle is the second barrier. The parasitism, poisonous chemicals, and enzyme methods used by nematophagous fungi to infect nematodes can be separated into three categories (Fig. 12.3).

Different nematophagous fungi infect nematodes and their eggs in a similar, general way. Infection of nematode eggs by *Pochonia rubescens*, as well as the zoospores of *Catenaria anguillulae*, which infect vermiform worms, demonstrate this. *P. rubescens* begins penetrating nematode eggs by contacting the egg with its hyphae and then forming an appressorium. The appressorium forms an extracellular matrix (ECM) or adhesive, which is disclosed by lectin Concanavalin A labeling. The fungus uses both mechanical and enzymatic components to enter the worm eggshell from the appressorium. Because the nematode eggshell is mostly made up of chitin and proteins (Bird and Bird 1991), chitinases and proteases are vital during the penetration of eggshell (Lopez-Llorca 1990a, b; Tikhonov et al. 2002). Eggshells are degraded as a result of proteolytic action.

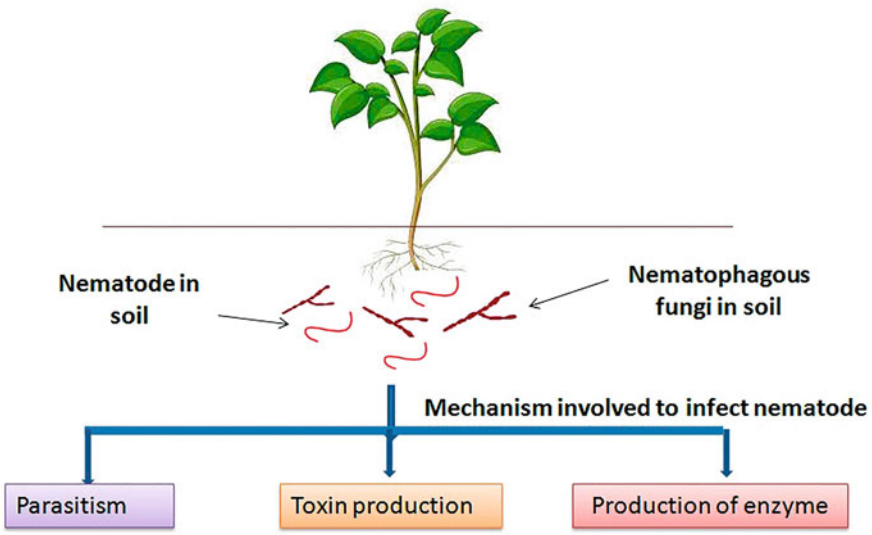

Fig. 12.3 Key mechanisms involved in the nematophagous fungi

12.8.1 Chemotaxis and Adhesion (Host Recognition, Host Specificity, and Infection)

The recognition phase of nematode begins, which includes chemotaxis of the host toward fungal traps, hyphae, or zoospore chemotaxis toward the host's natural apertures (Jansson and Nordbring-Hertz 1979; Jansson and Thiman 1992). It is unknown which chemicals are involved in the chemotactic events (Jansson and Friman 1999; Bordallo et al. 2002). After contact with a nematode, the adhesive on *A. oligospora* traps changes from amorphous to fibrillar, in contrast to the adhesive on *D. coniospora* conidia, which always appears fibrillar (Jansson and Nordbring-Hertz 1988). Lopez-Llorca et al. (2002) suggested that the adhesion on the appressoria of *P. rubescens* and *P. chlamydosporia* can be identified using the lectin Concanavalin A, indicating that it is a glycoprotein having glucose/mannose moieties. *A. oligospora*'s Gal-NAc-specific lectin and *D. coniospora*'s sialic acid-specific lectin have both been implicated in worm recognition (Nordbring-Hertz and Mattiasson 1979; Jansson and Nordbring-Hertz 1984). Infection events eventually trigger a signaling cascade that is required for nematode prey penetration and colonization (Tunlid et al. 1992). An extracellular substance is generated after contact, which keeps the fungus attached to the nematode surface. Proteins or carbohydrates are typically found in the adhesives of nematophagous fungi (Tunlid et al. 1991a, b). Carbohydrates on nematode surfaces are engaged in the lectin-binding recognition stage, but they also appear to play a role in nematode chemotaxis (Zuckerman and Jansson 1984; Jansson 1987). Major nematode sensory organs, such as inner labial papillae and amphids, are positioned around their mouth in the labial and cephalic region (Ward et al. 1975). Zuckerman (1983) and Zuckerman and Jansson (1984) proposed that carbohydrates play a role in nematode chemoreception. Lectins (Concanavalin A binds with mannose/glucose residues, and Limulin binds with sialic acid) could block the chemoreceptors, leading bacterial-feeding nematodes to lose their chemotactic behavior to microbial exudates (Jeyaprakash et al. 1985). Further, nematode chemotaxis was reduced when enzymes (mannosidase, sialidase) obliterated the terminal carbohydrates (Jansson et al. 1984), demonstrating the importance of carbohydrate moiety in nematode chemotaxis. An endoparasitic nematophagous fungus, *D. coniospora*, uses conidia to infect nematodes that cling to the host's chemosensory organs (Jansson and Nordbring-Hertz 1983). Both Limulin treatment of nematodes and sialic acid treatment of spores reduced conidial adhesion, implying that a sialic acid-like carbohydrate is involved (Jansson and Nordbring-Hertz 1984). Furthermore, it is evaluated that nematode adherent with spores lost their capacity to respond chemotactically to all the attracting sources, including hyphae, conidia, or bacteria, implying a link between chemotaxis and adhesion via carbohydrates on the surface of nematode (Jansson and Nordbring-Hertz 1983). *D. coniospora* conidia stick to *Meloidogyne* spp. chemosensory organs, but they do not penetrate and cannot infect the worms.

12.8.2 Differentiation and Signaling

When recognizing the surface of the host, or even synthetic surfaces, most harmful fungi distinguish appressoria. Appressoria formation in plant pathogenic fungi infecting leaves has been examined in depth (Lee et al. 2003; Basse and Steinberg 2004). St. Leger (1993) proposed a signaling hypothesis for the insect pathogen *Metarhizium anisopliae* during appressorium production, based in part on knowledge of plant pathogenic fungi. Appressoria on their hosts are differentiated by nematophagous fungi, particularly egg parasites (Lopez-Llorca and Claugher 1990). The signaling pathways that lead to nematode infection by nematophagous fungus are poorly understood. Using expressed sequence tag (EST) techniques, it was recently demonstrated that genes involved in the creation of infection structures and fungal morphogenesis were expressed during trap formation in the nematophagous fungus *Dactylellina haptotyla* (syn. *Monacrosporium haptotylum*) (Ahren et al. 2005). As a response to chemical and tactile inputs, fungi-infecting vermiform nematodes differentiate multiple trapping organs. The three ring cells that make up the trapping mechanism are inflated by the constricting ring traps. When a nematode comes into contact with the inner ring wall, an unknown mechanism causes the nematode to inflate and close, which takes around 0.1 s.

12.8.3 Nematodes Cuticle and Eggshell Penetration by NPF

Nematophagous fungi penetrate the worm cuticle or eggshell after a solid adhesion to the host surface. Both enzymatic and physical mechanisms appear to be used by the nematophagous fungus to penetrate host surfaces, as in many other cases of fungal penetration. Because the nematode cuticle is mostly made up of proteins (Bird and Bird 1991), proteolytic enzymes are necessary for penetration of the nematode cuticle. The PII serine protease of *A. oligospora* has been characterized, sequenced, and cloned (Ahman et al. 1996). The protein presence, such as nematode cuticles, increases PII expression (Ahman et al. 1996). The subtilisin PII has a molecular mass of 32 kDa and belongs to the subtilisin family (Fig. 12.4).

Another serine protease (Aoz1) was recently identified from *A. oligospora*, having 38 kDa molecular mass and 97% similarity with PII (Zhao et al. 2004). Other fungi have been isolated and characterized, including *Arthrobotrys microscaphoides* (Mlx) (Wang et al. 2006a, b) and *Arthrobortys shizishanna* (Ds1) (Wang et al. 2006a, b), both of which show significant similarities to the *A. oligospora* serine (Wang et al. 2006a, b).

Protein and chitin are structured in a microfibrillar and amorphous form in nematode eggshells (Clarke et al. 1967). As a result, extracellular enzymes that degrade such polymers were sought. Lopez-Llorca (1990a, b) identified, purified, and characterized P32, a 32 kDa serine protease from the egg parasite *P. rubescens* for the first time. P32 suppression by polyclonal antibodies and chemicals reduced the penetration and egg infection, despite pathogenesis being a complex process involving many variables (Lopez-Llorca et al. 2002). An extracellular protease

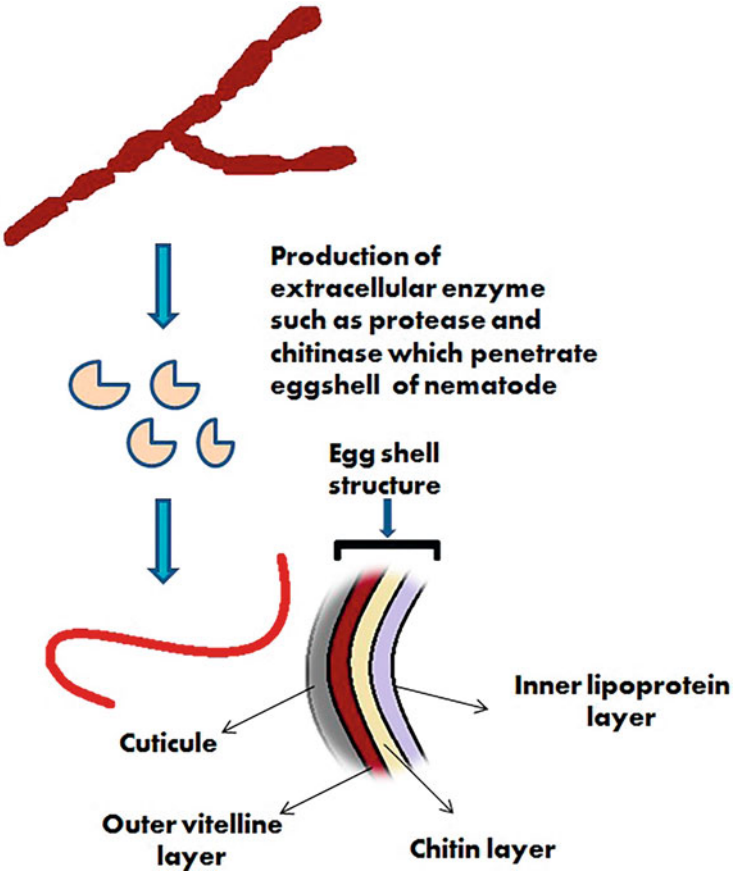

Fig. 12.4 Eggshell penetration by NPF

(VcP1) is produced by *P. chlamydosporia*, which is linked to P32 and similar entomopathogenic fungal enzymes (Segers et al. 1994). Eggs treated with VcP1 enzyme were more easily infected than non-treated eggs, implying that the enzyme plays a role in eggshell penetration by fungi that feed on eggs. Recently, a serine protease (Ver112) from *Lecanicillium psalliotae* was isolated and described, exhibiting approximately 40% homology with *Arthrobotrys* proteases (PII and Aoz1) and have 60% homology with egg-parasitic serine proteases (Yang et al. 2005a, b). Non-nematophagous fungi such as *Clonostachys rosea* and *Trichoderma harzianum* are additional sources of nematicidal serine proteases (Suarez et al. 2004; Li et al. 2006). Huang et al. (2014) reported that *Pochonia rubescens* and *Pochonia chlamydosporia* both have chitinolytic enzymes that have been discovered. A 43 kDa endochitinase (CHI43) was one of those responsible for the majority of the activity (Tikhonov et al. 2002). Damage to eggshells was more widespread when treating *G. pallida* eggs with both P32 and CHI43, which indicated that the two

enzymes work together to destroy eggshells (Tikhonov et al. 2002). A hydrolytic enzyme chitosanase from the egg-parasitic fungus *P. lilacinus* was recently identified and described (Chen et al. 2005).

12.9 Potential Application

For many years, nematophagous fungi have been tested for biological control of plant-parasitic nematodes, but the strategy had limited success due to the lack of understanding of these species' ecology (Stirling 1991) (Table 12.1).

The colonization of plant roots by endophytes is a significant element. By induced resistance or by the production of secondary metabolites, NF may protect plants from several fungal diseases and plant-parasitic nematodes. Plant growth can also be boosted by nematophagous fungi participating in the nutrient intake or modifying plant growth regulators. As a result, endophytic colonization must be taken into consideration while looking for nematophagous fungi as biocontrol agents. Combining numerous forms of nematophagous fungi, such as egg-parasitic and nematode-trapping fungi, that kill nematodes at different phases of their lives, could be a key requirement. When choosing the right fungi for biological control of plant-parasitic nematodes, interactions with other soil fungi, including plant-parasitic and biocontrol agents, are also crucial factors to consider. Larriba et al. (2015) demonstrated the egg-parasitic fungus *Pochonia chlamydosporia* having potential for biological control of plant-parasitic nematodes. They act as an endophyte in both monocot and dicot plants and have shown plant growth promotion in a variety of crops. Nematophagous fungus *Pochonia chlamydosporia* promotes growth of barley (*Hordeum vulgare*) plants by endophytic colonization of roots and provides defense against stresses. Escudero and Lopez-Llorca (2012) stated that endophytic colonization of tomato roots by *P. chlamydosporia* is important for plant growth and may influence root-knot nematode management.

12.10 Future Prospects

Plant-parasitic nematodes cause major yield and monetary losses in agriculture all over the world. The utilization of nematophagous fungi as endophytes is a potential technique for the biocontrol of nematodes in the soil. Larriba et al. (2015) observed that at the molecular level, plants colonized endophytically having growth-promoting effect by *P. chlamydosporia*, paving the way for more research into the fungus' ability to mitigate the negative effects of biotic and abiotic factors on plant crops. Bioproducts formulated with these NF have various advantages over chemical nematicides for more sustainable agriculture, including ease of application, environmental safety, little impact on soil biota, and no residues in harvested products. However, when producing a commercial bionematicidal product, there are various aspects to be kept in mind as it is a living system. As a result, new technology such as

Table 12.1 Some fungal biocontrol agents for the management of plant-parasitic nematodes

Name of fungi	Mixed with	Effective for nematode	Crop/ plant	References
P. chlamydosporia	Carbofuran + neem cake	*M. incognita*	Okra	Dhawan and Singh (2009)
Paecilomyces lilacinus	Groundnut cake, neem cake, castor cake, mahua cake, and linseed cake	*M. javanica*	Brinjal	Ashraf and Khan (2010)
T. viride	Compost	*Meloidogyne* spp.	Gotukola (*Centella asiatica*)	Shamalie et al. (2011)
T. viride	Neem cake	*M. incognita*	Tobacco	Raveendra et al. (2011)
Pochonia chlamydosporia	Combination of *P. fluorescens*, *T. viride*, and carbofuran	*Globodera* spp.	Potato	Muthulakshmi et al. (2012)
T. harzianum	Combination of neem cake and *P. fluorescens*	*M. incognita*	Brinjal	Singh et al. (2013)
T. harzianum	Carbofuran	*M. incognita*	French bean	Gogoi and Mahanta (2013)
P. chlamydosporia	Mustard cake and neem cake	*M. incognita*	Brinjal	Parihar et al. (2015)
T. harzianum	Lantana camara	*M. incognita*	Tomato	Feyisa et al. (2015)
T. harzianum	Carbofuran	*M. incognita*	Brinjal	Devi et al. (2016)
T. harzianum	Carbofuran and neem cake	M. incognita	Pea	Brahma and Borah (2016)
P. chlamydosporia	Neem cake	*Heterodera zeae*	Sweet corn	Baheti et al. (2017)
Paecilomyces lilacinus	Neem cake and Karanj leaves	*Heterodera zeae*	Sweet corn	Baheti et al. (2017)
P. fluorescens	Carbofuran	*Meloidogyne graminicola*	Rice	Narasimhamurthy et al. (2017)
Arthrobotrys oligospora, *Candellabrella musiformis*, and *Dactylella eudermata*	Carbofuran	*Meloidogyne incognita*	Tobacco	Hastuti and Faull (2018)
Drechslerella dactyloides	–	*Meloidogyne incognita*	Tomato	Singh et al. (2019a, b)
Dactylaria brochopaga	–	*Meloidogyne incognita*	Tomato	Singh et al. (2019a, b)
Duddingtonia flagrans	–	*Meloidogyne incognita*	–	Xiaoyu Mei et al. (2021)
Arthrobotrys oligospora	–	*Meloidogyne incognita*	Tomato	Soliman et al. (2021)

real-time quantitative PCR is used to quantify and track the biocontrol agent after its application into the soil. Biocontrol agents can be genetically modified to have their efficacy increased by increasing the expression of genes implicated in nematicidal activity or pathogenicity (Zhang et al. 2020a, b). To improve aggression and virulence against nematodes, expression of heat shock factors, UV protectants, immunological modulators, destroying enzymes of cuticle, and genetic modification techniques can be used. Several studies established the efficacy of applying a combination of treatments to manage plant-parasitic nematode populations under diverse conditions, including various cultural techniques (such as soil amendment and soil solarization), biological agents, and chemical nematicides (Zhang et al. 2014). Finally, the unpredictability of nematode antagonists against PPN in field circumstances, as well as their limited efficacy, are key barriers to using biocontrol agents to manage plant-parasitic nematodes. The intrinsic mechanisms governing ecosystem stability in field circumstances may be one of the causes for the disparities between the results of laboratory trials and field trials. Understanding interactions between nematodes and nematophagous fungi in native niches aids in the development of better applications for long-term crop protection approaches. The effects of combining various partners, such as NF, plant-pathogen mycoparasites, and plant growth-promoting microorganisms, could provide useful information for the development of biocontrol agents to reduce the impact of nematode and fungal pathogens on agriculturally important crops (Luns et al. 2018; Baron et al. 2020).

12.11 Conclusion

Nematophagous fungi are common soil organisms that may infect, attack, and consume nematodes at any stage of their development, including adults, juveniles, and eggs. To infect their nematode hosts, they use trapping organs, spores, and appressoria. In addition to infecting nematodes, nematophagous fungi can infect other fungi as mycoparasites and colonizing plant roots endophytically. Because of their various capacities, nematophagous fungi, in particular, may be a promising candidate for the biological management of plant root diseases. The use of nematophagous fungi as a substitute for synthetic chemicals used in the production of nematicides is fascinating. Obtaining bionematicides efficiently is a goal and a prerequisite for all agricultural researchers seeking sustainability in the system. Depending on the pathogenicity factor, some information is still lacking. Some NF enzymes such as serine proteases, chitinases, and toxins function as virulence factors and are especially interesting in the parasitic worm infection process. Some NF strains' success implies that they have different host preferences. Finally, we suggest that NF is a potential alternative to synthetic pesticides in the management of plant-parasitic nematodes, and that they may be more effective in making agriculture sustainable by replacing hazardous chemicals and mitigating the effects of their residues on the environment.

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Conflicts of Interest The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the content reported in this manuscript. The authors declare no conflict of interest.

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Rhizosphere Microbiome: Interactions with Plant and Influence in Triggering Plant Disease Resistance

13

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Abstract

The area of soil exposed to root activity is called the rhizosphere which harbors diverse microbes that can aid in plant growth and resistance against biotic and abiotic stresses. Rhizosphere microbiome is defined as all the microbial species found in the rhizosphere, which have one of the most complex and diverse ecosystems on the planet. These rhizosphere microbial communities interact with the plants as beneficial or detrimental interactions. Beneficial rhizosphere microbes promote plant growth through abiotic stress tolerance, absorption of nutrients in plants and antagonism against several phytopathogens, while parasitic interaction causes diseases of plants which are economically important, leading to challenges in food security and reduction in productivity. In this chapter, we have discussed in detail the various interactions on microbe-plant and microbe-microbe interaction and also the role of rhizosphere microbiome in plant health and resistance.

Keywords

Beneficial · Detrimental · Plant health · Microbes · Rhizosphere

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13.1 Introduction

The soil that surrounds the plant roots is amongst the most diverse and dynamic ecosystem. Rhizosphere is defined over 100 years ago as the zone or compartment of soil influenced by plant roots (Hiltner 1904; Lagos et al. 2015). Microbiome comprises of assortment of microbial genomes in a specific niche such as rhizosphere (Bulgarelli et al. 2013). It is a complex environment consisting of diverse microbial community associated with plants which is vital for plant growth and health. Around tens of thousands of microbial species live in the soil around plant roots (Berendsen et al. 2012; Bakker et al. 2013). These microbes intimate association with plants and allow them to compete for space, water, and nutrients (Hartmann et al. 2009).

Rhizosphere microbiome composition depends on complex interactions between the biotic inhabitants and its abiotic environment. Interactions between the plant-microbe occur on many different levels and ways (Schirawski and Perlin 2018). It harbors both beneficial and deleterious microorganisms consisting of fungi, oomycetes, bacteria, viruses, archaeal, and eukaryotes (Mendes et al. 2013). There are several rhizosphere microbiomes that interact with each other and the plants having many functions such as nitrogen-fixing bacteria, pathogenic microorganisms, microbial antagonist to control plant pathogens, plant growth-promoting rhizobacteria (PGPR) to enhance growth, nodulation, and Arbuscular mycorrhizal fungi association with plant roots (Barea et al. 2005). However, to date back most research on rhizosphere microbiome has been focused particularly on fungi and bacteria like symbiotic rhizobia, leaf pathogens, and mycorrhizal fungi. Such interactions are dynamic and under the influence of root exudates secreted by the plants which is a vital mediator of soil-microbes interactions with plants (Bever et al. 2012; Korenblum et al. 2020). The metabolic compounds secreted by the roots can suppress or activate the rhizosphere microorganisms (Doornbos et al. 2012). Plant metabolites in the microbiome exert selective pressure on the microbial community which results in different plant species having different communities of microbes (Hacquard et al. 2017; Zhang et al. 2017). Soil type, its pH, nutrient status, plant developmental stage, genotype, and species etc. are some key abiotic and biotic elements in a specific ecological niche that shape the structure and composition of rhizosphere microbiome communities (Broeckling et al. 2008).

Some rhizospheric microbes are helpful to plants, while others are harmful (Jacoby et al. 2020). Therefore, the interactions between rhizosphere microbiome and plants are crucial for fitness of plant by protecting against pathogen attack, better nutrient uptake, seedling vigor, growth of plant and its productivity (Berendsen et al. 2012; Mendes et al. 2013). Rhizosphere-microbiome (rhizobiome) can strengthen plant's immune functions (Vannier et al. 2019) by boosting defense of aboveground plant tissues against pathogens thereby rendering plant disease resistance (Zamioudis and Pieterse 2012). There are many defense mechanisms associated in suppression of pathogens by the microbes in rhizosphere which consist of nutrients competition, antibiosis, and induced systemic resistance (ISR) (Bakker et al. 2013). ISR primes the immune system of the plants to activate defense response.

Rhizospheric microbes such as mycorrhiza and rhizobacteria give broad-spectrum resistance by inducing systemic defense responses. Plants are able to detect microbe-associated molecular patterns (MAMPs) recognition system of beneficial microbes that leads to activation of defenses, disease resistance, and plant-beneficial microbe interaction (Van Wees et al. 2008; Hacquard et al. 2017). We now have a clearer picture of plant-microbe interactions in the rhizosphere thanks to omics technologies by enabling better study of community structures and its signaling (Pena and Vargas 2014; Dubey et al. 2020). Other tools such as Real-time PCR (RT-PCR), sequencing, phospholipid fatty acid (PLFA), chromatography, nuclear magnetic resonance, mass spectrometry (MS), and microscopy, etc. help in the study of plant-microbe interactions (Wu et al. 2009).

The knowledge and understanding about rhizobiome interactions with plants and its role in plant disease resistance can lead to the development of biological control agents or microbial inoculants either natural or synthetic to alleviate the deleterious effect of pathogens (Johns et al. 2016). It will also improve the plant health and ensure sustainable protection where the interactions of the microbiome with soil, plant, and environments are taken into account. However, there is still limited knowledge of rhizobiome precise effect and mechanism on plant health, growth, and diseases. Therefore, unraveling the rhizobiome is necessary to identify the microoganisms potential for exploitation for plant benefits. In this chapter, we have discussed the rhizosphere microbiome diversity and the factors affecting its occurrence, mechanisms of disease resistance induced by different types of rhizosphere microorganisms, and along with their growth promotion mechanisms. Finally, the tools to study such rhizosphere microbiome interactions are highlighted. This chapter mainly focuses on the rhizosphere microbes having disease suppression and growth promotion capabilities.

13.2 Microbial Diversity in the Rhizosphere

The plant microbiome is made up of the genomes of microbes that are firmly associated with plants (Berendsen et al. 2012; Bulgarelli et al. 2013). Plant microbiota, which includes both aboveground and belowground tissues, contains wide variety of microbes. The microbiome is involved in almost all soil processes, such as microbial composition, abundance, and activity that mainly regulate sustainable productivity of agricultural land (Barrios 2007; Van der Heijden et al. 2008; Philippot et al. 2013). Bacteria, fungi, oomycetes, archaea, and poorly surveyed viruses are among the organisms living in the rhizosphere (Swanson et al. 2009; Berendsen et al. 2012; Agler et al. 2016). The rhizosphere can consist of up to 10^{11} microbial cells per gram root (Egamberdieva 2008) and over 30,000 prokaryotic species (Mendes 2011). Fungi and fungus-like microbes are one of the most diverse classes in Eukarya, and they play an important role in soil microbial populations (Buee et al. 2009). *Aspergillus*, *Cephalosporium*, *Colletotrichum*, *Chaetomium*, *Fusarium*, *Phytophthora*, *Pythium*, *Penicillium*, *Rhizoctonia*, *Rhizopus*, and

Verticillium are amongst the fungal and oomycetes pathogen that exists in the crop rhizosphere.

Rhizobacterial species found in the plant rhizosphere have the capacity to improve plant growth and biological control capabilities. Endophytic rhizobacteria such as *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* that fix nitrogen form nodules by colonizing in legume plants, thereby, improving the growth of the plant directly or indirectly (Wang and Martinez-Romero 2000; Kumawat et al. 2019; Harman and Uphoff 2019). Plant growth-promoting rhizobacteria (PGPR) include genus such as *Agrobacterium*, *Azospirillum*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Caulobacter*, *Cellulomonas*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas*, and *Serratia* (Hossain et al. 2015; Duy et al. 2016; Disi et al. 2019; Hassan et al. 2019). Actinomycete genera such as *Micromonospora*, *Streptomyces*, *Streptosporangium*, and *Thermobifida* have been reported to inhibit fungal pathogens (Franco-Correa et al. 2010). In spite of the large diversity of soils, four bacterial phyla dominate the microbial population in the rhizosphere and endosphere of plants: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Archaea present in the soil are an essential group of ammonia oxidizers which has led to an increasing number of studies.

13.3 Factors Affecting the Occurrence and Diversity of Rhizosphere Microbiome

The microorganisms in the soil are diverse and they vary both qualitatively and quantitatively with difference in the biotic and abiotic factors. Details about the factors are described below:

13.3.1 Biotic Factors

13.3.1.1 Host Plant Factors

The microbiota linked with diverse plant species has been found to differ extensively. Despite the fact that numerous PGPRs, usually commercially available strains, colonize and have beneficial effects on a wide range of plants, their output varies enormously depending on the species or cultivar (Germida and Walley 1996; Montalban et al. 2017).

Plant Genotype Affecting Rhizosphere Microbiome

Differences in plant genotypes in a single gene, according to the current evidences, may have a substantial outcome on the rhizosphere microbiome. The microbial population on the roots of transgenic *Arabidopsis* was significantly changed by the production of a single exogenous glucosinolate (Bressan 2009). Bacterial rhizosphere population experiments in three cultivars of potato cultivars found 2432 operational taxonomic units in the rhizosphere of two separate soils (Weinert et al.

2011). These findings show that not only does the soil play a part in deciding rhizosphere communities, but also certain microbes have a particular affinity for specific genotypes. For separate genotype of the same species, plant genetic control over microbial communities in the rhizosphere has been studied (Jiang et al. 2017; Gallart et al. 2018).

Root Exudates Produced by Host Plant for Recruiting Microbial Diversity

Plant roots release variety of signals like root exudates which magnetize diversity of PGPRs (Dubey et al. 2019). The variety of chemicals secreted by different parts of the roots into the soil acting as chemoattractants are known as root exudates (Bulgarelli et al. 2013; Vyas et al. 2018). While root exudates release oxygen, ions, and water, they basically contain carbon-based compounds. Some root exudates serve as pathogen repellents, whereas others act as attractants, attracting useful microbes based on physiological status, species of plants, and microorganisms. In common, a plant root secretes root exudates either as diffusates by passive mechanisms or as secretions by active mechanisms. Exploring the process that guides the selection of the microbial community will provide new opportunities for cultivators to exploit rhizosphere microbiome of plant in order to increase its productivity.

Plant Metabolites and Their Role Played in Rhizosphere Microbiome

Numerous secondary metabolites are produced and delivered by plants into the rhizosphere, most of which play a vital part in plant-microbe interactions. These compounds are used by plants to draw attention to beneficial soil microorganisms and to protect themselves from pathogen attack. Phenolic compound plays an important role in shaping the rhizosphere microbial community. According to Neal et al. (2012), DIMBOA recruits a growth-promoting *Pseudomonas* strain into the rhizosphere. Flavonoids also function as chemoattractants attracting rhizobia to the root surface by controlling expression of the nod gene, which generate Nod factors (lipochitooligosaccharides). In a recent study, Camalexin, an indolic compound can regulate the functionality of root-associated microbial strains (Koprivova et al. 2019). Triterpenes, a class of secondary metabolites can regulate the bacterial root microbiota of *Arabidopsis* plant (Huang et al. 2019).

13.3.1.2 Microbial Factors

Microbial activity can be assessed in soil by measuring different parameters (Nannipieri and Badalucco 2003). Recently, the impact of plant diseases on the formation of rhizosphere microbiome was focused. Inoculation of downy mildew pathogen on leaves of *Arabidopsis thaliana* modifies the rhizosphere microbial community (Berendsen et al. 2018). An alteration of plant root exudates takes place, when leaves of *A. thaliana* were inoculated with *Pseudomonas syringae* by elevating the rhizospheric *Roseiflexus* genus. This shift in exudation patterns attracts a greater number of beneficial rhizospheric microbes which assist *A. thaliana* to resist pathogens that live aboveground (Yuan et al. 2018). *Pseudomonas fluorescens* inhibits the growth of pathogens such as *Fusarium oxysporum* and *Meloidogyne*

incognita by antibiotic secretion (2,4-diacetylphloroglucinol (DAPG)). In a study by Yadav et al. (2011), *Trichoderma harzianum*, *Aspergillus niger*, and *Penicillium citrinum* were found to improve chickpea growth along with potentials to be used as biocontrol agents.

13.3.2 Abiotic Factors

13.3.2.1 Structure and Soil Type

Out of all the factors, soil moisture qualities have the highest effect on microbial community composition, even more than the effect of on soil nutrients (Singh et al. 2009). During an analysis of soil bacteria diversity, Zhang et al. (2013) reported that Probacteria was the most presiding group and also notably connected with soil moisture quantity. The ability of an organism to migrate through the rhizosphere is directly related to soil moisture. Precipitation is related with the development of the specific soil structure and type. Bachar et al. (2010) discovered that bacterial diversity was independent of the precipitation gradient while studying the effect of rainfall in arid and semi-arid soils. According to the observation made by Egamberdiyeva (2007), *Bacillus*, *Mycobacterium*, and *Pseudomonas* are more effective in promoting the absorption of N, P, and K in corn plants, which are more likely to thrive in nutrient-deficient soils than nutrient-rich soils.

13.3.2.2 Soil pH

One of the most important elements deciding the composition of microbiome community is soil pH (Zhalnina et al. 2014). As soil microbes have a broad range of optimal pH tolerance, soil pH varies largely from a regional to a global scale, which can influence microbial communities. Phylum-level microbial diversity can be indicated best by pH of soil (Geyer et al. 2014). Zhang et al. (2014) reported that presence of *Acidobacteria* along an elevational gradient. When corn was grown in a low-pH, foliar lesions were greatly reduced on plants treated with a *P. fluorescens* strain producing 2,4-diacetylphloroglucinol (DAPG).

13.3.2.3 Soil Nutrients

The impact of soil nutrients and their influence on plants have been enormously studied in various parts of the world (Ryan and Sommer 2012). Nutritional factors like iron can influence the abundance of bacteria in the rhizosphere microbiome. The soil fertility is the product of diverse biotic and abiotic relationships with rhizosphere microbes playing an important role in the organic matter decomposition and making plants accessible to nutrients. Consecutively, increased plant growth lets nutrient acquisition through root exploration, letting soil microbes to bind to and occupy the roots. As a result, soil nutrients and their bioavailability influence the rhizosphere microbiome's diversity and abundance both directly and indirectly through plants (Berendsen et al. 2012). Carbon is also a vital determinant of structure and function of soil microbial community. In addition, phosphorus is another essential soil nutrient that acts as a modulator of the rhizosphere microbiome.

13.3.2.4 Effects of UV Radiation, CO_2, and Temperature

UV Radiation directly affects soil microorganisms (Formanek et al. 2014). UV-B radiation has the potential to alter pigment formation, content, and initiation of carbon assimilation in amino acid synthesis. Another identified UV defense mechanism is the pigmentation of phyllospheric bacteria due to exposure to UV-A radiation (320–400 nm). *Erwinia herbicola*'s carotenoid compounds play a significant role in cellular defense against UV-A radiation (Whipps et al. 2008). UV radiation reduces colonization of microbes and root biomass which results in decreased soil nutrients. The rate of microbial breakdown in pastures has been shown to be reduced by high CO_2 concentration in earlier studies. Increased CO_2 level reduces available N for microorganisms, which would increase plant growth, while decreasing microorganisms' degradation ability (Hu et al. 2001).

Similarly, when the air temperature rises, the soil heats up, modifying microbiome composition in the rhizosphere. Low-temperature environments are home to microorganisms that have developed to thrive under such conditions. While in high arctic with low temperature, native legumes can fix nitrogen and nodulate in comparison to those temperate climates' legumes. Microbial inoculants that promote plant growth under cold conditions are the main source of concern in horticulture and agriculture. For example, at low temperatures (4 °C), *Burkholderia phytofirmans* PsJN increased grapevine physiological activity and root growth (Barka et al. 2000).

13.4 Plant-Microbe Interactions in the Rhizosphere

Plant-microbe interactions are crucial for operation of an ecosystem and they interact continuously in order to maintain the health, development, and growth of the host plant. In some cases, such interactions are intimate and multifaceted. Microorganisms have developed two tactics, i.e., beneficial and pathogenic or detrimental interactions in order for them to acclimatize to the plant's environment and rhizosphere (Montesinos et al. 2002). Positive or negative nature of interaction is determined by the microbe's mechanism to adopt and type of microbial species associated (Nadeem et al. 2013).

13.4.1 Beneficial Interactions

In certain case, plant and rhizospheric microbes develop beneficial relations which are advantageous to the plant by enhancing plant growth and nutrient uptake along with improving its ability to resist diseases, pests, and abiotic stresses like sanility, drought, toxins, and nutrient stresses, etc. (Reid 2011; Harman and Uphoff 2019) where both the participants play a vital role in achieving such benefits. Such beneficial microbes can help in increasing the crop productivity as they can be developed into biological control agents and microbial fertilizers (Vryzas 2016).

Symbiotic and nonsymbiotic beneficial interactions caused by bacteria and fungi are given below.

13.4.1.1 Symbiotic and Nonsymbiotic Interactions

Beneficial microbe interaction also includes symbionts and nonsymbionts which defend the host against soil-borne pathogens and various other aggressions where the plants are exposed to. Nonsymbiotic soil microbes have an interdependent relationship and includes many beneficial microorganisms like plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) while common symbionts include Arbuscular mycorrhizal Fungi (AMF) and rhizobium legume association. Symbiotic plant-microbe interactions regulate the success of plants in an ecology by modifying the plant communities (Selosse et al. 2004). Microsymbionts need to first colonize the rhizosphere and invade the nearby nodule or plant roots in order to achieve the positive outcome and in return they utilize the root exudates' carbon compounds as nutrients (Tariq et al. 2017).

Arbuscular Mycorrhizal Fungi (AMF)

The arbuscular mycorrhizal fungi (AMF) symbiosis which is a mutualistic association formed between roots of 80% of terrestrial plant species and fungi (Schüßler et al. 2001), is by far the most important symbiosis on the planet. They also account for 50% of microbial biomass in soil and play a role in evolution of plants (Ryan and Graham 2002). AMF association is found in many agronomically important crops such as rice, wheat, corn, and all legumes (Wang and Qiu 2006). The rhizosphere around the AMF infection known as mycorrhizosphere stimulates and attracts plant growth-promoting rhizobacteria (PGPR) known as mycorrhiza helper bacteria (MHB) that have beneficial effect on the plant with disease suppression and growth enhancement capabilities (Heinonsalo et al. 2000; Frey-Klett et al. 2007). Chemical signaling between host plant and fungi occurs in order for the association to establish (Markmann and Parniske 2009) and allows better access of water and nutrients by the mycorrhizal roots in exchange of carbon compounds. The better uptake of nutrient like P, N, K, and trace elements by the host plants gives protective effect to the plants. They improve the plants fitness by providing resistance to biotic stress such as soil-borne pathogens and abiotic stresses like drought, heavy metals, and salinity like a prophylactic effect to the host plant (Plassard and Dell 2010; Begum et al. 2019). Changes in the metabolic and physiological processes in the plants upon colonization by AMF help to attain the above benefits (Auge et al. 2015; Fiorilli et al. 2018). They prime the plants to trigger systematic defense against belowground pathogens while mostly contrasting effects have been found for aboveground pathogens like virus (Miozzi et al. 2019). Furthermore, AMF endosymbionts play an important part in proper functioning of an ecosystem in order to enhance ecosystem stability (Gianinazzi et al. 2010; Diagne et al. 2020).

Rhizobium Bacteria

Rhizobia are gram-negative bacteria that are motile and rod shaped with subpolar flagella. They belong to the Rhizobiaceae family and form complex interaction with

leguminous plants thereby forming nodules on plant roots (Harman and Uphoff 2019). It is one of the best described endophytic and symbiotic relations without causing any symptoms of disease (Santoyo et al. 2016). Such rhizobia-colonized roots of legumes can fix nitrogen and play a vital role in agricultural production by enhancing the yield and growth of legume plants (Turan et al. 2017). Bacteria infect plant root hairs through cracks on surface and infection threads produced by the plants lead the rhizobium to plant roots through specific chemical signals called Nod factors as a result, root nodule formation and nitrogen-fixing bacteriod formation occur (Sprent and Platzmann 2001; Jones et al. 2007). The rhizobium bacteria inside the nodule convert nitrogen to ammonia thereby reducing the use and need for chemical fertilizer application (Gaurav et al. 2009). Furthermore, the phytohormones secreted by the Rhizobiaceae can act as biocontrol agents which can suppress many fungal pathogens in both leguminous and even nonleguminous plants (Dakora 2003). There are several strains of rhizobia which can induce plant disease resistance (Volpiano et al. 2019).

Plant Growth-Promoting Rhizobacteria (PGPR) and Plant Growth-Promoting Fungi (PGPF)

Beneficial free-living soil bacteria and nonpathogenic fungi that are present in the rhizosphere and also freely live on the surface or inside plant roots thereby promoting growth of the plants are termed as plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) (Herman et al. 2008; Hossain et al. 2017). *Pseudomonas* and *Bacillus* are the two major genera of rhizobacteria having plant growth-promoting capabilities (Podile and Kishore 2007) whereas, *Penicilllium*, *Phoma*, *Fusarium*, and *Trichoderma* are the varieties of PGPF that are being studied (Hyakumachi 1994). Apart from enhancing plant growth, they can effectively suppress and induce resistance against diverse plant pathogens by different mechanisms along with induction of innate immunity of plants (Murali and Amruthesh 2015). They can trigger induced systemic resistance (ISR) which is effective against foliar and soil-borne pathogens including insect herbivores (Van Wees et al. 1997). Phytohormones produced by the microorganisms, degradation of soil pollutants, and improving the nutrients uptake can directly promote the plant growth (Glick 1995). Both PGPR and PGPF are now being commercially exploited as a biocontrol agent to attain sustainable agriculture as an alternative to chemical pesticides (Verma et al. 2019). They are also vital for soil fertility as they are more versatile in mobilizing, solubilizing, and transforming nutrients present in the soil thereby helping in recycling the soil nutrients (Hayat et al. 2010; Glick 2012).

13.4.2 Pathogenic or Detrimental Interactions

Detrimental interactions such as plant-pathogen interactions are extensively studied and reduce the fitness of the host plant where the severity of damage on plants depends on host response and outcome of the interactions (Brown 2015). Pathogenic rhizospheric microbes include species belonging to oomycetes, fungi, bacteria, and

nematodes that impact the plant and soil (Binyamin et al. 2019). Soil-borne pathogens found in the rhizosphere which acts as an infection zone form a parasitic relation with the host plant and fight for sufficient microsites and nutrients with the rhizosphere microbiome (Chapelle et al. 2016). Such microbes interact negatively with plants due to their pathogenic nature and production of harmful compounds that cause detrimental effect like onset of diseases (Pamp and Nielsen 2007; Vacheron et al. 2013). The fungal and oomycetes pathogens (*Aspergillus*, *Collectrichum*, *Fusarium*, *Gaeumannomyces graminis*, *Magnaporthe oryzae*, *Phytophthora*, *Pythium*, *Verticillium*, *Rhizopus*, and *Rhizoctonia*), bacterial genera (*Agrobacterium tumefaciens*, *Dickeya dadantii*, *D. solani*, *Ralstonia solanacearum*, *Pectobacterium atrosepticum*, *P. carotovorum*, and *Streptomyces scabies*), and nematodes (*Heterodera* and *Meloidogyne*) are present in rhizosphere crops which are responsible for economic impact due to major crop losses around the world (Mansfield et al. 2012; Koberl et al. 2013; Dignam et al. 2016). Nematodes and fungi are being used by certain phytopathogenic viruses to get into the plant's rhizosphere (Rochon 2009). Fungi kill the plant by production of toxins and enzymes or by using haustoria to acquire nutrients from host whereas pathogenic bacteria produce harmful compounds such as cyanide, over production of auxin which has negative impact on plants (Lo Presti et al. 2015; Agbodjato et al. 2015). Plants on occasion interact with multiple species or gentoypes of pathogens often referred to as co-infection that leads to alteration of the course of disease and its severity and sometimes cause multiple disease outcomes (Tollenaere et al. 2016; Abdullah et al. 2017). In certain case, a pathogen infection can predispose the plant to infections by other secondary pathogens. For example, infection with phytopathogenic fungi *Fusarium verticillioides* and oomycete *Albugo candida* facilitates infection by several other avirulent and virulent pathogens by suppressing plant defense (Cooper et al. 2008; Saunders and Kohn 2008). The occurrence of opportunistic human pathogens has been documented in a range of research on the rhizosphere microbiome such as *Pseudomonas aeruginosa*, *Burkholderia cepacia* or *Stenotrophomonas maltophilia* in wild and cultivated plant species; and these pathogens are especially competitive for nutrients (Berg et al. 2005; Teplitski et al. 2009; Critzer and Doyle 2010).

13.5 Mechanisms of Rhizosphere Microbiomes in Inducing Plant Disease Resistance

Plants are subjected to various pathogenic fungal, bacterial, and viral diseases along with beneficial microorganisms and they respond to them by modulating their innate immune system thereby exhibiting suitable responses (Pieterse et al. 2014). Beneficial rhizosphere microbiome can boost plants resistance against various diseases through a variety of different mechanisms (Table 13.1) (Lugtenberg and Kamilova 2009). One of the significant approaches of plant disease control is plant disease resistance and there are different mechanisms employed by the beneficial microbes and host plants to resist the detrimental effect of the pathogens. Rhizosphere microorganism as biocontrol agent includes various beneficial PGPR, PGPF, and

Table 13.1 Mechanism of rhizospheric microbiome in inducing disease resistance and plant growth promotion

Rhizosphere microbes	Mechanism	Results	References
Enterobacter Cloacae, *P. putida*, *Serratia*, *Plymuthica*, *B. subtilis* (strain GB03), *Paenibacillus polymyxa* (BFKC01)	Siderophore	Increase metal ion assimilation in *A. thaliana* by activating iron acquisition machinery.	Saleh and Glick (2001), Ovadis et al. (2004)
P. fluorescens, *B. subtilis* GB03	Siderophore	Enhanced Fe nutrients in both Graminaceous and dicot plants	Zhang et al. (2009), Shirley et al. (2011)
Pseudomonas simiae WCS417	The activation of the marker genes IRT1 and MYB72 due to production of siderophore.	Increased the amount of Fe along with fresh weight of *A. thaliana* shoots	Verbon et al. (2019)
Burkholderia	Phosphate solubilization	Sunflower plants thrive in alkaline soils, where they dissolve $Ca_3(PO_4)_2$ and release phosphates for plant use.	Ambrosini et al. (2012)
Bacillus edaphicus	Potassium (K) solubilization	Increased K uptake to cotton and rapeseed plants grown in K-deficient soil	Sheng (2005)
Experiments performed in soil with *Sorghum vulgare* and in a growth chamber with *Triticum aestivum* and *Zea mays* with *Bacillus mucilaginosus*	K solubilization	Derived K from waste mica to plants	Basak and Biswas (2010), Singh et al. (2010)
Arthrobacter, *Burkholderia*, *Bacillus*, and *Pseudomonas Paenibacillus*	K solubilization	-	Sindhu et al. (2016), Sahu et al. (2019)
Pseudomona. putida, *P. oryzihabitans*, *Acinetobacter*, *Chryseobacterium* sp., *Calcoaceticus*, and *Pantoea brenneri*	P solubilization, production of IAA and ACC deaminase	Stressed Mediterranean habitats support local tomato cultivar and wild plant species, exhibited multiple in vitro PGP-associated traits	Leontidou et al. (2020)
Azospirillum sp.	N_2-fixation	Influenced maize growth positively	Garcia de Salamone et al. (1996)
Bradyrhizobium japonicum, *B. elkanii*	N_2-fixation	Increased soybean production	Alves et al. (2004), Torres et al. (2012)

(continued)

Table 13.1 (continued)

Rhizosphere microbes	Mechanism	Results	References
Beetroot rhizosphere protozoan species *Colpoda* sp., *Bodo* sp., *Oxytricha* sp., *Tachysoma* sp., *Vorticella* sp.	N_2-fixation	N_2 supply in rhizosphere soil during the initial growth of beetroot	Zheng et al. (2020)
B. subtilis RB14	Production of Antibiotics such as iturin and surfactin	Tomato plants defense against *R. solani*	Asaka and Shoda (1996)
PCL1612 strains of *B. subtilis* obtained from rhizosphere of healthy avocado	Iturin A antibiotic	Control *Rosellinia necatrix* and *F. oxysporum*	Cazorla et al. (2007)
Streptomyces sp. strain 385 and *Paenibacillus* sp. strain 300	β-1,3-glucanase	Cucumber wilt (*F. oxysporum* f. sp. *cucumerinum*) cell wall lysis	Singh et al. (1999)
P. fluorescens, *P. putida*, *Bulkholderia multivorans*, *Mezorhizobium ciceri*	Production of Protease, cellulase, chitinase, and siderophores	Effective against Ascochyta blight of chickpea, *Ascochyta rabiei*	Azizpour and Rouhrazi (2016)
Bacillus, *Lysinibacillus*, *Viridibacillus*, *Serratia*, *Klebsiella*, *Rahnella*, *Enterobacter*, *Raoultella*, and *Pseudomonas* from rhizosphere of Fe Quadrangle, Brazil	N_2-fixation, IAA, siderophore, Ammonium ion, HCN, cellulase, and protease production	Inhibit the growth of enteropathogens, *Staphylococus aureus*, *Klebisiella pneumonia*, *Shiguella flexneri*, and *Fusarium*	Felestrino et al. (2017)
P. fluorescens	ISR	ISR was triggered response to sugarcane red rot caused by *Colletotrichum falcatum*	Viswanathan and Samiyappan (2002)
Co-cultured grapevine with *Burkholderia phytofirmans* PsJN	ISR	Resistance to grapevine gray mold (*B. cinerea*), an in vitro enhancement of growth	Ait Barka et al. (2000)
B. pumilus SE-34, *B. amyloliquefaciens* 937b	ISR	Defense against *Tomato mottle virus* (TMV)	Sahoo et al. (2013)
Inoculation of *Nicotiana tabacum* cv. White burley leaves with *Peanibacillus lentimorbus* B-30488 in the soil	PR (pathogenesis-related)-gene expression, stress, and antioxidant enzyme production are all on the rise	Provide resistance against the *Cucumber mosaic Virus* in *N. tabacum* cv. White burley leaves by 91%. Also boost seeds and flowers production	Kumar et al. (2016)

(continued)

Table 13.1 (continued)

Rhizosphere microbes	Mechanism	Results	References
		along with plants physiology and health	
Enterobacter asburiae BQ9	ISR by expressing phenylalanine ammonia lyase, peroxidase, catalase, and superoxide dismutase	Induced resistance against *Tomato yellow leaf curl virus*	Li et al. (2016)
Streptomycetes cacaoi strain M-20 obtained from rhizosphere of *Avicennia marina*	ISR	Against phytopathogen, *F. oxysporum*	Janaki (2017)
Defense activation in *A. thaliana* due to attack of foliar by downy mildew pathogen (*Hyatonospora arabidopsidis*) specifically promotes *Xanthomonas*, *Stenotrophomonas*, and *Microbacterium* in the rhizosphere	ISR	Systemically enhanced the level of protection against downy mildew pathogen *H. arabidopsidis* in *A. thaliana*	Berendsen et al. (2018)
Yeast *S. cerevisiae* strains, *Rhodosporidium paludigenum*	ISR by increase in expression of PR genes and production of β-1,3-glucanase and chitinase	*Colletotrichum gloeosporioides* in grape planting and *Penicillium expansum* in pear fruits	Liu et al. (2018), Sun et al. (2018)
B. cereus, *B. subtilis*, *Paenibacillus* spp., *Providencia rettgeri*, *Providencia vermicola*	Disease suppression	Positive effects on the germination of the plants, promoted plant growth and suppressed bacterial wilt disease of potato, *R. solanacearum*	Chamedjeu et al. (2019)
Trichoderma strain isolated from a healthy rye rhizosphere	Auxin, gibberellins, and ACC deaminase	Inhibit the growth of *Fusarium* spp. and enhanced stem growth in wheat seedlings	Jaroszuk-Scisel et al. (2019)
Symbioses between *Glomus mosseae* and *R. leguminosarum*	BCAs	Clover damage caused by root hemiparasitic *Pedicularis* species was lessened	Sui et al. (2019)
Rhizobium etli	ISR	Reduce the lesions and colony-forming units in common beans caused by *Pseudomonas syringae* pv. *Phaseolicola* along with accumulation	Díaz-Valle and Alvarez-Venegas (2019)

(continued)

Table 13.1 (continued)

Rhizosphere microbes	Mechanism	Results	References
		superoxide anion (O_2^-) an callose deposition	
AMF, *G. deserticola* Trappe, Bloss & Menge, and *Glomus clarum* Nicol. & Sch.	Disease suppression	Antagonistic/control of *Fusarium napiforme* causing ear rot of maize	Olowe et al. (2020)
G. mosseae and *G. clarum*	BCAs	Reduce Damping-off disease (*R. solani*) severity on cucumber	Aljawasim et al. 2020

AMF that have been studied by several researchers in the last 30 years (Chandra and Singh 2016; Buttimer et al. 2017; Gramisci et al. 2018; Tian et al. 2019a, b). Beneficial microbes like AMF use mechanisms like competition, better uptake of nutrients, change in plants' chemical constituents, mycorrhizal-induced resistance, etc. (Huang et al. 2003) whereas PGPR, PGPF, and Rhizobia employ mechanism such as siderophores, antibiotics, lytic enzymes production, competition, mycoparasitism, and ISR to attain disease resistance (Compant et al. 2005). The plants' innate immunity averts growth of detrimental microbes whereas for beneficial microbes they make friendly interactions which subsequently boost plant immunity. Plant perceives the pathogen and triggers microbial effector-triggered immunity and molecular pattern-triggered immunity which induce numerous defense mechanisms to suppress the attack (Nishad et al. 2020).

13.5.1 Arbuscular Mycorrhizal Fungi (AMF)

13.5.1.1 Enhanced Nutrient Uptake and Morphological Alteration in the Root System

Enhanced nutrient uptake and increased biomass due to AMF colonization of host plant roots result in improved growth of the plant making them more tolerant or resistant to pathogen attack and drain host plant's carbon to the pathogen (White and Torres 2009; Singh and Singh 2017). The AMF hyphal network formed on the plant roots gives better access to nutrients like P, Mn, Ca, Cu, and Zn which get transported to the plant and also improve soil quality hence better plant health (Harrier and Watson 2004; Rouphael et al. 2015; Thirkell et al. 2017). Enhanced nutrient uptake is not the only sole factor contributing to enhanced disease resistance as nutritional and hormonal changes in AMF root also compensate further protection. AMF colonization changes the root morphology which could modify the development pattern of root diseases (Tahat et al. 2008). Morphological alterations due to lignification in the AMF-colonized root cells may slow down the infection and protect roots from penetration by pathogens like Fungi (Dugassa et al. 1996). But no clear correlation between changes in architecture and morphology of roots and bioprotection effect of mycorrhiza has been studied or found.

13.5.1.2 Competition

AMF and root pathogens compete for nutrients, infection sites, and carbon compound, i.e., photosynthate received by the roots (Smith and Read 2008a, b; White and Torres 2009) which may lead to the suppression of soil pathogen in mycorrhizal plants. In certain case, physical competition between fungal pathogens and AMF results in physical exclusion of the pathogens by preventing them from colonizing the mycorrhizal roots. There are also reports of arbuscules produced by AMF in mycorrhizal plants lowering the pathogens' infection sites (Vigo et al. 2000). Higher carbon availability in mycorrhizal plants due to AMF having primary access to the photosynthates could explain the pathogen inhibitory effect of AMF-colonized plants (Linderman 1994) as less carbon will be available for the pathogen (Xavier and Boyetchko 2004). But there is not enough evidence to prove the bioprotection ability of mycorrhizal plants by carbon competition.

13.5.1.3 Alteration in Chemical Constituents of Plant Tissues

Change in the plant roots due to mycorrhization can alter the composition of plant root exudates thereby resulting in inhibitory compound production and reducing the stimulatory compounds level (White and Torres 2009). Phytoalexins and wall-bound peroxidase are detected during later and early stages of AMF colonization (Azcon-Aguilar et al. 2002). Root exudates like strigolactones help in establishment of AMF symbiosis by inducing hyphal branching and enhanced root colonization by AMF (Vierheilig et al. 2003; Besserer et al. 2006). Germination and sporulation of pathogens like *Fusarium oxysporum* f. sp., *lycopersici*, and *Phytophthora fragariae* were suppressed by the exudates from roots (Norman and Hooker 2000; Scheffknecht et al. 2006). Morandi (1996) reported that AMF symbioses result in production of proteins that are involved in plant defense reactions like production of chitinase, β-1, phenolics peroxidases, 3-glucanase, pathogenesis-related proteins which also inhibit pathogens.

Mycorrhizal-Induced Resistance

Plants colonization by AMF leads to strengthening of plants defense against disease termed as mycorrhizal-induced resistance (MIR) that shares characteristics with induced systemic resistance (ISR) caused by nonpathogenic rhizobacterial colonization and systemic acquired resistance (SAR) due pathogen infection (Cameron et al. 2013). MIR gives systemic defense against an extensive variety of threats like biotrophic, necrotrophic pathogens, herbivorous arthropods, and nematodes.

Root exudate identified as strigolactones, a class of terpenoid lactones serve as a signal to recruit AMF which stimulates hyphal branching leading to facilitate infection and enhanced root colonization (Linderman 1988). Due to the AMF colonization of roots, plant immunity recognizes the microbial signature compound referred to as microbe-associated molecular patterns (MAMPs) by pattern-recognition receptors leading to series of signaling cascade resulting in MAMP-triggered immunity (MTI) and increased production of Salicylic acid (Zhang and Zhou 2010). MIR suppresses SA-dependent defense while biosynthesis and systemic priming of JA-dependent defenses increase. AMF symbiosis is established in

plant cells by perception of mycorrhizal Myc factors which subsequently counteracts MTI (Zamioudis and Pieterse 2012). The Myc factors trigger cytosolic calcium (Ca) to induce Mitogen-activated protein kinase (MAPK), ROS (Reactive oxygen species) generation, and alterations in G-protein. These ROS lead to biosynthesis of JA by inducing lipoxygenases while the MAPK and G-protein induce the production of defense genes of plant. These defense genes along with ROS and antioxidant enzymes attack the incoming pathogens and its infection sites leading to hypersensitivity reaction and inhibiting the pathogen (Khan et al. 2010). These defense signals prime the mycorrhizal plants making them response to pathogen attack much efficiently.

13.5.2 Rhizobium Bacteria

13.5.2.1 Mycoparasitism

Rhizobia present in the rhizosphere can parasitize and inhibit the fungal pathogens' growth by coiling the fungals hyphal tip, inhibiting the reproductive structures such as sclerotia or zoospores (Sharif et al. 2003). Rhizobia can reduce the fungal pathogens mycelial dry weight in in vitro condition (Chao 1990). Malajczuk et al. (1984) also found that the inhibition of zoospores of *Phytophthora cinnamoni* gives protection to the host plant. There are also reports of inhibition of growth of *Sclerotium rolfsii* by *Bradyrhizobium* (Yaqub and Shahzad 2011; Ghasemi et al. 2017).

13.5.2.2 Antibiotic Production

Antifungal antibiotic production by any beneficial microbes is among the different mechanisms involved in pathogen inhibition. Different rhizobia are able to produce different antibiotics that have negative impact on the attacking pathogens (Bardin et al. 2004; Chandra et al. 2007). Rhizobia like *Rhizobium leguminosarum* and *R. leguminosarum* bv. *trifolii* can produce the antibiotic bacteriocins and trifolitoxin (TFX), respectively (Robleto et al. 1998). Rhizobitoxine produced by *Bradyrhizobium* strains inhibits *Macrophomina phaseolina* infection in groundnut and soybean (Deshwal et al. 2003). *Rhizobium leguminosarum* bv. *viciae*, *R. meliloti*, and *Bradyrhizobium japonicum* can also produce antibiotics having inhibitory effect against many phytopathogens (Hafeez et al. 2005; Gopalakrishnan et al. 2015).

13.5.2.3 Siderophore Production

Some Rhizobia can produce siderophores, an iron-chelating compounds, that can limit the iron availability for pathogenic fungi (Martínez-Viveros et al. 2010; Datta and Chakrabartty 2014) which can subsequently inhibit the pathogens growth in both in vitro and in vivo situations (Chandra et al. 2007). Siderophore affects the proteins activity in plants specially causing metalloprotein modification, thereby activation of plant immune response if metalloprotein is shielded by a nucleotide-binding leucine-rich repeat (NB-LRR) (Aznar and Dellagi 2015). There are many

reports of production of several types of siderophores by rhizobia such as rhizobactin, trihydroxamate, vicibactin, citrate type, phenolate type, catechol type, anthranilic acid, cyclic, and dihydroxamate type siderophores (Das et al. 2017). Siderophore produced by rhizobia can inhibit pathogens like *M. phaseolina* and *Sclerotinia sclerotiorum* (Deshwal et al. 2003; Chandra et al. 2007).

13.5.2.4 Hydrolytic Enzyme Production

Rhizobia also secrete hydrolytic enzymes that antagonize fungal pathogens by causing lysis of fungal cell wall. They can produce both Chitinases and β-1,3-glucanases. Chitinase produced by *Rhizobium* sp. can inhibit pathogens such as *F. udum*, *F. oxysporum*, *Pythium* sp., *M. phaseolina*, *Curvularia lunata*, *Aspergillus niger*, *A. flavus,* and *S. sclerotiorum* (Sridevi and Mallaiah 2008; Mazen et al. 2008; Smitha and Singh 2014). β-1,3-glucanases secreted by *R. leguminosarum* can antagonize *F. oxysporum* by causing perforation, lysis, and degradation of hyphae (Kumar et al. 2011a).

13.5.2.5 Induced Systemic Resistance (ISR)

Many Rhizobia species such as *R. leguminosarum* bv. *Trifolii*, *R. leguminosarum* bv. *Phaseoli*, and *R. etli* can trigger Induced Systemic Resistance (ISR) in plants to provide protection against diverse pathogens (Patil et al. 2017). Plant defense enzymes such as phenolics, phytoalexins, and flavonoids are generated by Rhizobia species to trigger the plant's defense response when it is invaded by a pathogen; and rhizobiums' cellular components such as flagella, lipopolysaccharides, acetoin, homoserine lactones, and butanediol can also trigger ISR (Lugtenberg and Kamilova 2009; Das et al. 2017). Flagellin protein, a kind of microbe-associated molecular patterns (MAMP) produced by Rhizobium is recognized by plants pattern-recognition receptors (PRR) that subsequently trigger MAMP-triggered immunity (MTI)-causing jasmonic acid (JA) and ethylene (ET)-dependent defenses (Nishad et al. 2020). Exopolysaccharide (EPS) and Type III effectors secreted through the type III secretion system suppress MTI (zamioudis and Pieterse 2012). Rhizobial nodulation (Nod) factors that stimulate the legume rhizobium symbiosis also partly suppress MTI (Liang et al. 2013). The jasmonic acid (JA) and ethylene (ET)-dependent defenses play a vital role in ISR induced by rhizobium. ISR in rhizobial symbiosis also regulates the colonization density of the symbionts (Pieterse et al. 2012).

13.5.3 Plant Growth-Promoting Rhizobacteria (PGPR) and Plant Growth-Promoting Fungi (PGPF)

13.5.3.1 Competition

PGPR and PGPF compete with pathogens for nutrient, colonization on roots, root exudates utilization, etc (Duffy 2001). PGPR and PGPF in rhizosphere rapidly colonize the plant surface and compete for nutrients with pathogens by consuming all the substrates so that pathogens will not be able to utilize any of the available

substrate resulting in growth inhibition of the pathogen while stimulating the growth of the plants (Kundan et al. 2015). PGPF competes for infection sites on plant root whereas bacterial determinants and root niches competition are involved in successful root colonization by PGPR (Hossain and Sultana 2020; Compant et al. 2005). Root exudates secreted by plants to draw microbes can also have antimicrobial properties where the organisms having enzymatic activity to detoxify them (Bais et al. 2004) and PGPR can sense such exudates much easily than non-PGPR (Bacilio-Jiménez et al. 2003). The type IV pilli and lipopolysaccharides (LPS) particularly the O-antigen chain help in colonization of plant roots (Dekkers et al. 1998).

13.5.3.2 Mycoparasitism

Mycoparasitism is on the mechanism used specially by PGPF in order to antagonize the pathogen by recognition of the host by the mycoparasite, direct growth of the PGPF mycelium on the pathogen followed by coiling and dissolving cell wall of pathogen by hydrolytic enzymes (Woo and Lorito 2007). Mycoparasitism caused *Trichoderma harzianum* and *Sphaerodes mycoparasitica* prevent the root growth reduction and colonization by the pathogen (Vujanovic and Goh 2012). *Trichoderma*, *Penicillium* use this mechanism to kill phytopathogens such as *Rhizoctonia solani* (Nicoletti et al. 2006; Almeida et al. 2007). Specially, mycoparasitic activity of *Trichoderma* sp. was reported by various researchers against wide range of plant pathogenic fungi (Qualhato et al. 2013). *Trichoderma atroviride* uses MAPK and G-protein in order to trigger mycoparasitic response (Zeilinger 2017). Trichoderma produces chitinase that starts to degrade the cell walls of the fungal pathogen subsequently releasing oligomers that prompt the production of exochitinase and the attack starts (Gajera et al. 2013).

13.5.3.3 Antibiosis

Antibiotic produced by PGPR and PGPF can effectively suppress plant pathogenic microorganisms by having direct effect on the pathogens growth (Keswani et al. 2017). Based on their concentration, antibiotics can prevent pathogens development by supressing those enzymes involved in metabolism of nucleic acid, biosynthesis of cell wall, and repair or impair protein synthesis and membrane structure. BCAs such as *Trichoderma*, *Bacillus*, and *Pseudomonas* produce several types of antibiotics (Handelsman and Stabb 1996; Kumar et al. 2011). For instance, *Pseudomonas* sp. with antimicrobial activity, such as hydrogen cyanide (HCN), phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyoluteorin (Plt), pyrrolnitrin (Prn), pyoluteorin (Plt), and protein-type compounds (bacteriocins) and their effect on pathogen suppression are well reported (Haas and Keel 2003; Validov et al. 2005). Lipopeptides such as surfactin, iturin, and fengycin are an important types of antimicrobials antibiotics. Cazorla et al. (2007) found that iturin A secreted by *B. subtilis* strains PCL1612 recovered from rhizospheric soils of avocado could inhibit *F. oxysporum* and *Rosellinia necatrix* pathogens. Surfactin produced by *Bacillus mojavensis* RRC101 strain can lead to indirect antagonism of *Fusarium verticillioides* by triggering immune responses of the host plant, whereas fengycin is

also effective in inhibiting *Fusarium verticillioides* (Blacutt et al. 2016). Broad-spectrum activities were reported among the antimicrobial compound halocin and produced bacteria and archaea which can be exploited for biocontrol activities against soil-borne phytopathogens, as these antimicrobial agents can be ideal in extreme environments (Atanasova et al. 2013). Gliotoxin produced by *Trichoderma virens* can suppress *R. solani* (Wilhite et al. 2001). *Trichoderma* can also produce gliovirin and viridin with antimicrobial activity (Ghorbanpour et al. 2018). The capability to produce multiple antibiotics can enhance plant disease resistance by suppressing deleterious microbial competitors.

13.5.3.4 Lytic Enzyme Production

Lytic enzymes are mostly produced by PGPR to inhibit phytopathogens growth and it is a vital mechanism to induce plant disease resistance. Cellulases, lipases, chitinases, protease, and β-1-3 glucanases are amongst the enzymes produced by PGPR (Markowich and Kononova 2003). Such enzyme causes lysis and degradation of fungal plant pathogens cell wall components thereby suppressing the pathogen (Maksimov et al. 2011). In certain case, the lytic enzyme producing PGPR can hamper spore germination and destroy the oospores of phytopathogenic fungi (Frankowski et al. 2001). Lytic enzymes like protease, laminarase, and cellulose released by *B. subtilis* strain 330-2 can prevent the growth of *R. solani* thereby also degrading their cell wall (Ahmad et al. 2017). Chitinase and β-1,3-glucanase produced by PGPR can suppress *Fusarium* sp., *Cladosporium werneckii*, and *Colletotrichum gloeosporioides* (Vivekananthan et al. 2004; Compant et al. 2010). *Trichoderma* species use chitinases to antagonize *Rosellinia necatrix* (Hoopen and Krauss 2006).

13.5.3.5 Siderophore Production

Microorganisms produce siderophore which has strong affinity with Fe^{3+} ions (Sureshbabu et al. 2016). PGPR can acquire both rhizospheric metals iron and radioactive iron at even low concentration (Dimkpa et al. 2009). Such mechanism of PGPR can boost the persistence of beneficial bacterium, reduce competition for nutrient, and subsequently suppress pathogens in the rhizosphere. Siderophore produced by pathogenic fungi has low affinity with Fe, so it deprives them from this essential element whereas some PGPR can even take iron from different siderophore-producing microorganisms (Lodewyckx et al. 2002). Siderophore also enhances the growth of the plants and its adaptive capacity in stress condition. *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Rhodococcus*, i.e., both gram-positive and -negative bacteria can produce siderophore (Tian et al. 2009). Suppression of soil-borne and plant root pathogens by siderophore-producing PGPR has been reported (Dey et al. 2004). Siderophores produced by *B. subtilis*, *A. niger*, *P. citrinum*, and *T. harzianum* are effective BCAs of *Fusarium* wilt of pepper caused by *F. oxysporum* and also boost chickpeas growth (Yadav et al. 2011; Yu et al. 2011). To prevent the growth of *F. oxysporum* f. sp. *niveum*, *B. amyloliquefaciens* L3 strain is able to produce antifungal compounds, viz., 2-heptanone and 2-nonanone (Wu et al. 2019).

13.5.3.6 Induced Systemic Resistance

Several plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) strains can induce ISR in plants. Such defense is activated when the plant is attacked by pathogenic agent which makes the plant highly adapted and stronger species (Van Loon 2007). A signal transduction pathway for ISR is stimulated by ethylene and jasmonate signaling within the plant which provides defense against wide range of attackers such as pathogenic, bacteria, fungi, virus, and herbivorous insects (Verhagen et al. 2004) but independent of SA.

Pattern-recognition receptors (PRRs) of plant immunity recognizes specific inducers such as fungal chitin or bacterial flagellin from the beneficial PGPF and PGPR called as pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) (Boller and Felix 2009) thereby triggering the defense PAMP-triggered immunity (PTI), pathogens or beneficial microbes bypass this defense by using bacterial effector molecules to suppress PTI. The plants again attain a second line of defense that recognizes the specific effector proteins that cause effector-triggered immunity (ETI) leading to programmed cell death (Dodds and Rathjen 2010). ETI and PTI trigger an enhanced defensive capacity, i.e., induced resistance in healthy plant parts (Shah and Zeier 2013). There is a long list of PGPR and PGPF such as *Pseudomonas* sp., *Bacillus pumilus*, Enterobacteria, *Penicillium*, *Fusarium*, *Trichoderma*, *Pythium*, and also nonsporulating fungi which can elicit ISR and protect the plant from deleterious organisms (Jourdan et al. 2009; Hossain and Sultana 2020).

13.6 Mechanisms of Rhizosphere Microbiomes in Enhancement of Plant Growth

Rhizosphere microorganisms' ability to promote plant growth can be due to the provision of important plant nutrients that are scarce in the soil. Table 13.1 shows some mechanisms used by beneficial rhizosphere microbes to enhance plant growth. The main mechanisms to improve nutrient uptake are nitrogen (N_2) fixation, iron (Fe) binding through siderophore production, and phosphate (P) and zinc (Zn) solubilization (Richardson 2001; Miransari 2011).

13.6.1 Rhizosphere Microorganisms Promote Uptake of Mineral Nutrients

In sustainable agriculture, biological nitrogen fixation (BNF) by rhizobia-legume symbiosis can be used to replace chemical N_2 fertilizer (Galloway et al. 2004; Hunter 2016). Rhizobia inoculants that are derived from N_2-fixing rhizobacteria (*Azospirillum*, *Azotobacter*, *Achromobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, and *Pseudomonas*) have a beneficial effect on crop plants by improving both below and aboveground biomass (Igiehon and Babalola 2018; Miao et al. 2018; Do Nascimento et al. 2019). For example, rhizobacteria *Pseudomonas*

protegens Pf-5 X940, *P. stutzeri* A1501, and *B. japonicum* promote the available N_2 content and improve wheat and soybean production (Fox et al. 2016; Ronner et al. 2016).

P is another essential nutrition resource in agriculture. Bacterial and fungal genera such as *Azospirillum*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Erwinia*, *Enterobacter*, *Flavobacterium*, *Micrococcus*, *Microbacterium*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Serratia*, *Aspergillus*, *Chaetomium*, *Cephalosporium*, *Fusarium*, *Penicillium*, and *Sclerotium* are the most common phosphate-solubilizing microoganisms (Mehnaz and Lazarovits 2006; Pindi and Satyanarayana 2012; Sharma et al. 2013; Bakhshandeh et al. 2015). Bautista-Cruz et al. (2019) reported that phosphate-solubilizing bacteria, *Pseudomonas luteola* and *Bacillus* sp. have a synergistic impact on the growth promotion of *Agave angustifolia*. P absorption through AMF is influenced by a number of factors, including fungal species and plant root morphology (Smith and Read 2008a, b). Yadav et al. (2015) identified P-solubilizing haloarchaea from the rhizospheric soils of Gujarat, India belonging to the genera *Haloarcula*, *Halococcus*, *Halobacterium*, *Haloferax*, *Halolamina*, *Halostagnicola*, *Halosarcina*, *Haloterrigena*, *Natrialba*, *Natrinema*, and *Natronoarchaeum*.

Microorganisms in the rhizosphere also assist in the absorption of trace elements like Zn and Fe, which are essential for the growth and proliferation of plants. Fluorescent pseudomonads have been known to stimulate Fe nutrition in graminaceous and dicotyledonous plant species through siderophores (Shirley et al. 2011). By forming the biofilm or colonizing on cucumber roots, *Bacillus* spp. improve Fe acquisition (Xu et al. 2019). Rhizosphere microbes can both compete with plants for nutrients and also bear traits that help plants flourish. *Plantibacter*, *Curtobacterium*, *Stenotrophomonas*, *Pseudomonas*, and *Streptomyces* are all rhizosphere microorganisms known to mobilize Zn via the production of gluconic acid (Costerousse et al. 2018). Fungi such as *Pleurotus ostreatus*, *T. harzianum*, *Polyporus ostriformis*, and *Phanerochaete chrysosporium*, as well as bacteria such as *Chryseobacterium gleum*, *Sporocytophaga*, *Cellulomonas*, *Streptomyces*, and *Pseudomonas* have been found to digest biomass of plant, as a result nutrients are released for plant nutrition as well as their own (Mendes et al. 2013; Woo et al. 2014; Ahmed et al. 2018).

13.6.2 Secondary Metabolites and Phytohormone Production

Phytohormones synthesized by microorganisms are a valuable tool for altering physiology of plants resulting in plant growth enhancement to pathogenesis (Spaepen 2014). Phytohormones including indole acetic acid (IAA), auxins, gibberellins, and cytokinins are mainly produced as secondary metabolites because they are not needed for microorganism reproduction and growth (Shi et al. 2017). Stem elongation, fruit formation, seed germination, and sex expression are all influenced by Gibberellins (Bomke and Tudzynski 2009). Several bacterial and fungal genera, viz., *Bacillus*, *Herbaspirillum seropedicae*, *Azospirillum*, *Rhizobium*,

F. moniliforme, and *Acetobacter diazotrophicus* can produce gibberellin-like substances (Bottini et al. 2004; Meleigy and Khalaf 2009). *Aspergillus*, *Botrytis*, *Agrobacterium*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Rhizobium*, and *Rhizopus* are some fungi and bacteria which can synthesis auxin (Costacurta and Vanderleyden 1995; Hui et al. 2007). Many rhizospheric microbes can also synthesis 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Glick 1995; Belimov et al. 2001). VOCs are other major metabolites produced by rhizosphere microorganisms such as *B. cepacia*, *B. subtilis*, *S. maltophilia*, *P. fluorescens*, *P. trivialis*, and *S. plymuthica*, which can assist in interaction between the rhizobiome and host plants along with growth promotion capabilities (Saraf et al. 2014; Ali et al. 2015).

13.7 Rhizosphere Microorganisms Enhanced Abiotic Stresses Tolerance

Abiotic stress reponse can be induced by microbes which is referred to as induced systemic tolerance (IST). Plant abiotic stresses are mitigated by rhizosphere microorganisms, which have inherent genetic and metabolic abilities (Gopalakrishnan et al. 2015). Some genera of bacteria and fungi like *Achromobacter*, *Bacillus*, *Burkholderia Azotobacter*, *Enterobacter*, *Pseudomonas*, *Trichoderma*, *Rhizobium*, *and Methylobacterium* can promote plant growth through reducing several abiotic stresses (Atieno et al. 2012; Sorty et al. 2016; Meena et al. 2017). Phytohormonal substances produced by rhizosphere microorganisms regulate several physiological processes in plants to improve resistance and stress tolerance to detrimental situations (Glick et al. 2007). Under drought stress, IAA obtained from *Azospirillum* sp. induced growth of roots along with the lateral root development in wheat, which can increase nutrient and water absorption (Arzanesh et al. 2011). Similarly, IAA developed by *Bacillus* sp. SR-2-1 raises tolerance of potato to salt and tuber weight by having favorable impact of Na^+/K^+ efflux regulations (Tahir et al. 2019). Exopolysaccharides developed *Bacillus endophyticus* J13 and *P. aeruginosa* ZNP1 avoid osmotic stress in *A. thaliana* seedlings by increasing the water content (Ghosh et al. 2019). Collectively, microorganisms produced diversified compounds which are complex in nature. As a result, other beneficial and effective compounds generated by beneficial microbes, which form plant-associated microbiomes and improve plant health, need to be investigated. Plants can enhance the microbial community in the rhizosphere, boost the soil chemical and physical properties, and increase interaction between soil pollutants and microbes, leading to microbial bioremediation (Kuiper et al. 2004).

13.8 Tools/Techniques Employed to Understand the Plant-Microbe Interactions

13.8.1 Omics Technologies

Understanding the relationship of plants with the soil microbiota can be achieved with the omics technologies in the current scenario globally. These new approaches are able to explore contents of cause besides identify the structure and its function at gene level (Sarim et al. 2020; Sahu et al. 2020). Genomics, Metabolomics, Transcriptomics, Proteomics, Fluxomics, and Epigenomics are such new next-generation strategies that aid in unraveling the plant-microbe interactions and could explain an array of interactions (Singh et al. 2019).

Transcriptomic analysis of plant-associated bacteria using RNA sequencing (RNA-seq) technology, or gene expression microarray approaches, reveals genes that are differentially expressed under certain conditions. Transcriptome analysis paves a diverse opportunity and enhances the chance of exploring challenges on plant immunity in molecular level. Proteomics and metaproteomics approaches, mostly based on liquid chromatography-tandem mass spectrometry technology, reveal the diversity of bacterial proteins within an environment in a semi-quantitative manner (AbuQamar et al. 2016). Proteomics approaches provide a more precise snapshot of the active pathways within a sample. It can detect proteins differentially secreted by plant growth-promoting bacterial (PGPB) strains in response to root exudates (Kierul et al. 2015). For better diagnosing and characterizing of plant, human diseases, and their etiological agents, metabolomic methods have been widely used. They are used in agro-industrial investigation that emphasizes on tripartite interactions (plant—toxicogenic microbe—beneficial microbe). This novel technology supports well to identify specific metabolites and their role in plant-microbe interactions (Hacquard et al. 2017). Metagenomics is often used to discover novel antibiotic biosynthesis or resistance genes within soil metagenomes. Metagenome sequencing projects revealed genes that are enriched in the endosphere and rhizospheres of different plants, elucidated genes that are correlated with biocontrol activity, and even led to the discovery of novel metabolic enzymes. Epigenomics or Epigenetics explains the alteration in morphological or molecular phenotype of an organism without the change in the nucleotide sequence (Kalavacharla et al. 2014). Fluxomics refers to the study of total set of fluxes in a cell metabolic network (Cascante and Silvia 2008). The flux analysis captures and measures all metabolites in a biological sample and its functional interactions.

13.8.2 Sequencing Technique

High-throughput sequencing of marker gene amplicons is gradually being used in plant microbiome research to better understand the organization, spatial distribution, and structure of microbial diversities in the ecosystem (Knief et al. 2012). On the other hand, amplicon sequencing has the benefit of allowing it to target single classes

of microbes (e.g., Bacteria, Archaea) or even functional genes along with being highly specific (Herbold et al. 2015). Since field samples from plants contain a complex community of microbes, sequencing alone cannot reveal which subset of pathogen is responsible for pathogenesis. Biological knowledge will be essential in interpreting the data (Studholme et al. 2011).

13.8.3 Chromatography, Mass Spectrometry, Nuclear Magnetic Resonance

The advancement of medicinal studies of compounds of interest in the 1970s using gas chromatography-mass spectrometry, layed the groundwork for metabolomics (Sumner et al. 2003). The separation, detection, and identification of metabolites are the core parts of metabolomics research (Chen et al. 2019). Current metabolomics technology in plant-microbe interaction studies.

Nuclear Magnetic Resonance (NMR) spectroscopy or high-resolution mass spectrometry combined with ultra-high-pressure liquid chromatography which can collect and profile information on thousands of substances thereby offering unparalleled insight into the chemical phenotypes of sample species of organisms (Ward et al. 2007; De Vos et al. 2007). Development in gas chromatography combined with mass spectrometry enabled for more in-depth profiling of gaseous volatile compounds, resulting in the field of volatilomics emerging out of metabolomics (Cumeras and Correig 2018).

13.8.4 Phospholipid Fatty Acid (PLFA) Analysis

It is one of the techniques for evaluating microbial communities that does not focus on culturing microorganisms. It is commonly used to measure significant alterations in the makeup of the soil microbial population and to estimate overall microbial biomass (Buyer et al. 2010). In order to identify microorganisms, PLFA research uses phospholipids, which are the main lipids that make up cellular membranes (Hinojosa et al. 2010). To determine the individual microbes' types and quantities, PLFAs are converted to fatty acid methyl esters (FAMEs), and then subsequently processed using gas chromatography (GC) (Buyer and Sasser 2012).

13.8.5 Microscopy

Recent advances in live microscopy, such as the invention of fluorescent dyes, along with spectral analytical and microfluidics techniques and, have helped in the microscopic characterization of plant-microbe interactions. Live imaging of fluorescently labeled microbial strains can be used to study plant-microbe relationships on a microscale. Studies using this method have revealed chemotactic accumulation of microbes to plant tissue, such as with a *Bacillus subtilis* strain colonizing

Arabidopsis thaliana roots within a few hours (Allard-Massicotte et al. 2016). High-resolution techniques such as electron microscopy, fluorescence microscopy, and confocal microscopy, along with photoswitchable fluorophores for single-molecule localization microscopy (SMLN) and fluorescence in situ hybridization (FISH) were used to visualize the live or fixed microbe samples from the environment (Coltharp and Xiao 2012).

13.8.6 qPCR

Real-time polymerase chain reaction, also called quantitative real-time polymerase chain reaction is a laboratory technique based on the PCR. It is a more advanced version of traditional PCR that takes the benefit of technological advances to offer new innovative prospects for checking the amplification process and tracking product accumulation (Paplomatas 2006). There are a variety of methods that can be used to monitor the development of a PCR. Each technique involves the use of a fluorescent marker that binds to DNA.

13.9 Future Prospects

Current knowledge on rhizosphere microbiome and its complex interactions with plant is still at its initial stage. Better understanding of the microorganisms found in rhizosphere is vital to maintain plant health and productivity. So, the following future research can be taken into account on rhizosphere microbiome:

1. Uncovering other unknown beneficial soil microbes that have the potential to improve crop protection so that it can be utilized as a bioinoculant to control plant diseases.
2. In depth study of rhizosphere microbiome functions and their constituents found at different developmental stages of plant growth is required.
3. High-throughput and next-generation sequencing approaches can help to unravel the rhizosphere microbiome interactions.
4. And lastly, further study on the precise mechanism of rhizosphere microbiome colonization and their assembly will help in the use of beneficial microbiomes in development of agriculture.

13.10 Conclusion

Rhizosphere, rhizosphere microbes associated with plants have a long history dating back to 1904 as defined by Lorenz Hiltner, and still many more facets of the subject need proper understanding. Rhizosphere microbiome is important for agroecosystem processes along with positive effects on plant health such as disease resistance, growth enhancement, and abiotic stress tolerance. Microbiome is an outcome of

cooperative interactions between microorganisms, plant, and the biotic and abiotic factors occurring in hierarchical order. Such microbes can be beneficial or harmful to the plants while in some cases they develop symbiotic relation with their host. The plant rhizosphere is an area with great diversity of microorganisms mostly bacteria and fungi having an enormous potential for application in crop protection and improvement but there are fair share of challenges that need to be resolved. Studying the beneficial interactions will have benefit in agriculture practice by increasing fitness of plants by avoiding the use of chemical pesticides. Interactions between microbes and plants have been studied for AMF, rhizobium nodule, and PGPR and PGPF; however, there is limited knowledge about other rhizosphere microbes on plant health. More research on rhizosphere microbiome and plant-microbes interactions can be useful in protecting the plants from future diseases as bio prospect potential is the new avenue along with the identifying and inhibition of human pathogens found in the rhizosphere.

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14 Rhizospheric Functional Attributes of *Paenibacillus polymyxa* in Disease and Nutrient Management for Sustainable Crop Production

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Abstract

Paenibacillus polymyxa is a gram-positive, rod-shaped, endospore-forming, facultative anaerobic soil bacterium which performs important ecological functions, especially organic matter decomposition, nutrient recycling, and antagonism against harmful pathogens in agricultural ecosystem. It is often associated within the host plant as an endophyte and helps the plant through biofortification of nutrients and induced systemic resistance against pathogen attack. *P. polymyxa* possesses the plant growth-promoting (PGP) traits, which include nitrogen fixation, mineral solubilization (phosphorus, potash, zinc etc.), IAA, siderophore, and hydrolytic enzymes production (chitinase, cellulase, amylase, pectinase, lignocellulolytic enzymes, etc.). Besides, PGP traits, *P. polymyxa* protect the host plant against bacterial and fungal pathogens infestation through nonribosomal synthesis and extracellular secretion of small molecular weight peptides called lipopeptides. The molecular mass of these antimicrobial peptides is in the range of 800–3500 Da. The lipopeptides produced by *P. polymyxa* are grouped into four classes, viz., fusaricidin, polymyxin, tridecaptins, and paenilin (Lantibiotics). However, the first two groups are often reported. Different strains of *P. polymyxa* are reported for biofertilizer, biopesticide, biofuel, bioremediation application which shows the biotechnological potential of this bacterium in sustainable agriculture. The use of this beneficial bacterium in agriculture and related areas brings down the dependency on chemical inputs and can save the environment in the era of climate change.

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Keywords

Paenibacillus polymyxa · Sustainable crop production · Soil microorganisms · Biofortification · PGPR · Biological Control · Biofilm formation

14.1 Introduction

The large use of chemical in crop production resulted in environmental pollution, soil fertility deterioration, and toxicity to mammals. This forced researchers to look for alternative strategies to the use of chemicals in agriculture. Among the different strategies, the use of Plant Growth-Promoting Rhizobacteria (PGPR) attracts much attention due to its eco-friendly nature, efficiency in action, and safer to human beings and environment (Compant et al. 2005). Bacterial endophytes are the group classified under PGPR which resides inside the plant tissue and supports the plant growth through nutrient supplementation and antagonizes the pathogens effectively as they colonize the same niche of the pathogen (Sturz et al. 1997). Bacillus and *Paenibacillus* sp. are the predominant genera reported for PGPR activities which include disease suppression, nutrient uptake, plant growth promotion, and abiotic stress tolerance to the host plant. The antagonistic mechanism of the genera involves the production of hydrolytic enzymes, antibiotic production, siderophore, and volatile organic compounds production. They are also known to induce systemic resistance in host plant, they disrupt the pathogenic cell structure and compete out the phytopathogens (Gardener 2004).

P. polymyxa is an efficient PGPR and supports the plant growth through nitrogen fixation, solubilization of minerals (phosphorus, potassium, zinc, etc.) and auxin production, and antagonizes the plant pathogens through production of antibiotics and hydrolytic enzymes (Rosada and Seldin 1993; Heulin et al. 1994). The ecological and biotechnological potential of *P. polymyxa* in sustainable agriculture has been recently reviewed (Lal and Tabacchioni 2009; Grady et al. 2016). The colonization of *P. polymyxa* in different parts of host plant has been studied using modern techniques such as green fluorescent pigment (gfp) tagging, fluorescence microscopy, and confocal laser beam microscopy (Timmusk et al. 2005; Annapurna et al. 2013). *P. polymyxa* produces fusaridicin and polymyxin group of lipopeptide antibiotics which have ecological and economic significance in the era of modern agriculture and allied sectors too. In this chapter, the biotechnological potential of *P. polymyxa* as biofertilizer, plant growth-promoting rhizobacterium, biopesticide, etc. in sustainable agriculture has been discussed.

14.2 Ecology and Distribution

P. polymyxa is a gram-positive, facultative anaerobic soil bacterium which resides in soil and helps in soil organic matter decomposition, nutrient recycling, and protects the crops against harmful diseases. *P. polymyxa* invades plant roots and resides

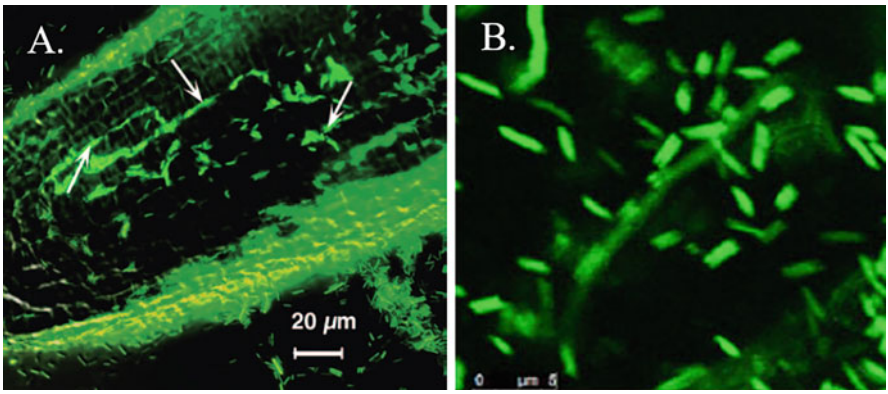

Fig. 14.1 Colonization of *P. polymyxa* in root tips of *A. thaliana* (**a**) (Timmusk et al. 2005) and root nodules of soybean (**b**) (Annapurna et al. 2013)

inside the plant tissue as endophyte. The endophytic *P. polymyxa* protects the host plant against biotic and abiotic stresses. In recent days, the colonization pattern of *P. polymyxa* in plant roots was established using the method of gfp (green fluorescent pigment) tagging and the use of sophisticated microscopy (confocal laser beam microscopy) to localize the endophytes association with the host plant (Timmusk et al. 2005; Annapurna et al. 2013). The localization of bacterial cells inside the root tissue is clearly visible by green fluorescence (Fig. 14.1). Timmusk et al. (2005) showed that *P. polymyxa* invades the root tips and forms biofilm in *Arabdiopsis thaliana*. Annapurna et al. (2013) reported that *P. polymyxa* HKA-15 invades the root nodules of soybean. The co-inoculation of *P. polymyxa* and *B. japonicum* increased the plant growth than the inoculation of *B. japonicum* alone.

The inoculation of gfp tagged *P. polymyxa* WL Y78 in crops, viz., wheat, maize, and cucumber seedling under gnotobiotic system and soil indicated the colonization of *P. polymyxa* in epidermal and cortical cells, intercellular spaces, and vascular system of root, stem, and leaf tissue (Hao and Chen 2017). The comparative, genomic, and functional analysis of *P. polymyxa* strains showed that plant growth-promoting traits are conserved in these bacteria while genes relevant to nitrogen fixation and antibiotic synthesis are evolved with the diversity (Xie et al. 2016).

14.3 Biocontrol of Fungal and Bacterial Diseases

Bacillus and *Paenibacillus* spp. are the major source of broad-spectrum peptide antibiotics active against various microbial and nematode pathogens (Govindasamy et al. 2010). *P. polymyxa* is reported for biocontrol of plant diseases caused by both fungal and bacterial pathogens. The list of *P. polymyxa* strains reported for biological control of several fungal and bacterial diseases and their causative agent (Table 14.1). *P. polymyxa* PKB-1 produces a number of secondary metabolites which were able to suppress *Leptosphaeria maculans*, the causative agent of

Table 14.1 Biological control of plant fungal and bacterial diseases by *P. polymyxa*

S. No.	Bacterial strain(s)	Disease suppressed	Causative agent	Reference
Fungal diseases				
1.	*P. polymyxa* PKB-1	Blackleg in canola	*Leptosphaeria maculans*	Beaty and Jensen (2002)
2.	*P. polymyxa* HKA-15	Charcoal rot in soybean	*Rhizoctonia bataticola*	Senthilkumar et al. (2009); Mageshwaran et al. (2010)
3.	*P. polymyxa* SR-19; GBR-462; GBR-508	Fusarium wilt in tomato	*Fusarium oxysporum* f. sp. *lycopersici*	Naufal et al. (2018); Son et al. (2009)
4.	*P. polymyxa* WLY78 and *P. polymyxa* NSY50	Fusarium wilt in cucumber	*F. oxysporum* f. sp. *cucumerium*	Li and Chen (2019)
5.	*P. polymyxa* APEC 136	Anthracnose in apples	*Colletortrichum gleosporoides*; *C. acutatum*	Shi et al. (2017)
6.	*P. polymyxa* A26	Fusarium head blight in wheat	*F. garminearum*	Timmusk et al. (2019)
7.	*P. polymyxa* YCP16-23	Phytopthora blight in pepper	*Phytopthora capsici*	Xu et al. (2020)
Bacterial diseases				
8.	*P. polymyxa* AC-1	Bacterial speck in tomato	*Pseudomonas syringae*	Hong et al. (2016)
9.	*P. polymyxa* Sx-3	Bacterial leaf blight in rice	*Xanthomonas oryzae* pv. *oryzae*	Abdallah et al. (2019)
10.	*P. polymyxa*	Bacterial common blight in soybean and French bean	*X. campestris* pv. *phaseoli*	Mageshwaran et al. (2012b, c)

blackleg disease of canola through the synthesis of fusaricidin group of cyclic depsipeptides (Beaty and Jensen 2002). *P. polymyxa* HKA-15 was active against *R. bataticola* causing charcoal rot disease in soybean (Senthilkumar et al. 2009). The antifungal property of this strain was found to be peptide antibiotics and the disintegration of fungal hyphae by HKA-15 cells was observed under light and scanning electron microscope (Senthilkumar et al. 2007a).

The endophyte, *P. polymyxa* SR-19 isolated from *Urtica dioica* protects the tomato plant against Fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici* (Naufal et al. 2018). *P. polymyxa* WLY78 produces fusaricidin antibiotic which antagonizes fungus *F. oxysporum* f. sp. *cucumerium* and induced systemic resistance via salicylic acid (SA) signal against fusarium wilt of cucumber (Li and Chen 2019). The PGPR, *P. polymyxa* APEC 136 significantly inhibited the mycelia growth of fungal pathogens and application of suspension of APEC 136 in harvested apples reduced the symptoms of anthracnose disease caused by *Colletotrichum gleosporoides* and *C. acutatum* (Kim et al. 2016). *P. polymyxa* strain HX-140 was

able to reduce the infection of Fusarium wilt of cucumber seedlings by 55.6% in a greenhouse pot experiment (Zhai et al. 2021).

P. polymyxa A26 produces biopolymer of D-glucuronic acids that aids in biocontrol of *F. graminearum* that causes Fusarium Head Blight (FHB) in wheat (Timmusk et al. 2019). *P. polymyxa* GBR-462, GBR-508, and *P. lentimorbus* GBR-158 reduced the Fusarium wilt symptoms by 90–98% in tomato plants (Son et al. 2009). *P. polymyxa* NSY 50 suppresses the growth of *F. oxysporum* (causing wilt disease) in cucumber rhizosphere and protects the host plant from pathogen invasion. Also, the inoculation of strain in soil modulates the microbial community and promotes the population of beneficial microorganisms thereby increased the soil biological property (Shi et al. 2017). *P. polymyxa* YCP 16-23 has the ability to control 70% infestation of Phytophthora blight (caused by *Phytophthora capsici*) in pepper (*Capsicum annum* L.). Besides, the strain has the plant growth-promoting traits, phosphate solubilization, enzyme production, siderophore production, IAA production, etc. (Xu et al. 2020).

The plant growth-promoting rhizobacteria, *P. polymyxa* AC-1, colonize leaf tissue of *Arabidopsis* and show the ability to control *Pseudomonas syringae* in tomato plants (Hong et al. 2016). The exposure of root knot nematode, *Meloidogyne incognita* to the culture filtrates of *P. polymyxa* GBR-1 reduced the egg hatch and substantial mortality to its juveniles (Khan et al. 2008). *P. polymyxa* SX-3 suppressed the bacterial leaf blight disease caused by *X. oryzae* pv. *oryzae* (Xoo) in rice and promoted the plant growth by nitrogen fixation, phosphate solubilization, etc. (Abdallah et al. 2019). The soybean seeds treated with *P. polymyxa* HKA-15 had lower percent disease incidence (PDI) of bacterial common blight disease caused by *X. axonopodis* pv. *phaseoli* and charcoal rot disease caused by *Rhizoctonia bataticola* as compared to untreated control (Mageshwaran et al. 2010, 2012b). Similarly, the application of crude metabolite of *P. polymyxa* HKA-15 @ 100 ppm resulted in suppression of bacterial common blight disease caused by *X. campestris* pv. *phaseoli* in French bean. The disintegration of *X. campestris* pv. *phaseoli* M-5 cells by crude metabolite of *P. polymyxa* HKA-15 was observed under Transmission Electron Microscope (TEM) (Mageshwaran et al. 2012c).

14.4 Mechanism for Biocontrol of Fungal and Bacterial Plant Pathogens

Bacillus and *Paenibacillus* spp. produce wide range of cyclic lipopeptides and identified as a major mechanism in biocontrol of plant pathogens (Kim et al. 2003). The identification and characterization of lipopeptides produced by different strains of *Bacillus* and *Paenibacillus* spp. have been classified into Bacillopeptins (Kajimura et al. 1995), fusaricidin (Beaty and Jensen 2002), mattacin (Polymyxin M) (Martin et al. 2003), Gaversin and Saltavalin (Pichard et al. 1995), and Polymyxin B (Selim et al. 2005). The lipopeptide antibiotics are initially concentrated and separated by employing different solvents. The most common solvents used for concentration of lipopeptides are methanol, hexane, chloroform, ethyl acetate,

butanol, etc. The extracted compound in crude form is purified by chromatography (mostly size exclusion). The different fractions collected are evaluated for bioactivity. The purity of the compound is tested by running thin layer chromatography and SDS-PAGE. The purified fractions are determined for the exact molecular mass by Mass Spectrometry (MS) and MALDI-TOF MS. Different researchers employed different methods of extraction and purification as their choice and it depends on the chemical nature of the compound and the available literature.

The purification and partial characterization of antifungal peptides produced by *P. polymyxa* strain HKA-15 showed they are cyclic peptide and depsipeptide and found to inhibit the growth of *Rhizoctonia bataticola* which causes charcoal rot disease in soybean. The antifungal compound was extracted with *n*-butanol and purified by hydrophobic interaction column (Sephadex LH-20) chromatography and reverse phase HPLC (Senthilkumar et al. 2007b). The determination of molecular mass of two antibiotic substances, viz., Gavaserin and Saltavalin produced by *B. polymyxa* revealed their molecular mass of 911 Da and 903 Da, respectively (Pichard et al. 1995). Hyun et al. (1999) extracted the antibiotic substance produced by *B. polymyxa* strain KB8 using the solvent, methanol and employed silica gel and Sephadex LH-20 column chromatography for its purification. Zhou et al. (2008) isolated an antifungal protein from *Paenibacillus* strain HT16 from locusts showed strong inhibition to *Penicillium expansum* and the molecular weight of the protein was found to be 4517 Da.

The fusaricidin peptides produced by *P. polymyxa* PKB1 were found to be with molecular masses of 883, 897, 948, and 960 Da (Beaty and Jensen 2002). *Paenibacillus* sp. strain B2 isolated from the mycorrhizosphere of sorghum colonized by *Glomus mosseae*, produced three different active compounds. The characterization of these compounds revealed that one of the compounds belongs to polymyxin B1 while the other compounds were found to be novel compounds with variation in the amino-acid sequence and molecular weight of 101 Da as compared to polymyxin B1. The study expanded the knowledge on newer compounds produced by *P. polymyxa* and the broad spectrum of antagonistic activity of the novel peptide compounds compared to that of polymyxin B (Selim et al. 2005). The antifungal metabolite produced by *Paenibacillus lentimorbus* strain WJ5, extracted with *n*-butanol and the crude metabolite was found to be thermostable and resilience to proteinase K, sodium dodecyl sulfate (1%), Tween-80 (1%), and glycerol (1%). The FT-IR spectrum of the antifungal metabolite confirmed the presence of the peptide and glycosidic bonds (Lee et al. 2008). Physicochemical characterization of antimicrobial metabolite produced by *Paenibacillus peoriae* strain NRRL BD-62 showed that the compound retained the activity even after autoclaving at 121 °C for 10 min. The compound was stable after the treatment with organic solvents, hydrolytic enzymes, and its activity was preserved at a wide range of pH (Weid et al. 2003).

P. polymyxa produces lipopeptide antibiotics and variety of enzymes like chitinase, pectinase, amylase, and catalase in the environment to restrict the growth of pathogenic microorganisms. *P. polymyxa* KT-8 produces fusaridicin group of antibiotics (Kajimura and Kaneda 1997). *Paenibacillus elgii* B69 produces two

antimicrobial compounds Pelgipeptins A and B. The molecular mass of Pelgipeptins A and B were 1072 Da and 1100 Da, respectively. The MIC of Pelgipeptin was 6.25–50 μg/mL and it shows strong antifungal activity against fungal pathogens. Thus, the antibiotics produced by *P. polymyxa* are an alternative source of chemical pesticides for the biocontrol of plant diseases (Wu et al. 2010). *P. polymyxa* M-1 suppressed the growth of phytopathogens, *Erwinia amylovora* and *E. caratovora*, the causative agent of fire blight and soft rot, respectively. The MALDI-TOF mass spectrometry revealed the molecular mass of 1190.9 Da and 1176.9 Da, two components of polymyxin, P1 and P2, respectively (Niu et al. 2013). *P. polymyxa* DSY-OF producing polymyxin E and Lantibiotic was isolated from food source. The Lantibiotic was active against broad spectrum of gram-positive bacteria which occurs as food contaminants (He et al. 2007). Beaty and Jensen (2002) reported that *P. polymyxa* KB-1 produces antifungal peptides. The antifungal peptides are in the range of 883–961 Da. The bacterium has the capability to inhibit the growth of *Leptosphaeria maculans* causative agent of blackleg disease of Canola (*B. napus* L. and *B. rapa* L.). The purification of lipopeptides produced by *P. polymyxa* was done with reverse-phase HPLC, Size exclusion chromatography, TLC, etc. In order to identify the new products produced by *P. polymyxa*, genome mining in combination with mass spectrometry is the method of choice. *P. polymyxa* harbors the gene clusters of A–D which produce nonribosomal peptide synthetases which are responsible for the synthesis of various lipopeptide groups of antibiotics. The gene clusters A and D code for fusaricidin and polymyxin group of antibiotics while gene clusters B and C code for synthesis of tridecaptins and paenilin (Lantibiotics) (Vater et al. 2018).

Paenibacillus sp. OSY-N produces Paenipeptins. The chemically synthesized Paenipeptins C had MIC of 0.5–4.0 μg/mL against gram-negative bacteria and 0.5–32 μg/mL against gram-positive bacteria. It has been demonstrated that peptide cyclization of the lipopeptide family is not essential parameter for their antimicrobial activity (Huang et al. 2017). Camptothecin, a complex pentacyclic cluster pyrroloquinoline alkaloid obtained from the tree *Camptotheca acuminata*. This drug has anticancer property. Pu et al. (2015) reported the camptothecin production from fermentation broth of *P. polymyxa* LY 214. The lipopeptide antibiotics, fusaridicin, and polymyxin produced by *P. polymyxa* have biosurfactant property and inhibited the biofilm formation by single and mixed bacterial species (Quinn et al. 2012). The antibacterial metabolite produced by *P. polymyxa* HKA-15, soybean bacterial endophyte was resilience to wide range of temperature, pH, surfactants, and organic solvents. A broad spectrum lipopeptide antibiotic having the molecular mass of 1347.7 Da was identified from *P. polymyxa* HKA-15. The lipopeptide was having strong activity against the phytopathogen, *X. campestris* pv. *phaseoli* M-5 which causes common blight disease in beans (Mageshwaran et al. 2011, 2012a).

14.5 Nutrient Supplementation and Plant Growth Promotion

The nitrogen-fixing ability of *P. polymyxa* is well established (Seldin 2011). Liu et al. (2019) identified five potential strains of *Paenibacillus* (JS-4, SZ-10, SZ-14, BJ-4, and SZ-15) that exhibited multiple plant growth-promoting attributes including nitrogenase activity, IAA production, and antimicrobial activity. *Paenibacillus polymyxa* E681, a potential microbial inoculant reported for eliciting biotic and abiotic stress tolerance in cucumber, barley, sesame, etc. (Jeong et al. 2019). The endophytic *P. polymyxa* SK1, isolated from *Lilium lancifolium* showed plant growth-promoting traits such as ACC deaminase production, IAA production, nitrogen fixation, phosphate solubilization, etc. besides, having antagonistic activity against set of fungal plant pathogens (*Fusarium*, *Botrytis*, etc.) (Khan et al. 2020). The consortium of bacterial inoculants, *P. polymyxa* ZM27, *B. subtilis* ZM63, and *B. aryabattai* S10 application in wheat crop biofortified the minerals, Fe and Zn in wheat grains (Hussain et al. 2020). *P. polymyxa* CR1 has multiple traits including biopesticide, biofertilization, biomass degradation, and biofuel production. It has potential uses in sustainable agriculture like control of plant pathogens, promotes plant growth promotion and postharvest degradation of crop residue (Weselowski et al. 2016).

14.6 Conclusion and Way Forward

The recent research outputs showed that the culturable microbe's exploration for use as microbial inoculants in agriculture often encountered with *Bacillus* and *Paenibacillus* spp. These genera have unique attributes such as spore forming, resilience to adverse conditions (biotic and abiotic), and multiple plant growth traits which make them suitable for use in agriculture. The different strains of *P. polymyxa* have been reported for multiple plant growth-promoting characters, biological control of fungal and bacterial diseases, eliciting induced systemic resistance, and abiotic stress tolerance to the host plants. *P. polymyxa* produces lipopeptide antibiotics which grouped into polymyxin and fusaricidin which are broad spectrum in nature, resilience to wide environmental conditions, and finds application beyond agriculture and especially in medical field due to increasing antibiotic resistance of the emerging diseases.

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Biochar-Mediated Suppression of Soil-Borne Pathogens in Agronomically Important Crops: An Outlook

15

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Abstract

Biochar is solid produce acquired by the heating of biological or carbon-based material in the complete or fractional presence of oxygen and is used as a soil amendment. The numerous valuable properties of biochar on the physical, biological, and chemical properties of soil as well as on plant condition and improvement are extensively acknowledged. The amendment of biochar has also been frequently debated for its properties of suppression of diseases. Nevertheless, the principal mechanisms for these properties are extremely complex and generally unidentified. It is anticipated that the composition of plant root exudate that alters the biochemical and microbial properties in the soil and the stimulation of defense mechanisms of plants due to the amendments of biochar are some critical reasons influencing pathogenic dominance. Further comprehensive studies are required for understanding the detailed connections of plant-pathogen coordination with various types of biochar that will support accomplishing maximum aid of biochar addition for the protection of plants from numerous soil-borne pathogens. In this chapter, the perspective of biochar for the regulation

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of pathogenic diseases is discussed, specifically the communications with plant pathogenic fungi under contradictory environmental circumstances. It is concluded that the amendment of biochar with soil could be an encouraging approach for the combined management of pests and pathogens.

Keywords

Biochar · Agronomically important crop · Soil-borne pathogens · Ecological fertilizer · Sustainable agriculture

15.1 Introduction

The modern agronomical scenario experiences a wide range of challenges with biosafety being the major one. Generally, plant diseases are chemically treated; though they are the main reason for the developed resistance in pathogens and environmental pollution. The pathogens residing in the soil destructively disturb the yield, quality, and production of various thrifts all over the globe. Since these microorganisms infect the system of roots in plants, first and foremost, infection occurs at the roots following in other parts of the plants (Wang et al. 2018). Bacteria, fungi, viruses, and nematodes and oomycetes belong to this association of microorganisms. Miscellaneous microbes possible share certain features for the survival and action in the soil. As a result, the biotic and abiotic mechanisms of the soil, along with agricultural methods such as soil preparation, irrigation, fertilization, and manure application, have a significant impact on them (Katan 2017). Presently, chemical soil disinfection is extensively used for the management of infections (Zhou et al. 2019). But the escalated fare of application, limitations on the use, and apprehensions about the impacts on the environment undoubtedly direct the necessity to search for other effective strategies for control management. Because soil harbors small intensities of pathogens, it is vital to administer strategies that can enhance health and sustainability of soil (Larkin 2015). Pathogens are suppressed directly as a result of the employment of management methods aimed at improving the soil's physicochemical and, biological qualities (De Medeiros et al. 2019; Jaiswal et al. 2019). Furthermore, because biochar has this capacity, the assumption of this work is that biochar could be a substitute method for unintended, operational management with less environmental effect against the pathogens residing in the soil (Wang et al. 2019a; Lima et al. 2018).

Biochar is the result of thermal degradation of biomass in the absence of oxygen, accomplished through pyrolysis, in which the feedstock is heated to elevated temperatures, causing chemical modifications and resulting in fine, carbon-rich porous particles (Pandey et al. 2020). Biochar is a product that is high in nutrients and improves the biological, chemical, and physical properties of soil (Li et al. 2019). Biochar has shown to have a favorable impact on the microbial population of soil because of its highly porous construction that serves as a habitation for a variety of microorganisms, altering the proportion of bacteria and fungus in the soil and, as a

result, increasing enzymatic activity (Wang et al. 2019a). As a result, biochar is extensively utilized as a soil conditioner, and more significantly, its addition to the soil is an effective option for the treatment of diseases, as it decreases the severity of disease and stimulates the systemic resistance of plants (Zhang et al. 2016).

Several studies have emphasized the capability of biochar in agronomical benefits, acting wholly or partly on disease control. Biochar has an observable effect because of its ability to improve soil quality. For an instance, Ali et al. (2019) found that under salt and water stress, the implementation of biochar boosted the development and production, along with nutrient absorption and photosynthesis in plants. As per Romdhane et al. (2019), the application of biochar improves the drought tolerance in corn by improving its chemical and physical properties and increasing the moisture capacity of soils treated by the substrate. Biochar enhanced the pH of soil, decreased the concentration of Al^{3+}, and elevated the exchange capacity of cation, which together had a substantial effect on the growth of maize plants (Xia et al. 2020). Ali et al. (2019) reported four variants of biochar having positive impact on the fresh weight biomass of Chinese cabbage and chives. Biochar can be considered an ecological fertilizer for sustainable agriculture, given the number of positive consequences it has on agricultural soils (Chen et al. 2018).

Furthermore, biochar suppresses disease through a variety of methods, including an increase in the density and activity of beneficial microbes such as plant growth-promoting rhizobacteria (Kavitha et al. 2018), N_2-fixing bacteria (Semida et al. 2019), mycorrhizal fungi, and Trichoderma species (De Medeiros et al. 2020). Biochar directly stimulates through fungi toxic actions, the establishment of resistance in pathogen-hosting plants, and the adsorption of biotic and abiotic phytotoxic compounds that can cause direct damage in the plant roots, encouraging pathogenic infections (Bonanomi et al. 2015).

15.2 Background on Plant Diseases

Plant pathogens embrace different types of bacteria, fungus, viruses, nematodes, phytoplasmas, viroids, oomycetes, and spiroplasmas. These pathogens are classified into two comprehensive groups, i.e., obligate and facultative parasites. Obligate parasites depend completely on the existing living host plant tissue for their sustenance and propagation, whereas, facultative parasites could cause great harm to the plants and also live as saprophytes on the organic material and vegetal remains. Infectious agents that communicate a disease to the root system and exist majorly in the soil are stated as soil-borne pathogens, while that infect plant organs above the ground are described as foliar pathogens.

The soil-borne plant microbes can survive in the soil atmosphere and in remnants on the surface of the soil for a prolonged period (soil resident) or for a short time (soil intruders) (Bruehl 1987). They can affect and can considerably decrease the produce and value of a wide range of plants, together with vegetables, fruits, trees, ornamental crops, shrubs, and annual plants. Soil-borne infections can have a critical effect on the breeding grounds and greenhouse production where plants are usually fledged

under monoculture (Katan 2004; Katan et al. 2002). Soil-borne plant microorganisms can live on the hosts, as saprophytes on the biological material and plant remnants, or in structures such as oospores, melanized mycelium, sclerotia, and chlamydospores up until initiated for propagation. In maximum circumstances, soil-borne pathogens directly infect the underground plant organs; on the other hand, the plant parts which are above the ground are also indirectly affected (Koike et al. 2003). Commercial harm due to soil-borne pathogens is probably at 10–20% of the achievable harvest for many crops (Pimentel et al. 1991). Critical diseases such as vascular wilt and take all in the cereals can be even more serious and sometimes put an end to the entire harvest. The loss of crops caused by soil-borne diseases in the USA only is estimated to exceed around US$4 billion/year (Lumsden et al. 1995; Mazzola and Reynolds 2010).

Comparative to the approaches engaged alongside the infection affecting plants' aerial parts, there are limited methods that show efficient disease control alternatives for the controlling of soil-borne diseases, and the ones that are being practiced do not generally result in complete control of the disease. Additionally, there are many works done for the regulation of soil-borne diseases that can have major effects on the social order and surroundings that outlay goes beyond the expenses of the disease to the cultivator and users. An example of this could be that when a soil fumigant (e.g., Dichloropropane) is used for the achievement of efficient control of diseases, it could result in a large environmental interference to the overall system of production. The root exudate contains amino acids, organic acids, carbohydrates, and phenols which help in stimulating the growth of soil-borne pathogens (Bais et al. 2006). Generally, microorganisms produce certain enzymes that reduce polymers in the host plant in the primary phases of infection, performing an essential part in the host penetration (Agrios 2005). Other than producing extracellular enzymes, nonenzymatic phytotoxins are also produced by pathogens (Bartz et al. 2012). The extremity of infection caused by plant pathogens has usually been deliberated by an operation of the interaction considering the component at the three vertices of the disease triangle (Agrios 2005): host exposure, pathogen virulence, and ecological situations. All these three factors need to be appropriate for an infection or disease to occur.

15.3 Biochar Production and Characterization

Biochar is a carbon-rich porous substance. It is prepared by the thermal decomposition of biomass in the absence or nominal presence of oxygen. Pyrolysis is a method of thermal degradation in which biomass is heated at high temperatures, resulting modifications in chemical conformation (Pandey et al. 2020). The composition and physicochemical qualities of the finished product are affected by the temperature range, heating time, and retention time utilized in the pyrolysis process. However, when the pyrolysis temperature raises the yields of biochar decrease, while carbon and ash contents are increased (Xiang et al. 2020). Pyrolysis at high temperatures releases hydrogen- and oxygen-containing groups that make a significant

contribution in increase the carbon concentration. The overall porosity, pore width, and surface area of biochar are affected by the pyrolysis temperature, as high temperatures cause pores to develop resulting in the discharge of volatile organic chemicals (Kavitha et al. 2018). Generally, pyrolysis temperature and feedstock source determine quality of biochar and its effects on soil characteristics and plant growth. Biochar formed at a relatively low temperature has more labile carbon, nutrients, and aliphatic compounds and is better for agricultural soil management; however, biochar produced by fast pyrolysis at an elevated temperature has more aromatic compounds and fixed carbon, as well as increased pH, carbon, pore size, surface area, ash content, and stability (Hassan et al. 2020). Cheng et al. (2018) reported that an increase in pyrolysis temperature decreases the nutrient dissolution and progresses the efficiency of fertilizer usage in a study involving the sandy loam soil amended with biochar generated at various temperatures. Adekiya et al. (2019) discovered an increase in soil pH, organic matter concentrations, nitrogen, phosphorus, sulfur, calcium, and magnesium, along with leaf nutrient levels and yield in radish after the amendment of biochar derived from hardwood by pyrolysis at 580 °C.

Since the qualities of biochar are impacted by the biomass resources, it is essential to choose the appropriate material that will eventually regulate the chemical and physical properties of the product. Generally, biochars have a large surface area, high porosity, a significant number of functional groups, and are high in organic carbon, making them an effective soil conditioner (Lima et al. 2018). Hassan et al. (2020) showed that biochar produced from wood derivatives has a higher production and stability than grass and manure-derived biochar in a meta-analysis examining the effects of feedstock sources and pyrolysis temperature on the attributes of biochar.

15.4 Effects of Biochar on Physical Characteristics of Soil

Addition of biochar in soil encourages constructive changes in its physical properties. Such as, the application of biochar decreases the bulk density of soil and hence, improves the soil structure and conditions for the growth of plants (Blanco-Canqui 2017). Concurrently, the reduction of bulk density of soil, the water holding capacity of soil are significantly increased as there is an escalation in the aeration in soil and water penetration (Razzaghi et al. 2020). Moreover, biochar consists of a large amount of pores that make it an effective material for uplifting the soil accumulation and increased water retention (Amoah-Antwi et al. 2020). Therefore, the major role in this development is played by the particle size of biochar, as the particles with smaller diameter help in retention of more water (Zhang et al. 2016). The amendment of biochar contributes in the soil aggregation ultimately helping in reduced erosion (Wang et al. 2016b). Besides the raw material and temperature of biochar, the kind of soil in which it is used with also affects the soil-biochar coordination. A study conducted by Razzaghi et al. (2020) concluded that the same biochar mixed with medium and sandy soils showed more efficiency in

the retention of water than that used with the clayey soils. The changes caused by the application of biochar on the physical characteristics of soil contribute in the intercommunication with soil microflora (Barros et al. 2014). A study reported by De Medeiros studied the aspects of soil involved in suppressing the black root rot disease in cassava plants caused by *Scytalidium lignicola* and concluded that the bulk density of soil had a progressive relationship with the severity of disease (De Medeiros et al. 2019), whereas, the soil containing clay showed the adverse interrelation with the disease severity by affecting some antagonistic agents and decreasing the community of soil-borne microorganisms (Sales Júnior et al. 2017). The increase in soil porosity, available water content, field capacity produced by the addition of biochar in the soil could play a significant part in increasing the flexibility of agronomical system to drought situations, consequently increasing the crops resistance against pathogens (Edeh et al. 2020). According to a study conducted by Zhang et al. (2017), the amendment of biochar produced from rice straw for the control of *Ralstonia solanacearum* causing tobacco bacterial wilt resulted in approximately 76.64% reduction of infection under field situations. Another study reported the decrease in possible crop infections of Fusarium and numerous operational taxonomic units after the addition of biochar in black soil for the 3 consecutive years (Yao et al. 2017).

15.5 Effects of Biochar on Chemical Characteristics of Soil

Biochar encouraged a wide-range of interest as an improvement for increasing the yield of agricultural production because of its capability to intensify the cation exchange capacity and neutralizing acidity, as these are the necessary characteristics for the improvement of soil chemically (Brassard et al. 2016). The addition of different concentrations of alkaline ash in the form of oxides of magnesium, calcium, potassium, carbonates, and hydroxides in the soil explains the elevated pH of biochars. Additionally, the surfaces of biochars contain numerous functional groups that help in interaction of soil cations and because of that, the capacity of pH buffering is increased (Han et al. 2020). Moreover, the type of biomass and production temperature affect the alkalinity of biochar (Gul et al. 2015). The cation exchange capacity of biochar determines its capability for the absorption of ammonium and calcium ion essential for plants. Therefore, the amendment of biochar with soil shows an elevation in the nutrient holding capacity and reduction in nutrient loss (Ding et al. 2017). There are certain studies reporting that addition of biochar helps in the increment of nitrogen content and reduction of inorganic nitrogen content in the soil (Xia et al. 2020). Studies have also suggested that biochar might contain fertilizing nitrogen, phosphorus, and potassium elements in its composition depending upon the raw material and pyrolysis conditions used (Brassard et al. 2016). The outcome of biochar on the chemical properties of soil could be seen after many years. A field study reported the increase in amount of total nitrogen and organic carbon after 8 years of biochar amendment. The extended effect of biochar in soil could be explained by the presence of extremely reduced structures providing

continuous benefits like increment of organic carbon in the soil by carbon sequestration, caused by the slow conversion of organic carbon dioxide (Luo et al. 2020; Zeeshan et al. 2020). The positive association was found in between the pH of soil, soil suppression capacity, and redox potential toward *Fusarium oxysporum* f. sp. *niveum* in watermelon Cao et al. (2016).

15.6 Effects of Biochar on Microbial Characteristics of Soil

Biochar can cause changes in the soil by modifying the microbial biomass and the composition of microbial community present in the soil. For example, the high porosity and large surface area of biochar help in creating a surrounding that encourages root growth and microbial community in plants. Subsequently, the nutrient cycle, microbial reproduction, and enzyme activity are affected positively (Wang et al. 2016a). Additionally, the structures in biochar consist of micropores, macropores, and mesopores, which are model spaces for the growth and development of several microorganisms, such as, bacteria and fungi (Palansooriya et al. 2019). The porous structures present in biochar turn into beneficial habitation for the microorganisms and help them in protection from the predators (Han et al. 2020). An additional factor explaining the effect of biochar on microbial characteristics is the rise in pH of soil that causes alterations in the microbial community (Herrmann et al. 2019; Zhu et al. 2019). Additionally, the elevated carbon content in the biochar encourages the development of biomass and microbial activity in the soil (Gomez et al. 2014). Numerous authors reported that there was an elevation in the quantity of Proteobacteria that embraces bacteria which are important in cycling carbon, sulfur, and nitrogen ultimately helping in disease management (Igalavithana et al. 2019; Zhu et al. 2019; Ali et al. 2019). Alterations in pH and the accessibility of easily available nutrients help in providing the essential circumstances for the development of Proteobacteria (Xu et al. 2016). Contrary to this, the application of biochar with soil enhances the microbial activity of soil and helps in changing the configuration of beneficial microbial populations. Biochar could make sure the effectual endurance of useful microbes in the soil (Jaiswal et al. 2019). The enzyme activity of soil due to its property of matter decomposition and energy accessibility plays an important role in the health of soil and is influenced by the amendment of biochar (De Medeiros et al. 2020). A number of studies presented that amendment of biochar directly changes the activity involving the metabolism that encourages the changes in biological, physical, and chemical environment along with various enzyme activities (Lehmann et al. 2011). The enzyme activities are intensely related with the destruction of infections caused by soil-borne microorganisms (De Medeiros et al. 2019).

15.7 Biochar for the Management of Plant Diseases

Allen first reported the positive outcome of biochar against plant diseases such as mildew and rust in various crops (Allen 1847). With the study of several pathosystems, Elan and his co-workers gained worldwide recognition (Elad et al. 2012). So far there are a large number of reports on the influence of biochar on soil-borne microorganisms (Table 15.1). Nevertheless, the evidence says that the influence of biochar on plant infections differs in the range of different biochar feedstock, properties of soil, implementation proportions, and the circumstances under the study (Gao et al. 2019). Frenkel et al. (2017) summed up that amendment of biochar at lesser concentrations (≤1%) repressed numerous infections, while the use of biochar at higher concentration (≥3%) was unproductive or even encouraged diseases in the plant. Bonanomi and his colleagues (2015) anticipated the five mechanisms of biochar contrary to diseases in plants as undeviating fungi toxic outcome of biochar, adsorption of phytotoxic and allelopathic compounds that can cause damage to the plant, stimulation of plant resistance, escalation of activities, and a large number of favorable microorganisms, alterations in quality of soil as nutrient accessibility and abiotic situations. They reported dominance in the plant diseases by biochar; however, 85% of the revisions showed a useful impact of biochar in diminishing plant illness seriousness, 12% made an unbiased difference, and exclusively 3% showed that biochar augmentations caused plant infections (Bonanomi et al. 2015). A study carried out reported a progressive effect of biochar and *Trichoderma harzianum* for the control of significant Phytopathogen, *Macrophomina phaseolina* (Araujo et al. 2019). In a different study, the amendment of biochar produced from rice husk was done for the improved growth of apple seedlings, and the profusion of *Fusarium solani* was also diminished (Wang et al. 2019a). Comparative interpretations were accounted presenting that the biochar manufactured from sawdust and poultry fecal waste efficiently regulated the ear rot in maize plants triggered by *Fusarium verticillioides* (Akanmu et al. 2020). The biochar from corncob was used for the suppression of *Fusarium virguliforme* causing root rot in soybean (Rogovska et al. 2017). A 58% progressive outcome of biochar for the concealment of dark leaf spots produced by *Magnaporthe oryzae* in perennial ryegrass was detailed (Wang et al. 2019b). Choudhary et al. (2018) stated vanquishing of wilt infection initiated by *Ralstonia solanacearum* using biochar. In another study, the application of biochar produced from the wood chips of the eucalyptus tree was found to diminish 71% of the damping-off disease in cucumber initiated by *Pythium aphanidermatum* (Jaiswal et al. 2019).

15.8 Possible Mechanism for the Control of Plant Disease by Biochar

Although the mechanisms for the suppression of diseases with the help of biochar are so far not completely understood but, it is suspected to be the outcome of a complicated interconnection in between soil condition, microorganisms present in

Table 15.1 List of the studies using biochar as an approach in the management of plant diseases initiated by pathogens residing in the soil

Source of biochar	Temperature for biochar production	Pathogen	Disease	Host	Reference
Wood	–	*Fusarium oxysporum* f. sp. *lactucae*	Gray mold	Lettuce	Bonanomi et al. (2022)
		Rhizoctonia solani	Collar rot	Tomato	
		Sclerotinia sclerotiorum	Root rot		
Rice husk	–	*Colletotrichum falcatum*	Ret rot	Sugarcane	Rose et al. (2022)
Tobacco stem	–	*Ralstonia solanacearum*	Bacterial wilt	Tobacco	Li et al. (2021)
Green waste	Pyrolysis (500 °C)	*Alternaria solani*	Early blight	Tomato	Rasool et al. (2021)
Wood					
Cornhusk	–	*Verticillium dahliae*	Verticillium wilt	Eggplant	Ogundeji et al. (2021)
Eucalyptus urophylla	Pyrolysis (350 °C) (400 °C)	*Fusarium oxysporum* f. sp. *lycopersici*	Fusarium wilt	Tomato	Silva et al. (2020)
Eucalyptus saligna					
Poultry faecal waste and saw dust	Pyrolysis (485 °C)	*Fusarium verticillioides.*	Ear rot	Maize	Akanmu et al. (2020)
Maize	Pyrolysis (600 °C) (210 °C)	*Fusarium solani*	Root rot	Blue lupin	Egamberdieva et al. (2020)
Sewage sludge	Pyrolysis (500 °C)	*Macrophomina phaseolina*	Gray rot	Bean	Araujo et al. (2019)
Wheat	–	*Ralstonia solanacearum*	Bacterial wilt	Tomato	Gao et al. (2019)
Rice husk	Pyrolysis (450 °C)	*Fusarium solani*	Crown rot	Chinese crab apple	Wang et al. (2019a)
			Foot rot		
			Fruit rot		
			Root rot		
Eucalyptus wood chips	Pyrolysis (600 °C)	*Pythium aphanidermatum*	Damping off and root rot	Cucumber	Jaiswal et al. (2019)

(continued)

Table 15.1 (continued)

Source of biochar	Temperature for biochar production	Pathogen	Disease	Host	Reference
Eucalyptus wood	Pyrolysis (600 °C) (350 °C)	*Fusarium oxysporum*	Crown and root rot	Tomato	Jaiswal et al. (2017)
Greenhouse pepper plant waste					
Corn stover	Pyrolysis (400 °C)	*Fusarium virguliforme*	Root disease	Soybean	Rogovska et al. (2017)
Hard wood	(500 °C)				
Mixed wood	(600 °C)				
Red oak					
Beech wood chips	Pyrolysis (500 °C)	*Fusarium oxysporum* f. sp. *lycopersici*	Fusarium wilt	Tomato	Akhter et al. (2016)
Green waste					
Holm oak	Pyrolysis (650 °C)	*Botrytis cinerea*	Gray mold	Lettuce	De Tender et al. (2016)
				Strawberry	
Hardwood biomass	–	*Fusarium oxysporum*	Crown and root rot	Asparagus	Elmer (2016)
		Fusarium proliferatum			
Greenhouse waste	Pyrolysis (450 °C)	*Botrytis cinerea*	Gray mold	Tomato	Mehari et al. (2015)
Eucalyptus wood	Pyrolysis (350 °C)	*Rhizoctonia solani*	Foliar diseases	Cucumber	Jaiswal et al. (2014)
Greenhouse waste	(600 °C)				
Animal bone	–	*Phytium aphanidermatum*	Crown and root rot	Tomato	Postma et al. (2013)
		Fusarium oxysporum f. sp. *radicis-lycopersici*			
Balsa fir bark	Pyrolysis (475 °C)	*Pythium ultimum*	Damping off and root rot	Bell pepper	Gravel et al. (2013)
Spruce bark				Lettuce	
				Basil	
				Garden geranium	
				Coriander	

Wood biochar	Pyrolysis (450 °C)	*Botrytis cinerea*	Gray mold	Strawberry	Harel et al. (2012)
Greenhouse waste biochar		*Podosphaera aphanis*	Powdery mildew		
		Colletotrichum acutatum	Leaf blight		
Pinus wood	Pyrolysis (550 °C)	*Phytophthora cactorum*	Stem rot	Red maple	Zwart and Kim (2012)
				Red oak	
Hardwood dust	–	*Fusarium oxysporum* f. *spasparagi*	Crown and root rot	Asparagus	Elmer and Pignatello (2011)
		Fusarium proliferatum			
Citrus wood	Traditional charcoal kiln	*Leveillula taurica*	Gray mold	Bell pepper	Elad et al. (2010)
		Botrytis cinerea	Powdery mildew		
Municipal bio waste	–	*Ralstonia solanacearum*	Bacterial wilt	Tomato	Nerome et al. (2005)
Coconut charcoal	–	*Fusarium oxysporum*	Fusarium root rot	Asparagus	Matsubara et al. (2002)

the rhizosphere, host plant, and the pathogen (Debode et al. 2020). It is stated that enhancements caused by the biochar amendment in soil help in creating the beneficial soil conditions for the development of plants (Graber et al. 2014). These enhancements also help in building up the resistance by increasing the systematic and microbial diversity of the soil, which triggers an antagonistic process between the infectious species and the natural microbiota for the available resources present in the soil. Therefore, the efficiency of biochar as a management policy is also connected with its capability to encourage the microbial population of soil, resulting in escalating the productive microorganisms that will help in protecting the plant and soil against the infection causing pathogens, and for the growth and development of Trichoderma genus fungi (Akanmu et al. 2020; De Medeiros et al. 2020). It is well known that the implementations of accomplishment of biochar in soil-borne diseases are considerably more diversified than the infections caused by the pathogens in leaf. The repression caused by the biochar is associated by the defense activation of plants that is ultimately delivered to the complete system of plants since it is extensively distant from the attack site of pathogen (Jaiswal et al. 2019). Additionally, a substantial diversity of microorganisms is colonized in the healthy and symptom-free plants by forming a complicated microbial accumulation affecting growth and productivity of plants (Hacquard 2016). The progressive modification of biochar in the microbial population encourages the improvement and growth of plants. This increase in the quantity and variety of the microbial community stimulated by the amendment of biochar in the soil takes place majorly because of its high organic carbon content serving as a substrate for the microorganisms (Fig. 15.1). The addition of biochar also provides the benefits in the physicochemical properties of soil resulting in an increase in the microbial activity and causing effects on the growth and virulence of the pathogens (Chen et al. 2020). For example, the pores present on the surface of biochar provide additional microhabitats for the growth of microbial population and the elevation in pH and nutrient accessibility are the supplementary features affecting the microorganisms' activity and biomass (Hernandez-Soriano et al. 2016). The disease caused by soil-borne pathogens can be influenced by biochar in various ways such as, changes in accessibility and supply of nutrients, variations in the physicochemical qualities of soil, modification in the functional and taxonomical assortment of the soil microbial population, alteration in growth, existence, virulence of the pathogen, and the release of pathogenic toxins. Furthermore, biochar amendment to the soil encourages the defense system in plants against the fungal pathogens by activating the systematic resistance against the pathogens with the assistance of defense-associated gene expressions (Jaiswal et al. 2019; Harel et al. 2012).

15.9 Future Perspective

Undoubtedly, the conception of effects and impacts of biochar in the advancement of soil-borne diseases is in its initial stages. It is expected that in the coming 5–10 years, development will be made in explaining the mechanism that governs the impact of

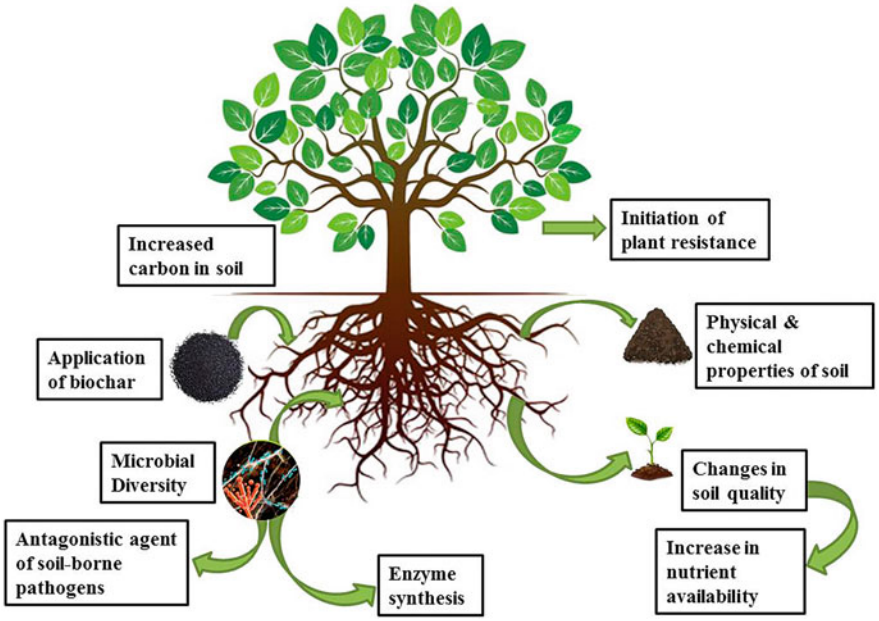

Fig. 15.1 Biochar as an approach for the management of plant diseases by the pathogens

biochar on plant diseases initiated by soil-borne pathogens. It is suspected that the positive influence of biochar addition on the microbial diversity of soil will be one of the main factors in the positive effects of biochar. It is also expected to see more attentiveness in biochar from the perspective of plants, and improved understanding of the effect of biochar amendment in the organism interaction chains, adaptation responses, cause-and-effect signaling, and physiological traits. The market of biochar is developing in many parts of the world and there is an essential necessity for continuous field tests for increasing the knowledge for making biochar an applied methodology for controlling the plant pathogens.

15.10 Conclusion

Biochar plays an important role as a soil amendment for the destruction of plant pathogens and elevating the resistance of plants to various diseases. Biochar generated from different biomasses could be effective against a variety of disease-causing microbes in plants. Though, the prevention of pathogens by biochar is not constantly associated with the variations in physicochemical properties. The addition of biochar helps in increasing the organic carbon content in soil and improves the vital nutrients for plant procurement and microbial propagation. This outcome was described by the effect of biochar on the suppression of disease and association for the stimulation of appropriate surroundings for increasing the activity of helpful

microorganisms. Furthermore, the application of biochar effectively improves the disease resistance by modifying the biochemical properties, physiological responses, and by inducing systemic resistance in plants. Therefore, the amendment of biochar can be a consistent method for the improvement of plants' health and protection against the fungal diseases, encouraging long-lasting agricultural sustainability.

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Harnessing Rhizosphere Microbiomes in Crop Productivity

16

Manish Mathur, Rajesh K. Tiwari, Parul Johri, and Mala Trivedi

Abstract

Chemical fertilizers and pesticides in present-day agriculture cause real damage to our environment and are a potential hazard to human health too. Enhancing crop productivity by using the potential of microbes is a new and opportune idea for sustainable agriculture. In plants, the microbial composition is arbitrary of biotic and abiotic factors. These include soil pH, structure, salinity, type, moisture, organic matter, and exudates, which are most pertinent for underground plant parts. The difference in communities of rhizosphere and phyllosphere because of plant-associated microbiota is another factor. Interactions between microbes either directly or indirectly with environmental factors have an impact on the host. The resistance against abiotic and biotic stress improves plant health which has an influence on the nutrient cycle by arbuscular mycorrhiza. Primary or secondary protection to the crop plants rapidly inhibits the rhizosphere apart from plant growth-promoting rhizobacteria (PGPR) which are a heterogeneous group of bacteria. The rate of seed growth was greatly accelerated by PGPR, and they also offer protection against harmful bacteria. The yield of many crops is substantially increased by the ability to uptake water and nutrients due to PGPR. PGPR work in symbiosis with other advantageous microorganisms, increasing the fixation of nitrogen and availability of primary and secondary micronutrients resulting in enhanced plant productivity.

Keywords

Microbiome · Rhizosphere · PGPR · Phyllosphere

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16.1 Introduction

Plants have a close link with microbes, representing the reservoir of the biological variety known in the world so far. Root excretions have an impact on the rhizosphere, which can have 30,000 prokaryotic species and billions of microbial cells per gram. The word "rhizosphere," coined by Hiltner (1904), refers to the soil where root system-mediated microorganism-associated processes occur. The fundamental and operational variety of microbiological species in the rhizosphere is well known. Rhizosphere is a compound word made up of two words, "Rhiza" meaning roots and "Sphere" meaning neighboring environment of roots. According to Hartmann et al. (2009), rhizosphere is the region of contact between a plant's roots and leaves. It is the area where plant roots influence microbiome in the surroundings by releasing some organic compounds, which promote the colonization of a plant and in turn promote growth and yield. Networking gives an understanding of rhizo-microbial metabolisms, including its niche area of research, to tackle the global challenges related to food security in the coming decades. Climate change, a boost in population, and improper application of chemical fertilizers are some of the significant obstacles affecting crop production. The focus of agricultural sciences has gone a little further with exploring the potential of plant microbiota or microbiome associated with plants above the ground (phyllosphere), within the plant (endosphere), and below ground (rhizosphere) (Vorholt 2012; Brader et al. 2017; Lemanceau et al. 2017). Diverse types of organisms including fungi, archaea, and prokaryotes exist among plant microbiota. To understand how the microbiome of plants is advantageous, it is essential to study various factors tangled in plant-microbe interactions (Hacquard 2016). There are plenty of reports of microorganisms being used as potential biofertilizers or biopesticides. Thus, they are used in agricultural operations as substitutes for chemical products (Mendes et al. 2013). Regarding biotic and abiotic stressors, plant genotype, and environmental factors in the plant microbiome might be possible to find suitable approaches for inoculation in the environment (Mitter et al. 2016).

16.2 Diversity of Microbes in Plant Rhizosphere

The rhizosphere is of central importance in a plant's well-being and excellence for microorganism-determined carbon confiscation, environment operational, and nutrient-pedaling in earth ecosystems. Effect of two factors—plant type and soil type—acts as a breeding habitat for microorganisms in the rhizosphere, which has an impact on their population. Enigmas of microbial life span have just been discovered due to molecular and microscopic techniques. Microorganism interactions are of enormous significance in the rhizosphere, effective in nutrient cycling in regular ecosystems along with agricultural and forest systems affecting the microbial populations in this habitat. Plant species, and soil type, had a significant influence on rhizosphere-associated microbial inhabitants. Rhizospheres are unique

microenvironments within ecosystems that come together to form a sophisticated microbial network.

Physicochemical and biological parts, as moderated by the dominant environmental conditions, are resolute by the many and diverse interactions among the complexity of the soil system. Soil functions have an acute impact on functional and the vast microbial communities' genetic activity, initiating metabolic processes involving precise enzyme actions. Sustainable agro-ecosystems are a collaboration between rhizobium and plant growth-promoting rhizobacteria (PGPR) for enhancing N_2 fixation. Associations between rhizosphere microbes and arbuscular mycorrhiza establish a useful mycorrhizosphere. Commercially available, genetically enhanced microorganisms are used to protect plants from diseases, promote plant development, and lessen the need for chemical fertilizers and biocides because they are environmentally friendly. There are more than thousands of varieties of microbes related to plant roots. Specific physical, chemical, and biological relationship in microbes leads to the development of microflora in root system of plants. Lynch (1990) has investigated the relationships between the rhizosphere's microorganisms in great detail.

Various methods are available to collect and identify root exudates which can be divided into the involvement of roots in aerated and sterile trap solutions and on the progression; most are based on the physical separation of the rhizoplane from the rhizosphere soil in a solid medium like sand or vermiculite. The membranes are porous that allow diffusion of root exudates and the diffusion of the root by hyphae in the soil compartment which may be horizontal or vertical.

16.3 Factors Affecting Plant Microbiota

Weather, pathogens, and human behaviors are examples of external environmental factors that have an impact on the microbiota above and in subsurface plant components. Plants growing in the environment are enlisted as different microbial communities in the rhizosphere (Balsanelli et al. 2013). The genotype of plant species involves the associated soil environment which contains root morphology, exudates, and type of rhizosphere deposits which play a major role in determining and designing the community structure of microbes in the rhizosphere of the plant (Agler et al. 2016).

By way of microbial colonization, host plants gain from resources derived from plants and develop community patterns that are taxonomically consistent. Microbiota may be produced by two distinct, mutually exclusive methods. Plant-microbe evolution may drive the plant selection process, resulting in active microbiota recruitment. It contributes to the shaping of plant community development. The idea that there are fundamental principles to community formation is supported by the consistency of microbial community patterns. Preliminary bacterial colonies are similar to their preceding; environmental variables, including soil and air, become increasingly plant-specific. Primary and secondary metabolites are derived from plants, as well as interactions between microbes and plants. Direct

contact and competition for existing occupied niches can lead to invasion and strain replacement.

The earth's soil is an incredibly rich source of microbes. It serves as both a catalyst for civic formation and a major bank for the rhizosphere's microbiota. The stepwise decline of microbial variety from the soil to the rhizosphere, rhizoplane, and roots indicates growing racial differentiation among microorganisms. This is in the plant, which grows on some microorganisms which are preferentially promoted or sometimes prevented.

The impact on microbiota is due to soil and plant-mediated factors, and temperature and the presence of herbivores and pollination insects are examples of environmental influences, UV radiation, water availability, and biogeography of microorganisms capable of plant colonization. Colonization of roots primarily powered by rhizo-deposits causes a change in the formation of the root community linked with the surrounding rhizosphere and bulk soil. The final community structure of the plant microbiota is influenced by microbial interactions and the host genotype.

A notable change in the make-up of a disease-preventing sugar beet rhizosphere community was brought on by the soilborne fungal pathogen *Rhizoctonia solani*. Chapelle et al. (2016) proposed a concept in which rhizosphere structure is altered by fungal invasion, either directly or indirectly, which results in stress reactions in the community. According to Suda et al. (2009) powdery mildew infection changed the bacterial community of the cucumber phyllosphere, to reduce the diversity. Through pathogenic effects, root-fungus commensalisms, and by driving the nutrient cycles, soil microbes have a significant negative or positive influence on plant growth and existence.

A major exotic plant that is native to Europe is *C. maculosa.* Callaway and Ridenour (2004) indicated that *C. maculosa* was capable of modifying soil microbiota in invaded soils to its advantage, thus favoring its invasion process to dominate many grasslands of western North America. Contrary to pathogenic germs' predisposition to infect only certain hosts, certain mycorrhizosphere fungi are more likely to infect a wide range of hosts. Due to the ability of the invader to amass mutualistic fungi in the absence of host-specific soil diseases, a striking weed's invaded area's soil microbiota response to the weed itself is probably neutral or favorable.

16.4 Relationship Among the Microbial Communities in Rhizosphere

Microbial interactions are regulated by precise molecules that are accountable for major environmental courses, such as the bio-geochemical cycle of matter and nutrients, plant health maintenance, and soil quality. The root-soil interface, plant roots, and soil elements are all interconnected by soil bacteria. The collection of interactions in root and microbe produces the emergence of an area around roots where microbes gather which is known as the dynamic arbuscular mycorrhiza habitat.

The rhizosphere is biased by roots at the zone of soil over and the release of materials that influence microbial activity. Rhizoplane is adhering soil particles on the root surface. Certain microbes are able to colonize root tissues; hence the root itself is a part of the system. Microbial colonization on the rhizoplane and root tissues is recognized as root colonization. The growing interest in improving the cooperative activities of arbuscular mycorrhiza and rhizosphere microbial inhabitants being applied to agriculture (Lucy et al. 2004) has been observed in recent decades. A wide variety of microorganisms is present among rhizosphere microhabitats.

Microbial plant symbiosis is exemplified by fungi which establish a mycorrhizal association with plant roots. The mycorrhizal fungi inhabit the root cortex biotropically, and then develop an external mycelium which is a bridge between the roots and the nearby soil microhabitats. The arbuscular mycorrhizae are unable to complete their life cycle in a host plant and are hence obligatory microbial symbionts. Identification challenges have limited the variety of arbuscular mycorrhizal fungi in natural habitats, which has impeded the progress in the enhancement of rhizosphere for the benefit of agriculture. Analysis of the ribosomal DNA sequence is a useful tool for interfering with the phylogenetic relationships of arbuscular mycorrhizae and a variety of natural rhizosphere populations.

The arbuscular mycorrhizal symbiosis increases the insufficiently mobile ionic forms of mineral nutrients or those with low concentrations in the soil solution in plants. An application in biological control of diseases that are carried by soil and in bioremediation of polluted soils is possible with applications of arbuscular mycorrhizae. Key procedures encouraging plant growth and health occur between individuals of various microbial kinds leading to the promotion and all interactions occurring in the rhizosphere and are mediated by plants. Improvement in nodulation due to N_2 fixation in legume plants can be done by rhizobacteria. Under field conditions, particularly N-based techniques support positive impacts of microorganisms working together.

The microbial populations are impacted by arbuscular mycorrhiza in plant physiology in both quantitative and qualitative ways. The rhizosphere of a mycorrhizal plant can vary from those of a non-mycorrhizal plant. Bacteria and fungi can be associated with both arbuscular mycorrhiza fungal and ectomycorrhizal structures (Frey-Klett et al. 2005). According to Barea et al. (2004), the creation of the rhizosphere is influenced by interactions between different microbial populations and their dependencies on one another. Interactions between many microbes including arbuscular mycorrhizal fungus rhizobium species and rhizobacteria affect plant health directly and/or indirectly. Effective plant growth, nutrient uptake, N_2 fixation, or the condition of the root system all exhibit particular and chosen functional compatibility relationships between arbuscular mycorrhiza and the microbial inoculates by several microbial combinations. Fungi in plant roots have been demonstrated to decrease arbuscular mycorrhiza caused by soilborne plant diseases while increasing plant tolerance. The biocontrol bacteria *Bacillus subtilis*, the fungal strains *Glomus mosseae*, *Pseudomonas fluorescens*, *Trichoderma harzianum*, and *Gliocladium catenulatum*, as well as other microbes were involved. Inoculated

microorganisms except *T. harzianum* and *G. mosseae* developed in the rhizosphere had no more growth-promoting effects than a single inoculation. The most promising rhizobacteria for boosting plant growth was *B. subtilis*.

16.5 Bacteria in Plant Nutrients

Synergetic bacteria in the roots cause root hair elongation and elongated bacteria escape at the hair tips, enhancing cell walls and emerging in the rhizosphere where they may obtain extra nutrients. Microbes proliferate on the rhizoplane in the exudate zone along with the root meristem. Most plants absorb dissolved inorganic fertilizers from soils to get their nutrition (Manetas 2012). Some plants have symbiotic relationships with prokaryotes that fix nitrogen with their roots and then transport that nitrogen to the plant (Pawlowski and Demchenko 2012). These nitrogen-transfer symbioses are intercellular in nature and diazotrophic actinomycetes are the colonizers. Common plant families with actinorhizal symbioses include Betulaceae, Elaeagnaceae, Fagaceae, Myricaceae, and Rosaceae (Santi et al. 2013). A few diazotrophic cyanobacteria can repair nitrogen imbalances in plant tissues.

Alpha-proteobacteria Bosea, Methylobacterium, Beta-proteobacteria Achromobacter, Burkholderia, Gamma-proteobacteria such as *Acinetobacter*, *Klebsiella*, *Pantoea*, *Pseudomonas*, *Bacillus* and *Paenibacillus*, and *Actinobacteria*, *Curtobacterium*, are among the kinds of Gram-positive bacteria found in nutrient-restrictive soils; it is known that plants increase exudate secretion. This increases microbial activity around roots and increases "microbial mining" for nutrients. Microbes that will thrive in the root exudates are attracted to these compounds (Ortíz-Castro et al. 2009). *Burkholderia* and *Klebsiella* are commonly observed to participate in the fixation of atmospheric nitrogen. In the tissues of plant roots, bacteria are entangled in the rhizophagy cycle and nitrogen fixation. Microbes were being consumed by plants, and rhizophagy moved nitrogen at a slower rate than soluble inorganic nitrogen. Root hairs may hold synergistic microorganisms and nutrients in the rhizosphere.

Endophytic bacteria due to their varying capacities to manufacture antioxidants, *Pseudomonas* spp. and *Micrococcus luteus*, two bacteria involved in the rhizophagy cycle, showed varying levels of resistance to oxygen destruction. Reactive oxygen produced by their hosts can break down rhizophagy bacteria. The growth of the grass host plants may be stimulated by pseudomonads' provision of a supply of nutrients, a cycle known as "rhizophagy" in which bacteria are regularly harvested by roots to provide minerals, vitamins, and other growth-promoting substances that can serve as a source of nutrition for plants. From *Froelichia gracilis* plant seedlings, the bacterium *Aureobasidium pullulans* was isolated. Based on the presence of fungi in the periplasmic spaces of the parenchyma, as well as after the tip and in root hairs, the initial colonization appears to be at the root tip. The root cell start producing defensive reactive oxygen as response to entry of microbes in root tip (White 2018).

16.6 Role of PGPR on Plants Productivity

The layer of soil around the root of plants is called rhizosphere; it is a habitat of many bacterial populations that are called rhizospheric bacteria. Of these, many would be categorized as plant growth-promoting rhizobacteria, as they play an important role in the growth and provide resistance to growing crop/plants against many pathogenic microbes. Bacterial consortium classified as PGPR can be used as biofertilizers for enhancing crop yield.

For sustainable agriculture research focus is now shifted towards PGPR for the improvement of crop productivity. There are many reports as well as evidence to prove the effective role of these microbes in growth promotion. PGPR are naturally occurring microbes residing in the rhizospheric regions and provide nutrients for plant development and protection from pathogens. Isolates of rhizospheric soil enhance crop productivity in two ways—by providing nutrients as well as providing resistance/tolerance to crop plants (Ali et al. 2014). PGPR follow two mechanisms, viz. direct and indirect, to enhance crop productivity. In the direct method, PGPR get involved in the production of plant growth promotors, vitamins, and essential minerals. However, the indirect method involves activation of the pathways that improve resistance in plants, e.g., lytic enzyme production, antibiotic production, and production of hydrogen cyanide and induced systemic resistance that, in turn, enhance crop productivity. The use of PGPR in place of chemical fertilizers, insecticides, and pesticides is safer for our environment too as it does not contain any hazardous components.

PGPR are very helpful in providing major nutrients, viz. nitrogen, phosphorus, etc., as both are insoluble and PGPR help in solubilizing them to crop plants (Yadav and Dadarwal 1997). Proteins, nucleic acid, and other vital nitrogenous compounds are composed of nitrogen and other essential primary nutrients (Venturla et al. 2013).

Iron is one of the important micronutrients for the growth of all the organisms. Low iron content in soil results in poor growth of plants too. PGPR help in providing iron to plant roots. Whipps (2001) reported that "PGPR produce siderophores to compete and attain Fe^{3+} (ferric ions) from surrounding under iron scarcity." PGPR provide iron in soluble form to plants (Kloepper et al. 1980). According to Wang et al. (1993) iron requirement of some of the economically important crops, viz. cotton, peanut, sorghum, and cucumber, is met by siderophores secreted by soil-borne beneficial microorganisms.

16.7 Mycorrhizal Fungi and Plant Productivity

Mycorrhizal fungi, generally called arbuscular mycorrhizal fungus (AMF), belong to the phylum mucoromycota. They are soilborne and are found in a symbiotic relationship with plants. They not only help plants in nutrient uptake and cycling, but also protect them from fungal infestations and help them cope up with abiotic stresses. According to Heijden et al. (1998), mycorrhizal fungi induce plant diversity and productivity. There are reports showing impact of mycorrhizal colonization on

the diversity, survival, productivity, foliar quality, clonal morphology, and fitness in the plants of various regions (Jonsson et al. 2001).

Physiological variants among mycorrhizal fungi are responsible for an increase in plant productivity with the diversity of mycorrhizal fungi. An extra functionally diverse mycorrhizal symbiosis involves the synchronized colonization of the host plant by both arbuscular mycorrhiza and ectomycorrhizal fungi.

Throughout numerous steps of their life succession, prokaryotes are linked with the extra-radical hyphae of mycorrhizal fungi, through mycorrhizal sporocarps and roots, the Ascomycota and Basidiomycota, that escort the interdependent fungi. Arbuscular mycorrhizal association was established to show a pivotal character in the conservation of the soil bacteriological structure. Rhizosphere of mycorrhizal fungi, and their partial spreading may be expounded by their short merit for non-external ectomycorrhizal fungi environment. Arbuscular mycorrhizal fungi are the main factor defining the ectomycorrhizal fungi on grass roots; they too institute that this essential ectomycorrhizal fungus gets affected by topsoil pH and for rhizosphere soils in particular is spatially structured.

A substantial quantity of microbial biodiversity in terms of bacterium/mycorrhizal fungus and plant association specifically that in the case of ectomycorrhizal fungi is reflected in the mycorrhizosphere. Transformation of the ectomycorrhizal fungi into biomass by ingestion of fungus-derived substrates elucidates the aptitude of bacteria to acquire nutrients via fungus. Recently three forms of bacteriological mycophagy: extracellular necrotrophy, endo-cellular biotrophy, and extracellular biotrophy (Leveau and Preston 2008). Necrotrophic and extracellular biotrophic actions of bacteria en route for mycorrhizal fungi are revealed by various studies. *Burkholderia* spp. penetrated the arbuscular mycorrhizal fungus, *Gigaspora decipiens* colonize senescing spores adhering to fungal hyphae (Levy et al. 2003). Electron microscopy exposed that the bacteria feed on the outside hyaline spore of arbuscular mycorrhizal fungi.

16.8 Archean Microbiota in Crop Productivity: A New Area of Research

Soil has a pivotal role to play in plant progression for the development of agricultural sustainability and management. The environment for the above can be affected to a great extent as soil is an important factor for uncertainty and renewability of the system. As we all know that the section of soil in or near the plant roots in which the chemical and microbial phenomenon is affected by their progression, breathing, and nutrient interchange. Various relations with plant hosts, bacterial diversity, fungi, archaea, and other microbes are exhibited in the rhizosphere. Many useful microbes including a variety of bacteria and different species of fungi and archaea have their specific functions in procurement of nutrients for plants required for their growth and expansion in the ecosystem as its integral component. The well-being of living beings is dependent on fibrous crops which improve the quality of food based on the quality of soil. Soil type is the deciding factor for the content of air and water

being utilized straight as it is a significant link to the atmosphere. Soil gradients are responsible for grounds of the climatic conditions and its uneven spreading or dispersal across the geographical sectors of the planet.

Archaea have more similarities with eukaryotes, however structurally more similar with prokaryotic microbes (Cavicchioli 2011). Archaea and eukaryotes stake more cohesion than eukarya and bacteria normally. Nucleus expansion occurred after bacteria and ancestral harmony were divided. Although archaea are coined as prokaryotes, eukaryotes are more correlated and henceforth cannot be considered under any of the two spheres (Bacteria and Eukarya). The survival ability of archaea in extreme environs which are salinity and temperature is commendable. Currently, in the biome, methanogens are the microbes that originate their liveliness from methane oxidation. Archaea are the only known microbes that produce methane and are the original methanogens in a particular environment. Deppenmeier (2002) revealed the role of archaea in the carbon cycle, as methane is manufactured from the flouting down of carbon which is the key gas of greenhouse.

Exclusive characteristics of archaea may present an alternate way of improving agricultural production. Archaea are cosmopolitan, including extremophilic areas. Archaea are found in diverse environments and are important in nutrient recycling which is very significant in agriculture as nutrients are required in large quantities to raise plants. This leads to nitrogen removal as denitrification, breathing centered on nitrate, and responses in the ecosystem as nitrogen fixation and assimilation. The enormous importance of archaea in responses to ammonia, mainly in the marine and soil surroundings, is discovered. Nitrite is produced by archaea which oxidizes in nitrate by additional variants of microorganisms to be consumed individually by microbes as well as plants. Authentication indicating persistent manipulation of plant development promoting rhizobacteria, various mycorrhizal fungi, and a variety of archaeal microbes may bring breakthroughs in agricultural sustainability. Metagenomics and computational biology have the capability to determine the entire variety and purposes of biotechnological benefits, for the purpose of agricultural production (Leininger et al. 2006).

Sulfur compounds are released and oxidized to get recycled into the atmosphere in large quantities through archaeal microbes which grow in the environment. Archaea enormously decompose biological matter by eliminating hydrogen from the carbon cycle. In anaerobic systems or environmental zones such as sewage treatment, marshes, and sediments, archaea act as decomposers. Archaea also produce novel antibiotics that are potentially powerful. Archaeocins have been identified, isolated, and characterized by the Sulfolobus and Haloarchaea class of archaea. Fand and Leyva (2008) isolated antibiotics that are different from those produced by bacteria with a different mode of action which needs an in-depth investigation.

16.9 Conclusion

The maximum portion of the national income of most of the developing countries including India meets up their need for food and employment through agriculture. In order to meet our future agricultural requirements, sustainable agriculture is potentially important in the current time. Microbial populaces in the rhizosphere are known to gain mycorrhizal symbioses and apply both to ectomycorrhiza and to arbuscular mycorrhizal associations. Soil microorganisms stimulate arbuscular mycorrhizal mycelia in the rhizosphere root diffusion which are accountable to create complexes that escalate the rates of root exudation. Some populations of bacteria and fungi inhabit the root and soil environments where they initiate cooperating deeds to profit plant development. In the rhizosphere, the count of microbes is always higher than in bulk soil as proved by plate counts analysis. Gram-negative bacteria are stimulated by rhizo-deposition whereas Gram-positive bacteria are inhibited. PGPR have shown potential as biofertilizers, contributing to plant development, amplified yield, solubilization of potassium and phosphorus, and intake of nitrogen and other elements via inoculation. A large amount of synthetic fertilizers are utilized to restock soil nitrogen and phosphorous, ending in high value along with enhanced environmental pollution in the absence of PGPR. Water accessibility, soil temperature, ultraviolet radiation along with macronutrient dispersal have been connected with communal changes regarding macro element availability in the soil. Concentration of nitrogen/phosphate produce by mycorrhizal fungi was quite higher than nitrogen fixing bacteria. Co-active microbial connections in the plant rhizosphere increase the greater indulgence of these processes, which enables their actual submissions in biotechnology due to new technologies.

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Microbial Management of Fusarium Wilt in Banana: A Comprehensive Overview

17

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Abstract

Globally, the production of bananas (*Musa* sp. L.) often suffers from various environmental challenges. Among them, biotic stress-induced disease caused by phytopathogenic soil microorganisms is the most threatening factor. *Fusarium oxysporum* f. sp. *cubense Foc* Tropical Race 4 (*Foc*-TR4) is an important soil-borne fungus triggering the severe disease, Fusarium wilt (Panama disease) in bananas. Following infection in a wide variety of bananas, strain *Foc*-TR4 harshly reduced their cultivation. Herein, we have summarized the present scenario of Fusarium wilt disease. Numerous challenges have been proposed by researchers to control the Panama disease as well as to improve banana production. Primarily aiming at increasing disease tolerance to bananas and improving their cultivation, various management strategies like crop rotation, burning of rice husks, biological soil disinfection, and use of chemical fungicides have been developed. However, these chemical and cultural practices have several drawbacks and therefore not often used. Plant growth-promoting (PGP) bacteria

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offer one of the most environmentally friendly, effective, safe, and economically sound solution to combat the Panama disease. Apart from growth promotion, this PGPR prevents phyto-pathogen-induced diseases. The recent chapter highlights the utilization of beneficial and antagonistic PGPR and their efficacy against diseases, and bacterial-mediated mechanisms involved in managing Panama disease. Induced systemic resistance (ISR), production of antibiotics, extracellular enzymes, cyanogenic compounds, siderophores, and other antifungal metabolites are the main mechanisms involved in PGPR-induced disease suppression. It will be possible to build or select sustainable management techniques to prevent or aid to minimize Fusarium wilt incidence in banana plantations using the scientific knowledge gathered in this research. The use of indigenous PGP rhizobacteria in plant disease control is gaining popularity as environmental and health concerns underscore the need for a more sustainable agriculture system.

Keywords

Panama disease · *Fusarium oxysporum* f. sp. *cubense* · Tropical Race 4 · Biological control agents (BCAs) · PGPR · Induced systemic resistance · Rhizobacteria

17.1 Introduction

Bananas are the world's utmost important fruit and rank among the world's top ten chief foods. The production of bananas is extremely affected by Fusarium wilt. This disease is caused by *Fusarium oxysporum* f. sp. *cubense* (foc) and is one of the greatest devastating banana diseases in history. Foc causes wilt, destroying xylem and killing banana plants. It can live freely in soil for an unexpected period. Firstly, this disease was found in Australia, and it has since then spread throughout the world through the casual interchange of planting materials and the association of spore-bearing soil (Dita et al. 2018). It is one of the most deleterious diseases in the world which is present in different types of soil. Several fungal diseases can reduce banana production worldwide, including aerial (e.g., Botryodiplodia finger rot, Anthracnose, fungal Scald and diamond spot brown spot, Cordana leaf spot, Cigar-end rot, Cladosporium speckle, pitting disease, black tip, and sigatoka leaf diseases) and soil-borne (e.g., root rot and Fusarium wilt). Panama disease, initiated by *Fusarium oxysporum* f. sp. *cubense* (Foc), is the primary hazard and limiting factor for various economically and strategically important banana cultivars around the world. Scientific interest in banana fusarium wilt has grown in recent decades, particularly in the major producing countries of bananas. This disease is the most common phytopathological problem in tropical banana crops. In any case, in spite of the staggering effect that Foc has had throughout the long term, and even though there is broad data concerning the science and hereditary variety of this microorganism, there are as yet restricted data accessible on its geographical distribution regarding soil and environment, and specifically, precision in the data on the agro-natural factors that influence

the study of disease transmission of this disease is still lacking. These data would be appropriate for a more extensive and further comprehension of the phytosanitary issues that Foc presents before banana plantations. Especially, it can furnish an understanding of its relationship with other major agronomic parts, which might be valuable for managing bananas and the infection. Consequently, this chapter aims to present an efficient, thoughtful review of the recent scenario of the major agricultural and environmental factors that affect the progress and escalation of Fusarium Wilt in bananas (Olivares et al. 2021).

Foc contaminates the roots of bananas, blocking the plant's vascular system and causing wilt and eventual death. The fungus reproduces asexually and can live for up to 30 years as chlamydospores in contaminated banana fields. It lives as a symptomless endophyte in alternate host plants such as weeds. Foc is dispersed territorially and locally with infected material of plants, soil, and water. Foc is an extremely different pathogen containing dissimilar evolutionary lineages. It is categorized into three biological races on the basis of its infectiousness to various host cultivars with Foc race (R1) affecting "Pisang Awak (ABB) bananas," "Gros Michel" (AAA), "Lady Finger" (AAB); Foc race 2 (R2) affecting "Bluggoe" (ABB) and related ABB clones; and Foc race 4 (R4) affecting the "Cavendish" banana subgroup (AAA), as well as the majority of Foc R1 and R2 vulnerable cultivars. However, when it comes to identifying Foc strains, the race structure is frequently unclear and erroneous. Heterokaryon development among the isolates of Foc is utilized to split the fungus into 24 vegetative compatibility groups, which aids in strain identification (vegetative compatibility groups) (Chittarath et al. 2022).

17.2 Symptoms

A typical wilt syndrome is caused by Foc that infests banana plant through the pseudostem vessels causing root rotting, rhizome, and necrosis. The most common signs become noticeable in vulnerable banana plants after the beginning of outer symptoms like light green lines on the petiole's base and a brown-reddish color staining of the vessels under the epidermis of the petiole. These symptoms appear 2–5 months after the roots have been infected. Foc and the other members of the *Fusarium oxysporum* (Fo) species produce fusaric acid (FA), a form of phytotoxin that has been linked to the symptom of leaf chlorosis (Jamil et al. 2019).

Banana wilt caused by *Fusarium* is a distinctive vascular wilt disease. "Tolerance" refers to cultivars that can withstand contamination by *Foc* without developing acute symptoms, while "resistance" is utilized for cultivars that loss somewhat the effects of *F. oxysporum* f. sp. *Cubense*. Remarkably, both the resistant and affected roots of banana cultivars are infected by *Foc,* but infection present in the vascularized sections of the rhizome is more severe in susceptible genotypes. The xylem lumena produces tyloses, gums, and gels in reaction to infection, while resistant cultivars produce host products prior to and more quickly than susceptible cultivars.

Fig. 17.1 Banana tree showing Panama disease

Tolerant cultivars do not experience infection of the pseudostem because the pathogen is obstructed by these host products. However, in sensitive cultivars, it occurs before these host reactions. *F. oxysporum* f. sp. *cubense* is found in surface waters, and utilization of such water for irrigation has facilitated the pathogen's rapid spread across river basins. *F. oxysporum* f. sp. *cubense* can also be transferred by infected tools, farm equipment, clothing, and footwear (Fig. 17.1).

17.3 Epidemiology

Plant disease epidemiology is a critical component of plant health. Epidemiological data can be utilized to plan and shape policy decisions for evidence-based management strategies in a strategic way. Agriculture is fighting new and recurring epidemics all the time, and there is a group of researchers who have come up with

a novel approach to solving a problem. Globally, Panama disease is mostly seen in tropical and subtropical regions. This disease was originally reported from banana plants of the sugar (Silk AAB group) type in Eagle Farm, Brisbane, Queensland, Australia, in 1876 (Bancroft 1876). Through transportation of infested planting materials, the disease got spread to different parts of the world. Except for the South Pacific Islands, Papua New Guinea, and several nations bordering the Mediterranean, this disease has spread to virtually every banana-growing region, including Asia, Africa, Australia, the South Pacific regions, and Latin America.

Due to the sexual reproduction in the pathogenic fungus being uncommon in nature, Cavendish varieties can resist Fusarium wilt for decades, preventing the spread of new virulent pathotypes. Cavendish variants that were banana industry's only chance after the Gros Michel epidemics were infected with *Foc* race 4, infecting all other known banana cultivars. The *Foc* race 4 strain is destroying Cavendish types in Australia, China, and South Africa, Taiwan, Spain (Canary Islands). *Foc* tropical race 4 strains are primarily found in tropical Asia and northern Australia, while *Foc* subtropical race 4 strains are found in subtropical nations such as Australia, Taiwan, South Africa, and the Canary Islands. Only under severe conditions can *Foc* subtropical race 4 contaminate race 1 resistant Cavendish cultivars, whereas Foc tropical race 4 can contaminate both stressed and unstressed Cavendish cultivars. If *Foc* race 4 establishes itself in the significant banana-growing locations such as South America, Brazil, and India, the banana's export potential may be restricted, as there is currently no recognized equivalent for the commercial significance Cavendish cultivars (Ghag et al. 2015).

17.4 Distribution in India

Fusarium wilt disease by Foc race 1 strain caused crop reductions of up to 50% to 70% in India. Monthan, Ney Poovan, Rasthali, Amrithapani, Karpuravalli, and Virupakshi were the main varieties affected. In India, some banana varieties, for example, red banana and the Nendran, have been discovered to be unaffected by the race 1 infections. Because the race structure of Foc is undefined to such an extent that the vegetative compatibility method was utilized to classify the Foc, cross-compatibility exists between some vegetatively compatible groups, resulting in VCG complexes. Furthermore, no new plants can grow in the contaminated field since *Foc* remains dormant in the soil for quite a time. This will ultimately lead to the transition of cultivation of another crop or the relocation of farmers to other places. As a result, Panama disease has appeared as a severe danger to the long-term viability of banana cultivation in India. It is now critical to restrict the expansion of this disease by entirely separating the affected field and prohibiting the activity of the cultivator(s) and movement of any material from the infected field that can contribute to the spread of the pathogen to newer areas. The contaminated plants must be eradicated. Usage of pathogen-free planting material will help stop the infection from spreading. To stop the spread of Fusarium wilt disease, farm

employees must be educated on the disease's importance, as well as how to identify, diagnose, and prevent it. In the long run, the current situation necessitates a long-term remedy, for instance, the plantation of resistant cultivars, identification of antagonist microorganisms, and identification of the botanicals, for the minimization of severity of disease.

17.5 Disease Cycle

The signs of Panama infection in banana plants are similar to those of any other Fusarium wilt disease, including yellowing and wilting of plants. By passing the plant's defense mechanisms, the fungus gets access to the plant system through the roots. Chlamydospores in the soil attach themselves to the root caps and germinate, colonizing the host plant's root surface. Foc reaches the small lateral roots epidermal cells by breaching the cell wall directly or entering through wounds or damage areas. The mycelia go in the intercellular gaps of the root and epidermal cells, following the production of many chlamydospores, macroconidia, and microconidia in a parasitic monokaryotic stage.

When hyphae infiltrate the roots, they develop intercellularly and intracellularly, invading the cortex tissue before crossing the endodermis to spread the xylem arteries. The fungus grows rapidly inside the plant's vascular system. Intense mycelial propagation and sporulation block the xylem vessels, causing less water uptake efficiency in the host plant, resulting in water insufficiency, and eventually causing wilting. The fungus moves acropetally via the conducting vessels to get around the obstacles, alternating between the sporulating and germination phases. Microconidia germinate when they are inhibited by the conducting tissue's sieve cells, resulting in the mycelium that passes through it. The transport of *Foc* in xylem vessels is constrained in *Foc* race 1 resistant cultivars due to gel deposition in the Cavendish cultivar. The fungus spreads through the corm tissue and to the pseudostem through the sap flow, appearing as purple or brown strands. The older leaves of the infected plant yellow and the base leaves wilt and fall, leaving a skirt of dead leaves around the pseudostem. As the infection progresses, the discoloration of rhizome worsens, and the plant entirely wilts. The fungus then nourishes saprophytically on dead plant parts like leaves, pseudostems, and the roots, producing a large number of spores that form the soil flora. Chlamydospores in the soil survive for three to four decades, causing a wilt outbreak in freshly planted vulnerable banana varieties.

As a result, cultivating bananas on Foc-infected soils is risky. The physico-chemical properties of the soil have an impact on the germination of chlamydospores. In suppressive soils with actinomycetes and bacteria, chlamydospores germinate poorly making them favorable soils with a large amount of filamentous fungus and yeast. Soil topography and rhizosphere microorganisms influence the amount of chlamydospore germination and the progression of Panama disease. Wind, run-off waters, and unintended distribution via animals, birds,

humans, and even agricultural equipment transfer these spores from contaminated soils to disease-free soils.

In the absence of banana plants, Foc has been observed colonizing the roots of non-host plants such as *Chloris inflate, Euphorbia heterophylla, Tridax procumbens, Commelina diffuse,* and *Cyanthillium cinereum* to complete part of its life cycle. These plants do not show symptoms of Fusarium wilt, but they frequently carry a high inoculum of Foc, which is enough to trigger epidemics. The interface between Foc and non-host plants may also affect the pathogen's epidemiology and evolution potential. Transportation of infected asymptomatic secondary hosts across regions, countries, and continents is thus a possible cause of *Foc* transmission. The interaction between Foc and the banana root system is a complex process that requires extensive research. The wilt phenotype is caused by an accumulation of high molecular weight polysaccharides, degraded plant cell debris, secondary metabolites, and conidia, which obstructs water supply to the plant. Later, this infection defeats the plant's defense mechanism and causes cell death. In the later phases of infection, Foc promotes pathogen-induced necrosis. *Fusarium* spp. is a soil-borne necrotrophic fungus, according to most studies. However, it goes through a brief biotrophic phase before changing to a necrotrophic phase, thus being a hemibiotroph. Plant cell death is also triggered by potent phytotoxins such as fusaric acid and beauvericin, which are produced by Foc during infection (BEA). BEA inhibits cholesterol acyltransferase and causes conventional programmed cell death, whereas fusaric acid is a known powerful phytotoxin that causes senescence in infested banana leaves. In biological membranes, BEA also causes pore development. Banana plants produce H_2O_2 and defensive enzymes such as superoxide dismutase, polyphenol oxidases, peroxidases, and catalase in response to Foc infection (Ghag et al. 2015).

17.6 Mechanism of Biocontrol

Disease suppressive areas have been found to exhibit increased microbial community richness and diversity (Shen et al. 2015), as well as likely a high number of antagonistic individuals, as demonstrated for streptomycetes (Jauri et al. 2018). Furthermore, changes in the makeup of these groups were linked to whether the soil was disease suppressive (presence of Acidobacteria) or conducive (abundance of Bacteroidetes) (Shen et al. 2015). In China, manipulating the microbiota of the banana rhizosphere by introducing known antagonists unaccompanied or in arrangement with biological changes (bio-organic fertilizers) has previously achieved good outcomes against Foc TR4 (Shen et al. 2013; Xue et al. 2015). Changes in the structure and content of the microbial community are also a result of this method, which can be used to control the Fusarium wilt of bananas better (Shen et al. 2015; Fu et al. 2016).

The biocontrol mechanisms behind biocontrol agents are numerous and diverse (Fig. 17.2). It is critical to understand the biocontrol mechanisms of action, including their limitations and necessities, to maximize their potential for disease treatment

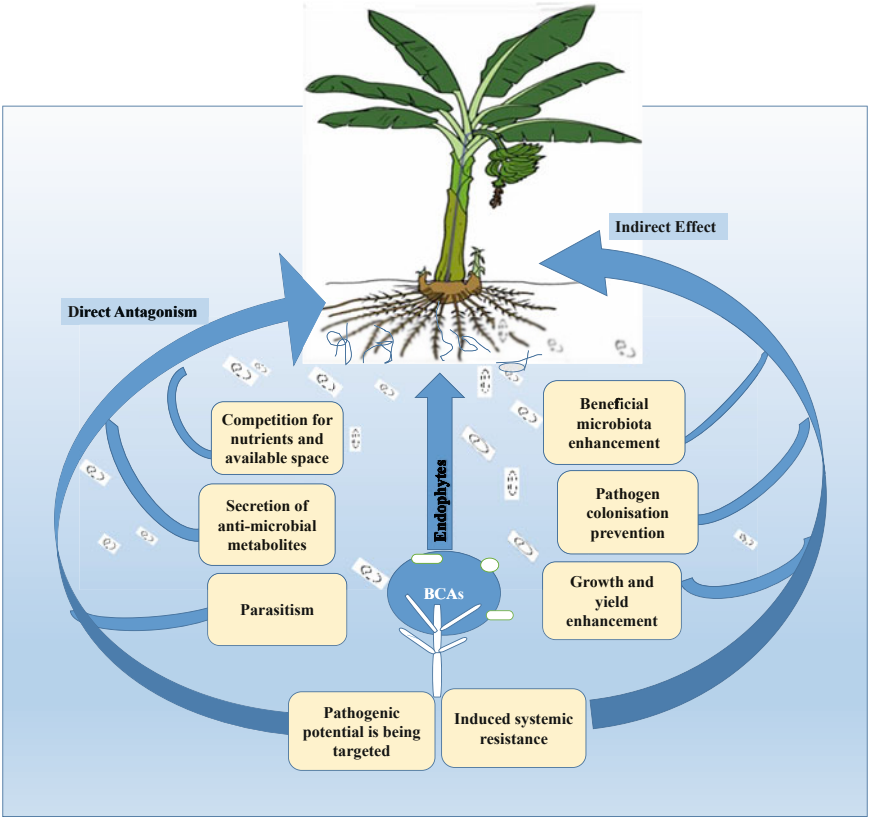

Fig. 17.2 BCAs and their potential modes of action

(Narayanasamy 2013). Furthermore, combining BCAs with diverse mechanisms of action may effect in improved biocontrol due to additive, or even synergistic, interactions among biocontrol activity (Parnell et al. 2016; De Vrieze et al. 2018) and how biocontrol activity work either directly or indirectly against Foc. Antibiosis (antibiotics, lytic enzymes, volatile organic chemicals, and so forth), parasitism, and competition (for space and/or nutrition) all can cause direct antagonism (Table 17.1). Plant growth promotion, induction of local/systemic resistance, and changes in soil/plant microbiota in favor of more useful microbial taxa are all examples of activities that function indirectly against the pathogen, or at the very least aid to decrease infection or disease. Biocontrol agents have several primary processes, one of which is antibiosis. Indeed, in vitro selection of new biocontrol activity frequently focuses on the pathogen's sole antimicrobial activity, with other pathways examined afterward, possibly after the BCA's efficiency has been established at least under controlled settings.

Table 17.1 Some examples of biological control agents (BCAs) involved in the management of Panama disease of banana

PGPR applied as BCAs	Mechanism involved	Best disease control obtained (%)	Effective remarks	References
Bacillus amyloliquefaciens strain W19	Secreted growth-regulating essential metabolites such as phytohormones (indole-3-acetic acid, gibberellin, zeatin, ethylene, abscisic acid) and antifungal compounds	–	PGPR strain enhanced the plant height (23.64%) and pseudostem diameter (26.57%) and other growth features of banana	Wang et al. (2013)
Streptomyces morookaensis strain Sm4–1986	Synthesized indole-3-acetic acid and antifungal metabolites	Approximately 50%	Reduced the severity of disease index and occurrence of disease in banana	Zhu et al. (2021)
Pseudomonas sp.	Produced siderophore and other antifungal compounds	21–73–50-38%	Suppressed the mycelial growth of the pathogen Controlled the disease incidence	
Bacillus amyloliquefaciens strain NJN-6	Secreted PG-promoting hormones indole-3-acetic acid gibberellin (GA3), and some important antifungal lipopeptides iturin A	–	Applied strains potentially inhibited the occurrence of wilt disease and considerably enhanced the growth and production of bananas	Yuan et al. (2015)
Pseudomonas fluorescens	Antifungal metabolites and several extracellular	60%	Significantly increased the growth and yield efficacy of banana	Selvaraj et al. (2014)
PAB-2 (*bacillus* sp.)	Antifungal metabolites and growth-regulating substances	46.9%	By synthesizing the antimicrobial substances and reducing the disease severity, strain increased the length, biomass, and pseudostem diameter of plants	Li et al. (2011a, b)

(continued)

Table 17.1 (continued)

PGPR applied as BCAs	Mechanism involved	Best disease control obtained (%)	Effective remarks	References
Bacillus licheniformis CSR-D4	Secreted secondary antifungal metabolites like iturin, fengycin, surfactin, and bacillomycin	77.59%	The incidence of disease was potentially reduced and antioxidant defensive enzymatic (POD, PAL, PO chitinase, and β-1,3 glucanase) activities were enhanced	Yadav et al. (2021)
Bacillus amyloliquefaciens	Antifungal compounds, such as iturin, surfactin, fengycin, and bacillomycin	70%	BCAs increased the plant height, stem diameter, and leaf area in banana	Wang et al. (2015)
Bacillus siamensis Gxun-6	Antifungal metabolites such as siderophore and extracellular enzymes	68.8%	Both under in vitro and in vivo, strain strongly suppressed the fungal pathogen and improved the growth of banana	Shen et al. (2022)
Bacillus amyloliquefaciens S185	Produced antifungal compound iturin A5	–	The biocontrol agent completely reduced the severity of disease in vitro and augmented the banana growth by efficiently colonized the plants	Singh et al. (2021)
Streptomyces malaysiensis 8ZJF-21	Produced several extracellular enzymes and novel antifungal metabolites	–	Under in vitro, strain inhibited mycelial growth and spore germination, increased the growth of banana	Zhang et al. (2022)
Pseudomonas fluorescens strain Pf10	Secretion of antifungal protein and fusaric acid	50%	Suppressed the incidence of diseases, controlled the	Thangavelu et al. (2001)

(continued)

Table 17.1 (continued)

PGPR applied as BCAs	Mechanism involved	Best disease control obtained (%)	Effective remarks	References
			disease in bananas, and increased plant growth	
Bacillus velezensis H-6	Secreted antifungal metabolites	63.3–66.7%	Suppressed the mycelial growth Reduced the disease incidence in plants and improved the production of banana	Huang et al. (2019)

17.6.1 Endophytic Bacteria

The use of endophytic bacteria as BCA is a smart strategy because it is environmentally beneficial. Antibiotic endophytic bacteria have been identified from various plant hosts that can influence the growth and survival of their pathogens. *Burkholderia* sp. generates the antifungal drug pyrrolnitrin (prn), a common broad-spectrum antibiotic, as well as volatile chemicals that impede the growth of phytopathogenic fungi. All plants include a wide range of beneficial or neutral bacteria that live within their tissues without harming the host (Hardoim et al. 2015). Helpful endophytes can increase plant health and growth through a variety of methods including phosphate solubilization, nitrogen fixation stimulation of defense responses, phytohormone synthesis, reduction of abiotic stress by lowering ethylene levels, etc. (Compant et al. 2016) They have significant agro-biotech potential that has yet to be completely exploited (Mercado-Blanco and Lugtenberg 2014). Endophytes have evolved ways to exist inside the plant interior (for example, nutrition availability and evasion/modifying host defensive responses), where they can also employ BCAs against diseases. As a result, there is a growing body of knowledge about the isolation, characterization, and evaluation of definite culturable individuals of indigenous endophytic communities as biocontrol agents. In the banana plant, endophytic bacteria and fungi have been studied for their biocontrol activity against Foc and other biotic limitations (Ortiz and Pocasangre 2012).

The fact that various experiments using endophytes against Fusarium wilt of banana have been undertaken in recent years is fascinating. In semi-field and field circumstances, *P. aeruginosa* FJAT-346-PA reduced Fusarium wilt of banana by 82–84%, according to an early study. The strain has been shown to colonize banana roots and stems, as well as boost plant growth (Yu et al. 2010a, b). Cao et al. (2004) studied the actinomycete communities present inside healthy and damaged banana plant leaves and roots.

Similar to Foc and *Colletotrichum guaranicola*, three endophytic *Bacillus* spp. identified from *Musa* cultivars in Brazil demonstrated antagonistic action (Souza et al. 2014). Endophytic bacteria from the genera *Brevibacterium, Corynebacterium, Arthrobacter, Curtobacterium, Kytococcus, Kocuria, Naumannella, Rothia, Micrococcus, and Tessaracoccus, α- and γ- Proteobacteria (Enterobacter, Brevundimonas, Klebsiella, Serratia, and Pseudomonas* (Sekhar and Thomas 2015). *Klebsiella variicola, Pseudomonas aeruginosa,* and *Enterobacter cloacae* strain displayed varying antagonistic activity against Foc in this study's collection, but their potential as impressive biocontrol activity remains to be proven. Other plants, such as weeds and medicinal plants, have been found to have fungal and bacterial endophytes (Ting et al. 2009). He et al. (2002) suggest that they could be useful repositories of Foc antagonists. Finally, some research goes a step further by investigating the mechanism behind the antagonistic impact. Ting et al. (2010), for example, looked into the effect of volatiles formed by different fungal endophytes in Foc R4 growth inhibition. Even if disease control was inconsistent, the same authors suggested that *Penicillium citrinum* BTF08 isolated from banana internal stem tissues induced host resistance as a mechanism implicated in control of Fusarium wilt of banana (Ting et al. 2012).

Remarkably, some of the investigations concentrated on inoculating banana plants with biocontrol agents during in vitro propagation stage. In vitro co-culturing of banana plants with *Pseudomonas fluorescens* Pf1*, Bacillus subtilis* EPB 10, and EPB 56 resulted in effective control of Fusarium wilt in the field and improved leaf nutritional status, vegetative development, bunch production, and fruit quality (Kavino et al. 2016). The endophyte and rhizobacterial strain resulted in 78% control of Fusarium wilt of banana and a significantly higher bunch weight in two field trials (Kavino and Manoranjitham 2016). Moreover, when using a range of naturally occurring uncultivated endophytes from healthy banana plants from a commercial plantation to inoculate banana tissue culture plantlets, this technique was found to be effective (Lian et al. 2009). In this work, endophytes were reintroduced to the banana tissue culture, which resulted in a 67% reduction in Panama wilt disease caused by Foc R4 (artificial inoculation) and growth promotion under greenhouse circumstances. Under greenhouse conditions, 10 non-pathogenic *F. oxysporum* isolates isolated from healthy micro-propagated "Cavendish" banana roots were capable of considerably reducing Fusarium wilt, but none of them, nor *P. fluorescens* WCS417, provided disease protection (STR4) in the field (Belgrove et al. 2011). Some scholars attempted to increase the probabilities of obtaining effective biocontrol activity by isolating from Fusarium suppressive soil, such as non-pathogenic *F. oxysporum* strains (Forsyth et al. 2006), or even healthy banana plants growing in *Foc*-infected soil, such as *Erwinia chrysanthemi* E353 (Forsyth et al. 2006; Yin et al. 2009). Moreover, endophytes that are effective against Fusarium wilt of banana are not only found in banana plants. For example, Ho et al. (2015) recovered *Burkholderia cenocepacia* 869 T2 from vetiver grass (*Chrysopogon zizanioides*) roots that had been surface-sterilized. Under field conditions, banana tissue culture plantlets treated with 869 T2 demonstrated a decreased disease occurrence (86% reduction in disease occurrence) as well as

considerable plant growth enhancement. *Serratia marcescens* ITBB B5–1, an endophytic strain, was identified from the rubber tree (*Hevea brasiliensis*) and found effective against wilt pathogen (Tan et al. 2015). Inoculation of banana plants with Foc R4 strain resulted in a significant reduction in disease severity in both greenhouse (79%) and field environments (70%). Furthermore, it has been claimed that chitinase and glucanase activities are implicated in its antifungal activity (Tan et al. 2015). Finally, effective control of Fusarium wilt of banana and increased production (number of banana hands and bunch weight) were stated under field environments when different mixtures of endophytic (*P. putida* C4r4, *Achromobactrum* sp. Gcr1, *Rhizobium* sp. Lpr2, and *B. flexus* Tvpr1) and rhizospheric bacteria (*B. cereus Jrb1, P. putida* Jrb2, *Bacillus* (Thangavelu and Gopi 2015a, b) were inoculated. In the present scenario, bacteria obtained from various banana accessions were used to disinfect a naturally contaminated soil. Combined treatments of the endophytic *T. asperellum* prr2 and the rhizospheric *Trichoderma* sp. NRCB3 resulted in a 47% reduction in Fusarium wilt of banana incidence and a 45% rise in bunch weight in another field study (Thangavelu and Gopi 2015a, b).

17.6.2 *Bacillus* spp.

The number of *Bacillus* spp. strains that suppress plant diseases caused by soil-borne phytopathogens has rapidly increased. The interested reader now has access to a wealth of information on the biocontrol mechanisms involved, as well as their application and efficiency under different climatic conditions (Fira et al. 2018; Aloo et al. 2019). *Bacillus* species have a significant benefit over other advantageous microbes in the realm of biological control due to their ability to generate spores. On the one hand, this feature allows these bacteria to survive in harsh environments. On the other hand, it supports the progress and manufacturing of commercial formulations that are more constant over time from an agro-biotech standpoint. Furthermore, several *Bacillus* species have fast growth rates and the capability to produce a large amount of secondary metabolites, which are significant in antibiosis against a variety of harmful pathogens (Radhakrishnan et al. 2017). Some species, such as *B. subtilis*, *B. pumilus, B. amyloliquefaciens,* can also secrete volatile organic compounds, which are useful for promoting plant growth and activating plant defense systems by inducing systemic resistance (Raaijmakers et al. 2010; Cawoy et al. 2015). *Bacillus*-mediated plant growth promotion may also be owing to the bacteria's ability to enhance phytohormone production (i.e., indole-3-acetic acid gibberellic acid), which improves nutrient uptake in the host and stimulates plant defense responses to biotic and abiotic challenges (Chen et al. 2007; Harman 2011). *Bacillus* species can secrete lytic enzymes like chitinase and 1,3-glucanase, which are involved in the breakdown of the fungal cell wall, in addition to producing antibiotics and eliciting systemic resistance in plants against infections (Kumar et al. 2012). Combining multiple strains of *Bacillus* spp. (or other biocontrol agents) with different biocontrol mechanisms appear to be an attractive way to improve

biocontrol efficiency under various cropping situations and environmental conditions, given their compatibility and adaptability. *Bacillus* spp. are prevalent in the rhizosphere of banana plants (Xue et al. 2015), and several species of this genus have previously been examined for their biocontrol activity for a number of Fusarium-induced plant diseases (Khan et al. 2017). The literature contains representatives of *B. subtilis, B. amyloliquefaciens, B. pumilus,* and *B. thuringiensis. Bacillus subtilis* is known for its antifungal and antibacterial properties against a variety of bacterial and fungal plant pathogens. Its biocontrol activity is mostly related to the generation of antibiotics (Cawoy et al. 2015) and the enzymatic products are particularly effective against a variety of fungal infections. In Brazilian fields, the biocontrol effect of the plant endophytic *B. subtilis* strain TR21 against Fusarium wilt of banana was investigated with promising results (74% effectiveness) (Yu et al. 2010a, b). Similarly, in pot trials under greenhouse circumstances, *B. subtilis* strain N11 isolated from the rhizosphere of a healthy banana plant displayed biocontrol action (Zhang et al. 2011). Plantlet weight, bud multiplication, pseudostem height, and Foc conidia and toxin resistance were all improved when 10% (v/v) culture filtrate from the endophytic *B. subtilis* strain EBT1 was added to the plant development medium (Yang et al. 2010).

Bacillus subtilis strain B25, discovered from Hainan banana rhizosphere soil, is an alternative active antagonist, not only against Foc but also against other plant pathogenic fungi such as *Alternaria solani, Botrytis cinerea, Corynespora cassiicola, and Colletotrichum gloeosporioides* (Tan et al. 2013). The research contains effects on its ability to control Fusarium wilt of banana in greenhouse and field conditions, though they are not easily accessible (Liu 2011). Mycelium and spore tumescence, as well as aberrant pathogen growth, were generated by B25's antifungal protein, which was identified as a disease-resistance protein (Tan et al. 2013). While effectiveness *in planta* and under field conditions has not yet been established, the chitinolytic and heat-tolerant strain *B. subtilis* TSA3 inhibited Foc growth in vitro (Nawangsih and Purba 2019). *B. subtilis* strain S-1, like previous strains, inhibited not only *Foc* growth in vitro but also had shown deterrent effects on fungal infections such as, *C. gloeosporioides, Curvularia lunata, Gibberella zeae,* and *Verticillium dahliae* (Sun et al. 2008). In a Fusarium wilt of banana suppressive soil, the *B. amyloliquefaciens* strain NJN-6 was isolated from the rhizosphere of a healthy banana plant. Plants pre-treated (in nursery pots) with a bio-organic fertilizer colonized by NJN-6 reduced disease incidence by 68.5% in field plots, resulting in doubling the yield (Xue et al. 2015). The mode of action is based on numerous metabolites secreted by them. Numerous *Bacillus* strains, including NJN-6, produce the lipopeptide iturin A, a potent antifungal surfactant (Yuan et al. 2011). HPLC/electrospray ionization mass spectrometry was used to identify two bacillomycin D homologs and three macrolactin family homologs in NJN-6. Bacillomycin D and macrolactin were found to have strong antagonistic effects against Foc and *R. solanacearum*, respectively (Yuan et al. 2012a, b). Lastly, 11 of the 36 VOCs found in NJN-6 reduced Foc development completely (Yuan et al. 2012a, b). Wang et al. (2013) identified 57 bacterial strains from the rhizosphere of healthy banana plants growing in a diseased field, all of which showed anti-Foc activity. In

greenhouse studies, 6 strains (W2, W10, W14, W15, W17, and W19) with the strongest rhizosphere survival capacities were examined, with *B. amyloliquefaciens* W19 being the most efficient against Fusarium wilt of banana. Moreover, the biocontrol efficacy of a *B. amyloliquefaciens* W19-colonized bio-organic fertilizer was later demonstrated in a naturally infested area, where it reduced Fusarium wilt of banana by 44% while improving yield by 35% (Wang et al. 2016). The strain W19, like *B. amyloliquefaciens* NJN-6, produces several antifungal metabolites such as lipopeptides (e.g., bacillomycin D, surfactin, and iturin), 18 VOCs (Wang et al. 2013), and indole-3-acetic acid (Wang et al. 2016). Surfactin synthesis in banana root exudates appears to improve this strain's ability to colonize roots by enhancing bacterium biofilm formation (Wang et al. 2016).

17.6.3 *Pseudomonas* spp.

Similar to *Bacillus* spp., *Pseudomonas* spp. strains are endemic to the plant endosphere, rhizosphere, and/or phyllosphere, where they thrive as commensals. Some of them have been successfully utilized as plant inoculants to inhibit the negative effects of certain phytopathogens, resulting in improved plant growth and health (Pliego et al. 2011; Schreiter et al. 2018). A huge number of *Pseudomonas* spp. strains have been investigated as Foc antagonists, with the majority of the research focusing on *fluorescens* species. *P. fluorescens* Pf1 was revealed after screening fluorescent pseudomonads were isolated from the rhizoplane of several crops for antagonistic activity (Vidhyasekaran and Muthamilan 1995). In banana roots, *Pseudomonas fluorescens* Pf1 produces siderophores, hydrogen cyanide, and the antibiotics 2,4-diacetylphloroglucinol (DAPG) and pyoluteorin, as well as resistance-associated enzymes like PO and PPO (Akila et al. 2011; Selvaraj et al. 2014). *P. fluorescens* Pf1 has also been shown to be effective against Fusarium wilt of banana in multiple field studies employing a variety of application techniques and formulations. Though liquid formulations of *P. fluorescens* are known to have several advantages over solid formulations, including a higher cell count, longer shelf life, zero contamination, greater resistance to environmental stressors, and increased efficacy under field conditions (Hegde 2002), various chemicals were tested for the development of a liquid formulation, including trehalose, polyvinylpyrrolidone, and glycerol, with glycerol providing the best Pf1 survival until 6 months of storage. In several trials conducted at different locations reported that a liquid formulation of Pf1 was found effective against FWB, Saravanan et al. (2004) found a substantial in vitro inhibitory impact on Foc R1 when they tested five distinct *P. fluorescens* strains (Pf1, Pf2, Pf3, Pf4, and Pfm) obtained from banana rhizospheres, with strain Pfm having the strongest antagonistic effect on pathogen growth. A substantial reduction in vascular staining of the banana rhizome was reported in greenhouse studies employing a talc-based formulation of strain Pfm (Saravanan et al. 2004). Furthermore, *P. fluorescens* Pfm systemically caused the accumulation of three essential defense enzymes (PAL, PO, and polyphenol oxidase or PPO) in roots, which contributed to the development of resistance to Foc

(Saravanan et al. 2004). Sivamani and Gnanamanickam (1998) looked at the probability of decreasing Fusarium wilt of banana by bacterizing, citrus (Pfcp), peanuts (Pfgn), bananas (Pfb), black gram leaves (Pfbg), rice roots (strain Pfrl3), and cotton with different *P. fluorescens* strains (Pfco). The capacity of these strains to antagonize Foc R1 and R4 was investigated in vitro. The strain Pfcp displayed the greatest reduction of Foc mycelial growth, so that it was chosen to bacterize *Musa balbisiana* seedlings. Under greenhouse circumstances, seedlings treated with Pfcp displayed less wilting symptoms and internal discoloration. They also demonstrated improved root development and total plant height. In other study, 11 strains of *P. fluorescens* isolated from the rhizosphere of bananas were examined in vitro for their ability to inhibit Foc. The strain Pf10 was the most efficient in inhibiting the pathogen's mycelial development among the isolates tested (Thangavelu et al. 2001). The outcome of strain Pf10 treatment and Foc inoculation on the induction of banana plant enzymes and chemicals linked to defense responses (e.g., POX, chitinase, 1,3 glucanase, PAL, and phenolics) was investigated (Thangavelu et al. 2003). With *P. fluorescens* strain IIHRPf12, in vitro growth suppression of Foc was also found. This strain reduced Foc colonization and Fusarium wilt of banana, severity under greenhouse trials utilizing banana cv. Neeypovan. Remarkably, structural changes in cortical cells near the spot of the fungal entrance were detected, implying that bacterized root cells were somehow "alerted" to organize some defense structures aimed at halting pathogen progression (Mohandas et al. 2004).

17.6.4 *Trichoderma* spp.

Trichoderma belongs to a class of asexually reproducing fungi. It is distributed in mostly all the temperate and tropical regions. This fungus shows sexual teleomorph (genus *Hypocrea*) frequently, but the sexual stage is not known in many strains, like in most biocontrol strains, *Trichoderma* spp. belongs to a wide genetic diversity. They are known for producing several enzymes like chitinases and cellulase, approx. 100 metabolites and antibiotic activities, and it also produces several extracellular proteins. This genus can parasitize other fungi like *R. solani*. The induction of plant resistance controlled the *Trichoderma*-mediated biocontrol including antibiosis and mycoparasitism (Harman et al. 2004). *Trichoderma* spp. are well-known for their biocontrol activity (Harman et al. 2004; Vinale et al. 2008) because of their metabolite arsenal, rhizosphere-competence, and capability to stimulate plant growth and are widely studied against Fusarium wilt of banana. They colonize the plant roots, where they perform intense interactions with roots (Vinale et al. 2008). They form a colony where they damage only the upper epidermis of the plant root, but not the internal epidermis (Yedidia et al. 2002), but some of the authors claimed it being endophytic in their studies, like in bananas (Caballero Hernández et al. 2013; Thangavelu and Gopi 2015a, b; Chaves et al. 2016).

17.6.5 *Arbuscular Mycorrhizal* Fungi

The symbiotic relationship formed by the fungi (phylum Glomeromycota) with the roots of higher plants inter- or intracellularly is called mycorrhiza. This formation of relationship results in the infected root is called arbuscular mycorrhiza, which develops morphological structures called arbuscules. These AMF abstract several nutrients from the root and in return give back some other nutrients which help in plant growth. AMF also protect plants against other phytopathogens and abiotic stresses (Parniske 2008; Bonfante and Genre 2010; Lenoir et al. 2016). AMF is considered a plant growth promoter while it is less remarkable as a pathogen antagonist. It has several benefits for bananas although it has an inconsistent result. It is interesting to see how Arbuscular mycorrhiza inoculated in the banana nursery can provide protection from Fusarium wilt of banana in the field for several weeks in certain circumstances. An initial study found that either *Glomus intraradices* or *Glomus* spp. encouraged the development of the banana plant cv. Grande Naine and that rhizome necrosis and external FWB symptoms were reduced (Jaizme-Vega et al. 1998). Fusarium wilt of banana reduces in pot-grown plants cv. Maca, by inoculation with *Gigaspora margarita* especially observed under low concentration of Foc inoculum (Borges et al. 2007). Also, the population of FOC in the roots of banana plants was dramatically reduced after 7 months of treatment with a combination of *G. mosseae* and *T. harzianum*, as evaluated by ELISA (Mohandas et al. 2010). In the field, the treatment of banana plants with *G. clarum* again had higher biomass than the plant which is untreated with *G. clarum*. When compared to the untreated control (88%), it showed a lower incidence of Fusarium wilt of banana (67%). The incidence and severity of Fusarium wilt of banana in plants pre-treated with *G. clarum* and then inoculated with a commercial product based on *P. putida* and *T. asperellum* at transplanting were not different from the untreated control (Lin et al. 2012). Due to the mineral fertilization plant colonization by Arbuscular mycorrhiza is hindered and stimulated by the soil organic matter. In banana cv. Maca, the use of a bio-fertilizer helped mycorrhizal colonization abundantly and lower expression of Fusarium wilt of banana symptoms, as compared to the use of the Hoagland solution at three different concentrations (non-fertilized control was not established) (Sampaio et al. 2012). However, the result obtained with Arbuscular Mycorrhiza fungi has mostly been inconsistent. In a factorial experiment, the efficacy of a product based on a commercial Arbuscular mycorrhizal fungus including two other commercial Biocontrol agents was context-definite.

17.7 Conclusions and Future Perspectives

Panama disease is the most severe disease affecting commercial and subsistence banana crop worldwide, among other production restrictions. The antagonistic Foc TR4 strain, initially discovered in Asia in the 1990s, is now found in most of the world's banana-growing regions, including Central America. As a result, worldwide banana production is under serious menace, threatening the livelihood and food

security of millions of smallholders who raise more than 85% of the crop. Because not a single approach exists to effectively contain the disease, the combination of many management strategies such as creating awareness and sensitization among all the stakeholders, including plant tissue culture firms; quarantine and sanitation techniques to prevent disease spread to uninfected areas; crop rotation, intercropping, cover cropping, and fertilizer application based on need, proper management of this disease, use of effective microorganisms and soil amendments such as cakes, organic manures, ashes, and banana waste recycling must be used; resistant cultivars and disease-free planting material must be used, and sound agricultural practices must be maintained (Thangavelu et al. 2019). Exclusion and biosecurity measures are immediately needed, with a focus on disease-free planting material and avoiding the spread of infected soil and water. This must be taken into account at all levels, from farm to international, and should include not only TR4, but also more virulent Foc populations across countries (Raza et al. 2017). Farmers and scientists cannot afford to be complacent just because they are growing an allegedly resistant variety. At present, the mechanism of biocontrol cannot be fully explained by applying a single approach; it requires a combination of multi-high-throughput techniques. The obtained information will increase our understanding of wilt control in bananas caused by Fusarium.

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Soil Health Management and Microorganisms: Recent Development

18

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Abstract

One of the essential components for sustaining life on Earth is soil. It provides a diverse range of ecosystem services that are supported by soil processes and tasks

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carried out by soil biodiversity. One of the key elements in maintaining plant health and biomass output is the soil microbiome in particular. The control of soil microbial populations, both targeted and untargeted, seems to hold promise for enhancing food crop productivity, nutritive value, and sustainability over the long term. The acquisition of indicators that can be employed to assess the soil's existing status and afterwards create sustainable agricultural systems is one of the main goals of assessing soil health. This is because during the past few years, tremendous progress has been achieved in the creation of particular biomarkers and macromolecular probes, allowing for quick and accurate assessments of soil microbial populations. Recent years have witnessed an increase in the use of omics techniques, which enable the assessment of microbial phylogenetic diversity and functional information, to research changes in soil microbial diversity brought on by agronomic practices and environmental conditions. The study of soil microbial diversity, plant health, and the quality of derived raw materials will benefit from the application of these high-throughput technologies, strengthening the relationship between soil health, food quality, food safety, and human health.

Keywords

Soil microbiome · Functional microbial diversity · Sustainability · Soil health management

18.1 Introduction

Soil is fundamental to the functioning of the Earth's ecosystems and to human life. In fact, in addition to constituting the base of the food chain, healthy soils also provide several ecosystem services that are essential to our survival, e.g., nutrients cycling, bioremediation, provision of clean drinking water, pest control, and contribution to plant growth (Wall et al. 2004). It is nowadays clear that soil is to be considered not only a substrate that is physically supporting the growth of naturally growing plants and cultivated crops but rather as a living ecosystem (Ponge 2015) to preserve and keep functional. Over the last decades, the scientific community, as well as international institutional bodies, have recognized the importance of promoting soil health at local, national, and international levels and have engaged in soil health awareness actions. The Council of Europe (1972) clearly stated the importance of soil as a fundamental asset to anthropic activities, the need to protect and monitor soil resources, and called for a soil conservation policy. Another, more recent example of international initiatives oriented to soil health awareness promotion the World Soil Day, which was instituted by the Food and Agriculture Organization of the United Nations (FAO 2022), with the aim of fostering human well-being and functioning ecosystems through soil health improvement.

The realization that soil health is a fundamental component to secure ecosystems stability and food security has also driven the demand for a set of internationally standardized measures of soil quality (Nortcliff 2002) to compose a common framework to be used as a reference and to produce effective indicators that could serve as soil health monitoring tools also for policymakers (Stone et al. 2016; Paleari 2017). In order to achieve this objective, a common definition of "soil health"—a matter that has been an object of research and debate—was necessary. Some argue that soil health and soil quality should be considered distinct concepts, in that soil health encompasses a broad range of sustainability components that include planetary health as a whole, while soil quality more strictly refers to ecosystem services important to humans (Lehmann et al. 2020). Within the present manuscript, following Doran and Zeiss (2000), the term "soil quality" will be used in relation to the ability of a given soil to absolve to a specific function, while "soil health" will be used, in a broader sense, to refer to the capacity of soil to ensure biological productivity, promote environmental quality through ecosystem services, and maintain plant and animal health (Doran and Parkin 1994). Soil health has also been defined as a function of ecological characteristics, such as disease suppression capability (Van Bruggen and Semenov 2000). One of the main drivers of soil quality and fertility is soil organic matter (SOM) content. In the last few decades, there strong evidence supporting the major role of the soil microbiome in the synthesis of SOM has been provided (Kallenbach et al. 2016). Hence, the approach to soil health assessment has to necessarily keep the soil microbiome in due consideration. How soil microbiome responds to different agricultural management systems (Mann et al. 2019) is also of high interest in that the agricultural practices farmers decide to implement should take that into account (Nunes et al. 2020), in that improper agricultural practices can be detrimental to soil health and jeopardize future harvests (Manda et al. 2020a).

In order to evaluate the effect of different agricultural practices on the soil microbiome, it is necessary to develop analytical tools and indicators that can provide information on the soil microbiome status and evolution in response to those practices. This information is supposed to not only enrich the shared scientific understanding of the mechanisms underlying soil fertility and plant–microorganism interaction, but also to help farmers, extension islands, and farming consults making better management decisions. The aim of the present work is to offer an overview on the recent developments on soil health management within agricultural systems, with a specific focus on the role of microorganisms and analytical tools and indicators used to monitor soil microbiome and on strategies for soil microbial diversity management in agricultural settings.

18.2 Biological Indicators and Standard Analytical Procedures Used to Determine Soil Health

In the attempt to provide international standards for the assessment of soil quality, a Technical Committee of the International Organization for Standardization (ISO) was set up (Hortensius and Nortcliff 1991). The ISO/TC 190 started their activities in 1985 and dealt with six topics: terminology, sampling, chemical analysis methods, soil quality and biological systems (including the effects on microorganisms), physical investigation of soil, and radionuclides and radioactivity determination methods. It is important to observe that those were not meant to be indicators of soil health but rather "a set of proven, widely used standard methods which can be reliably used by those seeking to evaluate soil quality" (Nortcliff 2002). As discussed earlier, soil health is a broad concept that may be defined in different ways. This poses a challenge to standard measurement method identification. Nevertheless, there has been some effort to identify a shared set of soil health indicators. The present chapter will discuss physical indicators, chemical indicators, and molecular techniques measuring microbial biomass and genetic and functional biodiversity that have emerged as informative and effective in providing information on soil health and quality. Lastly, an overview of -omics approaches will be provided. -omics approaches allow to assess microbial diversity and functionality and have increasingly been used in recent years. These approaches make use of promising technologies that could help studying the soil microbiome in a faster and more thorough way. Genomics, transcriptomics, proteomics, enzynomics, and metabolomics (Bertola et al. 2021) are the main -Omics approaches currently used to study soil microbial communities.

Although the pivotal role of microbial communities in the achievement of soil health has emerged as prominent (Sherwood and Uphoff 2000), microbial and biological indicators are not always able to provide the entire picture (Fierer et al. 2021). Moreover, the search for effective biological indicators, as highlighted by Bruggen and Semenov (2000), has not been systematic.

In fact, one of the major challenges in the use of biochemical properties to assess soil quality is the lack of reference values to interpret the proposed biochemical indicators (Bünemann et al. 2018a, b), as well as the contradictory behaviour shown by some of these indicators and regional variations in expression levels (Gil-Sotres et al. 2005). Therefore, standard analytical procedures, such as water infiltration rates, bulk density, pH, electrical conductivity, ion-exchange capacity, aggregate stability, and soil slaking which have been used and studies for decades, are well known among soil scientists, and have been tested under different environmental conditions in different areas of the world have to be kept as a valid reference and integrated with the newest analysis techniques with the aim to compose a complete picture of the health status of any particular soil.

18.3 Role of Soil Physical and Chemical Indicators for Microbial Sustainability

Daniel (2004) documented that 1 g of soil nearly contains 10 billion microorganisms with hundreds of various microbial communities. The presence of numerous microbes within side the soil is crucial for balanced plant growth as a maximum of the nutrient cycles are managed with the aid of using those microorganisms such as natural remember decomposition, nitrogen fixation, and conversion of ammonia to available plant nitrate. The physical, as well as chemical, signs of soil play a major role in the detection of microbial sustainability in a particular soil type. Polysaccharides and polyuronides launched at some point of decomposition allow for fostering the aggregation of soil particles and hence affect the physical condition of the soil in a particular location. Besides, the chemical compounds produced by fungi mycorrhizal are also crucial in the promotion of soil aggregation (Wright et al. 1999).

18.4 The Effect of Soil Water Infiltration Rate on Microorganisms

18.4.1 Water Infiltration in the Soil

Normally, a huge amount of water is stored in soils. Crops and microbial populations in the soil depend extremely on the presence of water in the soils, besides water is necessary for nutrient cycling. The infiltration rate of water is more in uncompacted soil rather than in a soil which is highly compacted though of the same type (Hamza and Anderson 2005), whereas soil water infiltration rate differs both in time and in space, thus controlling dual effects: availability of water to microbes and plants; and has a dominant effect on the rate of diffusion of solutes and gases (Adl 2003). The infiltration rate of a soil is the highest rate at which the soil of a region under a given set of specific conditions can absorb rain (Richards and Moore 1952). Besides, a quantitative definition for infiltration rate could be explained as the amount of water percolating into the soil per unit area per unit time. The status of soil infiltration rate is explained in two major approaches: the soil water infiltration rate, which explains the quantity of water present in the soil, and soil water potential associated with the energy level by which the water is held in the soil including matric, osmotic, and gravitational potential (McKenzie et al. 2002). Processes dealing with water balance are more related to water infiltration rate whereas processes related to water movement are related to soil water potential (Warrick and Or 2007).

18.4.2 Effect of Water Infiltration Rate on Microbes

Water is an essential participant in hydrolysis processes, and it is also an important transport medium for the substrate. As a result, the water infiltration rate regulates

the activity of microbes and is a paramount component which regulates the rates of mineralization (Paul et al. 2003).

Low Water Infiltration Rate A decrease in water infiltration rate causes a reduction in the water that reached the living organisms in the oil, thus leading to a reduction in the activity and growth of microbes (Bottner 1985; Kieft 1987), mineralization of C and N (Sleutel et al. 2008), and also shifts the structure of microbial community (Hueso et al. 2012; Sorensen et al. 2013). By maintaining a higher osmotic potential (more negative) in the cytoplasm than that of the environment, cells are able to retain enough water for cell turgor and metabolism (Martin et al. 1999). Soil microorganisms can build up organic and inorganic compounds at low water infiltration rates (high water potential), which raises the osmotic potential within their cells. Therefore, deposition of osmolytes serves as the primary mechanism of tolerance for both high salinity and low water infiltration rate. Additionally, because the pores drain and water films present around the aggregate as soils dry up, the substrate reservoir shrinks dramatically in size, resulting in weaker and unconnected aggregates (Ilstedt et al. 2000).

High Water Infiltration Rate In this case, excess soil water infiltration rate leads to a decrease in oxygen diffusion because oxygen diffusion in water is much lower (about 104 times) than in air, which causes a reduction in the functioning of aerobic microbial communities (Skopp et al. 1990), whereas this environment also leads to the increase in the viability and activities of anaerobic microorganisms. Gram-negative bacteria are less able to withstand high matric potential than fungi, gram-positive bacteria, and archaea because they possess weaker cell walls (Fierer et al. 2003; Martin et al. 1999; Schimel et al. 2007; Vasileiadis et al. 2012). Variations in the pace of water infiltration's impact on soil microorganisms temperature, the length of irrigation periods (for farmlands), and seasonal cycles of rainfall all affect soil moisture and the distribution of water within a soil profile (Manda et al. 2021). In semi-arid and Mediterranean ecosystems, the soil on the topmost layer commonly experiences long dry periods, which are followed by relatively frequent and fast wetting (Fierer and Schimel 2002). Research on the effects of drying and rewetting on soil microbial populations and their functions has been conducted (Griffiths et al. 2003; Herron et al. 2009; Schimel et al. 2007; Xiang et al. 2008). The result was that the concentration of available substrate and microbial activity reach its highest point in the initial 24 h after rewetting (Fierer and Schimel 2003).

Rewetting causes sensitive bacteria to lyse, which causes this to happen (Manda et al. 2020b). Meanwhile, other microbial communities release the organic solutes that these strains have gathered during the dry phase (Halverson et al. 2000). Additionally, the soil aggregates disintegrate, exposing the organic material that was previously shielded and allowing for further decomposition. An increase in the quantity of dry and rewetting cycles leads to a decline in the microbial biomass, activity, and nitrification (Mikha et al. 2005; Wu and Brookes 2005). Because of faster microbial biomass turnover and carbon loss during the flush in respiration

upon rewetting, the microbial biomass declines as the number of drying and rewetting cycles increases (Fierer and Schimel 2003). According to Jin et al. (2013), the interaction of soil moisture and soil type, aggregation, and the concentration of potentially accessible soil organic matter affects how microbial activity responds to drying and rewetting (Anderson and Ingram 1993). Nevertheless, drying and rewetting kill microbiome and change the makeup of the microbial community, which may have an effect on nutrient cycling (Fierer et al. 2003; Schimel et al. 2007). The study found that rewetting and drying resulted in an increase in Gram-positive bacteria and a decrease in fungus (Butterly et al. 2009).

18.4.3 Effect of Bulk Density on Soil Microbes

Bulk density reflects the ability of the soil to function for structural support, nutrient and microbial life movement, and water and soil aeration as a very compact soil has few large pores which are less hospitable to various organisms like springtails, mites, and earthworms. In contrast, lower levels of oxygen present in compact soils may influence the forms of nutrients and their availability; e.g., significant quantities of NO_3 may be lost under anaerobic conditions (Wai et al. 2020).

18.5 Effect of Bulk Density on Bacterial Population

18.5.1 Bacteria

Pupin et al. (2009) concluded that soil microbial biomass is adversely affected by the increase in bulk density. Average bacterial cell density was observed to be 174 cells mm^{-2} and 99 cells per square metre with a bulk density of 1.3 and 1.5 g cm^{-3} in the soil, respectively (Juyal et al. 2021). The propagation of bacteria and their colonization of the pore space at lower bulk density are influenced by soil porosity and solid–pore interfaces, leading to substantially higher bacterial populations in bigger pore spaces. At lower bulk density, soil porosity and solid–pore interfaces affect the spread of bacteria and their colonization of the pore space which leads to relatively higher bacterial densities in larger pore spaces (Juyal et al. 2021). For example, there was a decline in the rate of the spread in pseudomonas with an increased bulk density of soil (Juyal et al. 2021). The compaction caused by an increase in bulk density leads to a reduction in the soil aeration of because of the decline in the air-filled porosity by 13–36% which further results in the reduction of microbial biomass nitrogen and microbial biomass carbon (Tan and Chang 2007). In addition to this, Tan et al. (2008) found the decline of microbial biomass phosphorus with the increase in soil bulk density, thus leading to an increase in compaction. Shestak and Busse (2005) concluded that the soil strength values ranging from 75 to 3800 kPa change the physical properties of the soil but cause no effect on the biological indicator of the soil including microbial biomass and enzymatic activity.

18.5.2 Enzymatic Activity

Any disturbance or stress to the soil can impact the enzymatic activities of the soil (Buck et al. 2000). The increase in bulk density alters the physical as well as chemical attributes of the soil and induces a reduction of phosphatase, amidase, and urease. Anaerobic conditions in the soil lead to changes in the microbial community, thus favouring the microbial populations which are capable of tolerating these conditions. Lower the eukaryotic/prokaryotic ratios, more is the iron and sulphate reducers, and thus, higher methanogens were found in compacted soils in comparison with uncompacted soils (Schnurr-Pütz et al. 2006).

To conclude, a high soil bulk density can adversely affect soil physical properties and can limit microbial activity and biochemical processes which are crucial for nutrient availability.

18.5.3 Soil pH

The pH of any soil determines its acidity or alkalinity as it represents the concentration of hydronium ions [H_3O^+] available in the soil. Sources of H^+ ions in soil include carbonic acid formed when carbon dioxide (CO_2) from root respiration, decomposing organic matter and the soil atmosphere is dissolved in the soil water. Nitrification of ammonium (NH_4^+) from fertilizers and organic matter mineralization, the reaction of aluminium ions (Al^{3+}) with water, rainwater, the reaction of sulphur compounds, and acid rain are the other sources of H^+ ions. Lauber et al. (2009), Andrew et al. (2012), and Zhalnina et al. (2015) observed that pH is one of the biggest influencers affecting the soil microbial community. Furthermore, pH was currently reported to be the best predictor of microbiome diversity at the phylum level (Geyer et al. 2014). Hence, there are numerous studies conducted globally to focus on the effect of pH at different scales. To illustrate, continent-wide research clearly demonstrated an association between soil pH and the presence of certain microbial communities (Fierer and Jackson 2006; Lauber et al. 2009), demonstrating that pH was the key factor accountable for this variation of diversity and richness of the soil bacterial communities (Fierer and Jackson 2006). This is because it greatly controls the abiotic factors, namely carbon availability (Andersson et al. 2000), availability of nutrients (Kemmitt et al. 2006), and metal solubility (Flis et al. 1993). Besides, soil pH may possibly impact biotic factors, for instance, the biomass composition of bacteria and fungi (Fierer et al. 2006), in both forest (Bååth and Anderson 2003) and agricultural soils.

The beneficial microorganisms present in the soil prefer an approximate pH scale of 6–7; hence, alteration in the soil acidity could lead to shifting in the species, quantities, structure, and functions of various microbes living in the soil. The effects on two principal microbial decomposer groups—bacteria and fungi, when there are changes from the neutral pH are as follows:

Decrease in pH Acidic soil indicates high H^+ ion concentration, which impacts the microbial community in numerous processes, namely decline in the reproductive ability, cell membrane distortion, and fluctuation in the release of enzymes. The overall microbial function decreased in the health and productivity of soils due to these reasons (Birgander et al. 2014). Besides, soil fungi prefer a low pH environment to flourish; hence, soil with a low pH has an unbalance between the fungi and bacterial concentrations; the fungi population being the dominant (Rousk et al. 2010). This could further lead to the high probability of fungal pathogen infections due to the favourable environment and decrease the mineralization of nutrients at pace by soil microbes into plant-available forms. The reason for the latter is the imbalance in the microbial composition because various microbes release different nutrients after decomposition, potentially limiting plant mineral uptake. Therefore, this imbalance causes the immobilization of soil carbon and nutrient release (Rousk et al. 2009). Generally, Fungi show a wider range of pH tolerance in comparison with bacteria.

18.5.4 Bacteria

Nodulation of Legumes Leguminous crops fix their own nitrogen through a symbiosis from the air with specialized bacteria (Manda et al. 2020c). Under favourable conditions, nitrogen-fixing bacteria maintain a symbiotic relationship with crops and pasture legumes in root nodules (Weese et al. 2015). Acidic soils limit both root growth and rhizobia survival, which reduces the chances of roots contacting the bacteria to form a nodule which results in inhibition of the performance of nodules (Weese et al. 2015). Essentially, low pH leads to failure in the formation of nodules. In the case of acidic soils, the failure of a functioning symbiotic relationship results in a deficiency of plant nitrogen (Weese et al. 2015). Species of rhizobia bacteria have variable tolerance to soil acidity like medic rhizobia are very sensitive to low pH and may fail to survive in such soils. In acidic soil, grass-dominated pastures can result from the failure of pasture legumes (Weese et al. 2015).

When soil pH is around neutral (6 or 7), roots of the leguminous plants naturally form an alliance with rhizobia bacteria in the soil and fix nitrogen symbiotically, which was earlier in an unavailable form to the plants. Whereas the use of ammonia-based fertilizers decreases the efficiency of this symbiotic relationship effectively and increases the availability of Nitrogen (N) to their host plant (Weese et al. 2015). Even though some rhizobia can survive in an acidic environment, it can drastically diminish the number of nodules, their functions, and the ability of leguminous plants like lentils, chickpeas, and soybeans to fix Nitrogen (Tang and Thomson 1996). This causes a decline in the plant vigour and productivity and consequential yield loss in a region where soil pH plummets. In soils, where the pH remains below 5, nodules per soybean crop can decline by an average of 50%, in comparison with a soil with a pH of more than 6 (Lin et al. 2012). The lower value of pH can inhibit nodulation by

limiting the legume's ability to secrete the signals required into the rhizosphere which attracts the rhizobia and the formation of the root nodules (Hungria and Stacey 1997). Besides, calcium ($Ca^{2+)}$ and molybdenum (Mo) ions become unavailable below pH 5, as both are known to be necessary for root nodule formation and nitrogen fixation thus limiting rhizobia N fixation. Moreover, as pH lowers in soil solution metals like aluminium (Al) and manganese (Mn) become increasingly available, these are toxic to the legume–rhizobia symbiosis (Bordeleau and Prévost 1994).

Fungi There is a higher proportion of fungi in acidic soil communities because numerous strains of soil bacteria are not able to survive in acidic conditions well (Sylvia et al. 2005). Research by Ritz (2011) concludes that hyphal length can be as long as approximately 176 miles per ounce when fungi predominantly account for nearly 75% of the soil microbial biomass in agroecosystems. Many of these soil fungi microbiome function primarily in decomposition processes as well as nutrient cycling, but they may also aid with remediation of metals such as Al in acidic conditions. Fungi assist in ameliorating soil and plant health in the acidic environment by fungal-driven binding of aluminium (Gadd 2007). Plant–symbiotic fungi, known as mycorrhizas, have been found to protect plant root systems against stresses ranging from nutrient depletion to drought and disease, as well as metal toxicity (Seguel et al. 2013). These fungi escalate the access to limiting nutrients, including phosphorus (P), which is predominantly essential in low pH soils because of the reduced P availability in acidic conditions (Seguel et al. 2013).

Increase in pH Bacterial growth is favoured by soil having neutral or high pH, in contrast to acidic soil, which prefers fungal growth (Rousk et al. 2010). To illustrate, in a study it was found that treatment of forest soils with lime and ash resulted in an increase of pH from about pH 4 to 7, which further escalated bacterial growth by about five times, as measured by TdR incorporation (Bååth et al. 1995). Similarly, another research that included more than 15 different soils from regions with different land uses, spanning a pH range from 4 to 8, demonstrated that there was a rise in bacterial growth by four times at higher pH as measured by Leu incorporation (Bååth et al. 1998).

In conclusion, by regulating the chemical forms of the soil components, soil pH is highly correlated with the availability of nutrients for plants (Reddy et al. 2020). This has also been seen as an indirect limiting factor for the populations of soil-borne microorganisms (Zhalnina et al. 2015). While acidic soils typically exhibit lower diversity indices, neutral soils generally have a greater diversity of bacteria or microbes (Fierer and Jackson 2006; Lauber et al. 2009; Rousk et al. 2010).

In light of this, soil pH would only affect a few microbial species' survival and is not a universal determinant for all species. Instead, numerous studies have not discovered a connection between soil pH and the ecosystem's bacterial diversity. For instance, numerous abiotic factors, including soil pH, were examined in a biogeographic study of the nitrogen-fixing rhizobacterium *Sinorhizobium meliloti*

across various regions of Croatia; however, only soil type and other geographical characteristics were recognized as being necessary for defining the genetic diversity of the 128 isolates analysed. Surprisingly, soil pH did not have to be present in order for genetic diversity to exist. The conclusion is that pH is therefore essential in affecting the nutrients.

18.5.5 Electrical Conductivity (EC)

The capacity of water present in the soil to carry electrical current quantifies as the electrical conductivity (EC) of the soil. Electrical conductivity is an electrolytic process which occurs chiefly through water-filled pores in the soil. The major soluble salts are cations: K^+ (potassium), Na^+, Ca^{2+} (calcium), Mg^{2+}(magnesium), and NH_4^+ (ammonium) as well as anions, namely Cl^- (chloride), NO_3^- (nitrate), HCO_3^- (bicarbonate) and SO_4^{2-}(sulphate), transfer electrical charges and conduct the electrical current because these salts are dissolved in water-filled pores (Shi and Wang 2005). Therefore, the EC of soils is determined by the concentration of ions. EC is expressed in Deci Siemens per metre (dS/m). Electric conductivity is mainly used to measure soil salinity while in non-saline soils it can be used to evaluate soil attributes such as soil moisture and soil depth. The soil of an area is considered to be saline when the electrical conductivity (EC) is 15 dS/m, according to World Soil Resources Reports (2007) in contrast to Soil Taxonomy (2010) which considers the reference value at 30 dS/m. Soil that has excessive salts is known as salt-affected soil. The USDA system classifies the soils into three distinct categories as saline, sodic, and saline-sodic soils.

The size and the activity of soil microbes in biomass are impacted by the salinity of the soil (Rietz and Haynes 2003), which in turn plays an important role in biogeochemical cycles. Most of the microbes are sensitive to high electrical conductivity. Bacteria, except for halophytes (salt-tolerant bacteria), are more susceptible in comparison with Actinomycetes and fungi. Microbial processes, such as respiration and nitrification, decline as the salinity increases.

There are two main mechanisms namely the osmotic effect and specific ion effects that occur in the soils having a high concentration of soluble salts, affecting soil microorganisms.

Osmotic Effect The soluble salts (cations and anions) increase the negative osmotic potential of the soil water, thus leading to plasmolysis by desiccating the cells of water which may kill microbes and plant roots. Salinity reduces microbial biomass (Rietz and Haynes 2003) predominantly because the osmotic stress results in drying and cell lysis (Yuan et al. 2007). Low osmotic potential makes it challenging for roots and microbes to draw water from the soil, on the other hand (Oren 1999). The synthesis of osmolytes requires a lot of energy, which inhibits the growth and functioning of flora and microfauna. Plants and microorganisms can adapt to low osmotic potential by storing osmolytes (Wichern et al. 2006).

Specific ion effects: A high concentrations of certain ions, namely sodium, chlorine, and bicarbonate, lead to a toxic environment for numerous plants. There are specific ions that will affect a certain species of organisms. The sensitivity of soil enzyme activities to salinity (EC) may alter the activities of urease, β-glucosidase, and alkaline phosphatase (Pan et al. 2013). However, catalase and dehydrogenase were less affected (Garcia and Hernandez 1996).

Research by Andronov et al. (2012) showed that salinity decreased microbial biomass, and microbial activity and changes microbial community structure (Setia et al. 2011). Besides, an increase in soil EC declines soil respiration rate (Adviento-Borbe et al. 2006; Wong et al. 2009). To illustrate, it was reported that soil respiration may be reduced by more than 50% at EC (1 ratio 5) more or nearly equal to 5.0 dS 1/m (Setia et al. 2010). However, in 2003, Rietz and Haynes found out that soil respiration was not remarkably correlated with EC. As EC increased, the metabolic quotient which is respiration per unit biomass found to be increased. There are microbes that occur in naturally saline (with higher EC) habitats that are supposedly meant to share a strategy for resisting high salt concentrations (Sagot et al. 2010). They have developed a number of adaptations for maintaining their population active while surviving in extreme environmental conditions. According to the aforementioned information, microbes have the ability to adapt or tolerate salinity stress (Sagot et al. 2010), by accumulating osmolytes (Zahran et al. 1992). Proline and glycine betaine is the prime organic osmolytes while potassium cations are the most common inorganic solutes which are used as osmolytes accumulated by salinity tolerant microbes (Oren et al. 2001), whereas the synthesis of the organic osmolytes requires high amounts of energy, thus causing a decline in the activities of microbes and plants (Killham 1994). Accumulation of osmolytes as inorganic salts can be very toxic; therefore, it is confined to halophytic microbes evolving salt-tolerant enzymes to survive in highly saline environments (Gros et al. 2003). From the genetic point of view, the salt-tolerant species which display an under, overexpression of peculiar genes and metabolites allow them to cope with osmotic stress (Dion and Nautiyal 2008). It is important to understand the elusive dynamics of microbial communities in saline soil as it would shed light on the depth of selection mechanisms operative in the environment (Parkin 1993). The bacterial communities opting to wait for favourable conditions rather than developing metabolic adaptations for survival in a niche of higher salinity and instability represent the structure of microbial heterogeneity and taxa spatial composition in these soils (Pereira e Silva et al. 2012).

The bacteria/fungi ratio seems to be higher in saline soils as fungi tend to be more salt-sensitive than bacteria (Sardinha et al. 2003; Wichern et al. 2006). Therefore, the community structures in saline soils are impacted due to the difference in tolerance of salinity among microbes (Gros et al. 2003) in comparison with proportionate populations in non-saline soils (Pankhurst et al. 2001). According to the recent meta-analysis conducted on microbes, salinity has a major role in impacting the global composition of microbes in saline soils than any other chemical attributes like pH or temperature (Ma et al. 2013).

In conclusion, the understanding of the relationship of microbial communities in soils with varying salt concentrations will help to harness the biotechnological potential of these microbes which could be used in the conservation or restoration of the saline environments apart from being a genetic reserve for further applications. This would also help in exploring the diversity and mechanisms operative at the level of soil in limiting conditions (Nacke et al. 2011; Roesch et al. 2007)

18.5.6 Effect of Ion-Exchange Capacity on Soil Microbes

Ion-exchange capacity is an inherent soil chemical property that estimates the total capacity of a soil to clutch exchangeable cations. It impacts the soil's ability to grip important nutrients and acts as a buffer against the acidification of soil. The ion-exchange capacity is directly related to the amount of organic matter present in the soil. Besides, the changes in pH and salt concentrations affect the ion-exchange capacity, which is specially referred to as cation-exchange capacity.

Virus There is a diversity of viruses in the soil. According to a research (Lipson and Stotzky 1983), the addition of cations (as chloride salts) to distilled water enhanced the adsorption of reoviruses, with divalent cations being more effective than monovalent cations and 10^{-2} M resulting in more adsorption than 10^{-3} M. Potassium ions suppressed the adsorption of such viruses to montmorillonite, probably by collapsing the clay lattices and preventing the expression of the interlayer-derived cation-exchange capacity. Higher quantities of the virus were adsorbed by montmorillonite, which converted homo-ionic to various mono-, di-, and trivalent cations than by comparable concentrations of kaolinite clay which are homo-ionic to the same cations. The sequence of adsorption amount to homo-ionic montmorillonite was $Al > Ca > Mg > Na > K$ while the sequence of adsorption to kaolinite clay was $Na > Al > Ca > Mg > K$.

Fungi Das et al. (1991) stated that actinomycetes and fungi in soil showed a positive correlation with available K^+, exchangeable Ca^{2+}, Mg^{2+} and the cation-exchange capacity of the soil.

Bacteria Ciccolini et al. (2016) explored the composition of microbial communities involved in nitrogen cycling in Mediterranean peaty soils drained for farming activity and found that ammonia-oxidizing communities like AOA (ammonia-oxidizing archaea) were shaped by clay content, AOB (ammonia-oxidizing bacteria) were shaped by bulk density, and both AOA and AOB were controlled by exchangeable calcium content.

18.6 Aggregate Stability and Soil Slaking

Soil structure is an essential soil property as it controls numerous biological and physical soil processes. Soil structural properties in addition to soil aggregation are affected by changes in agricultural management practices (Six et al. 2006; Tiemann et al. 2015). Numerous techniques have been recommended to fragment soil into various aggregates with each fragment having its own inherent positive and negative attributes (Schutter and Dick 2002; Lützow et al. 2006; Dorodnikov et al. 2009). It was found that microbial diversity and community structure are more affected by particle size than other factors like pH or organic nutrient content (De Fede et al. 2001). Aggregate stability is a measure of the soil aggregates' vulnerability to outer destructive forces. The plenty of microbial groups and their functional diversities in soils are strongly influenced the type and amount of available organic substrates (Grayston et al. 2001). Besides, recent progress in isotopic, spectroscopic, and ecogenomic (DNA/RNA) techniques assists in measuring the changes and distribution in specific microbial genera (including active and total as well as functional and taxonomic groups) in different parts of aggregates and their pore spaces. These techniques also help in determining the role of these microbes in soil functional processes with reference to organic matter composition (Davinic et al. 2012). Slaking is the fragmentation process that occurs when soil aggregates are suddenly immersed in water (Chan and Mullins 1994) due to their resistance to withstand the stresses of rapid water uptake. At fast rates of wetting, internal stresses developed from differential swelling and the air entrapment in the soil aggregate. Soil slaking is affected by the rate of wetting, texture, water content, clay mineralogy, and organic matter content.

Soil structure regulates soil physical and chemical heterogeneity, therefore playing an important role in the distribution of microbial communities, and their activities among different aggregates (Vos et al. 2013). Aggregates of different sizes and their stability in the soil produce niches with different physicochemical and structural characteristics. These niches foster the colonization and maintenance of various microbial assemblages in each aggregate (Davinic et al. 2012; Vos et al. 2013; Tiemann et al. 2015).

According to research by Ling et al. (2014) and soil aggregates have a significant impact on the composition and structure of the microbial community (2014). It was shown that when aggregate size reduced, the enzymatic processes associated to carbon breakdown increased (Qin et al. 2010; Lagomarsino et al. 2011; Ling et al. 2014; Nie et al. 2014). Previous research that found that soil aggregate size mostly influences soil microbial activity and carbon dynamics supports this finding (Elliott 1986; Schutter and Dick 2002).

According to a study by Trivedi et al. (2015), farm management only altered the enzymatic activities of soil fractions in macroaggregate and cultivation methods that led to an increase in soil fertility. Enzymatic activity was greatly increased by carbon. The distribution of bacteria in different aggregates and subsequent effects on microbiological activities and diversity at small scales can be influenced by soil structure (Six et al. 2006; McCarthy-Neumann and Ibáñez 2013; Vos et al. 2013;

Ling et al. 2014). According to Nannipieri et al. (2012), variations in microbial populations' enzymatic activity can also depend on the type of plant inputs, including humic substances in the soil. Microbial mucilage and polysaccharides released by some species of bacteria (e.g., Acidobacteria and Actinobacteria) and several fungi play a major function in the stabilization of various aggregates (Tripathi et al. 2015).

18.6.1 Bacteria

Ranjard et al. (2000) and Sessitsch et al. (2001) and illustrated differences in bacterial community structure and composition by utilizing microbial community profiling methods in various aggregate size classes. Normally bacterial proportion within soil changes with aggregate size. Though a larger population of bacteria is connected with microaggregates, a smaller population is with macroaggregates (Monreal and Kodama 1997; Neumann et al. 2013). Interaction between the organic matter, microbe, and clay particles is necessary for the survival of bacteria as they provide nutrients and habitat to bacteria (Van Gestel et al. 1996; Sessitsch et al. 2001). For example, silt clay fractions demonstrate higher bacterial populations as compared to other aggregates (Trivedi et al. 2015). Besides, crop management practices influenced carbon content and microbial biodiversity more prominently in the larger-sized aggregate fractions than in fine clay silt fractions (McCarthy-Neumann and Ibáñez 2013). Poll et al. (2003) observed that differences in the bacterial community abundance were very less for the similar particle size fractions as compared to coarse sand fractions. The reason could be the lower C content in macroaggregates as compared with microaggregates because there is an increase in enzymatic activities related to carbon decomposition for smaller aggregate sizes (Nie et al. 2014).

Additionally, cloning-sequencing analysis procedures used by Momma et al. (2006) concluded that the colonies of Alpha-proteobacteria, Actinobacteria with subdivision rubro-bacteriaceae, and Gemmatimonadetes within micro-aggregates had a huge population; however, the population of acido-bacteria was comparatively more profuse in macroaggregate fractions. In addition, there are differences in bacterial diversity and population within micro-aggregates. Similarly, according to the research by Remenant et al. (2009) closely related bacterial genotypes/communities survived in rhizosphere aggregates in contrast to the non-rhizosphere aggregates. This also illustrates that plant roots may allow certain strains of soil bacteria to survive and grow in the soil matrix. Besides, the roots of distinct plant species release different exudates, and the composition of microbial communities surrounding those roots will be different. Moreover, the spatial heterogeneity, as well as complex soil structure, produces different habitats for bacterial diversity, thus sustaining various different microorganisms (Vos et al. 2013).

18.6.2 Fungi

In 1982, Tisdall and Oades generalized that plant roots and soil fungi bound the smaller aggregates into stable macroaggregates. The authors claimed that both entanglement and adhesion processes accumulate small roots and mycorrhizal hyphae accumulate small aggregates and soil particles. Hattori et al. (1976) worked on the distribution of bacteria and fungi in various aggregate size fractions and at different locations of an organism and its metabolic functions. In addition to this, culture-based research by McCalla et al. (1957) found that soil aggregates get stabilized by fungi.

Recently, in 2011, Ruamps et al. claimed that fungal use of carbon substrates present in small pores of soil has more fungal use as fungi can spread easily as compared to bacteria through the growth of hyphal and mycelial.

Effect of Management Practices Soil carbon and microbial communities are influenced by the various crop management practices because they are affected by aggregate size distribution. These impacts are more prominent in macroaggregate as compared to micro-aggregate sizes.

Ghimire et al. (2014) observed a mechanism by which crop management practices could affect the soil microbial community is the influx of easily changeable carbon. According to Tripathi et al. (2015), the quantity of labile carbon declines with the increase in aggregate size, thus causing an impact on the microbial community of soil. Carbonetto et al. (2014) and Ghimire et al. (2014) observed the microbial communities of copio-trophic utilize the higher amounts of labile Carbon, thus proliferating within such management practices thus further leading to the increase in the availability of easily degradable carbon in the soil system.

Microaggregates are distinguished by an increase in the amount of recalcitrant carbon and these environments effectively decline the microbial responsiveness to crop management practices (Lal and Kimble 1997). According to Pankaj et al. (2015), the research indicates that smaller aggregates are less affected by microbial responsiveness to crop management practices declined.

Cultivation disrupts aggregates, thus changing the proportion of macroaggregate and macroaggregates. Furthermore, an increase in the microaggregates leads to significant reductions in various chemical and biological properties like organic carbon, fungal biomass, respiratory activity, and enzyme activities like arylsulfatase and acid phosphatase in comparison with macroaggregates, whereas in macroaggregates aggregates became destabilized due to disrupted fungal hyphae were mineralized and their binding properties destroyed.

Soil architecture (aggregate hierarchy and slaking) imparts various habitats like aerobic and anaerobic microsites that are essential to support the survival and activities of a diverse microbiome. It is evident that soil cultivation affects soil microbial habitats due to the changes in particle size distribution and structure of pores. A major result of this disturbance in the soil initially with protected and

undisrupted organic matter becomes available for microbial metabolism and impacts populations and functions of microbial communities (Six et al. 2006).

Studies, activities, and research of microbial communities in different microenvironments including aggregate size are limited as this study has essential implications for increasing crop production and agricultural sustainability (Grundmann 2004). Besides, the researchers have limited understanding of the importance of soil aggregates in structuring microbial communities and not much is documented about the localization of microbial communities and their functions. The scientists need to study in-depth soil aggregate structure and the location of various microbial communities which have impacts on microbial community resilience to environmental stress.

18.7 Molecular Techniques to Measure Soil Health: Microbial Biomass

18.7.1 Fluorescence Microscopy

Estimating the populations of soil bacteria, as well as their biomass, cell volumes, and cell division frequencies can be done with the help of fluorescence microscopy and computerized image processing (Bloem et al. 1995). Some photoreactive molecules have a property known as fluorescence. This property is characterized by the absorption of energy at a specific wavelength (λ), which causes the electrons of the fluorescent molecule to move into an excited state. After a certain amount of time (also referred to as the fluorescence lifetime), a portion of the energy that was absorbed, is then emitted, which causes the electrons to return to their stable state (Herman 1998). Only at particular wavelengths, which are exclusive to a given molecule, can the fluorescence molecule's energy absorption and emission take place. Fluorescence microscopes are constructed with this very principle of the emitted wavelength fluorescence given off by the emitted fluorophores in mind.

For the purpose of researching and quantifying the microbial biomass in soil, intact soil samples are required. The use of procedures that involve resin embedding will preserve both the structure of the soil and the spatial link between the soil microorganisms and the matrix of the soil. Fixation, staining and de-staining, dehydration, resin embedding, and thin sectioning of the soil sample are the steps outlined in the technique for preparing a thin soil section (Altemüller and Van Vliet-Lanoe 1990). When imaging bacteria in thin sections, selecting an appropriate fluorochrome is the step that carries the biggest importance. Staining with fluorescein isothiocyanate can be utilized as a method for estimating the biomass of soil microbes. However, when using a fluorescence microscope, it might be challenging to differentiate between different types of bacterial cells in soil matrices because of autofluorescence and background staining. There are two different categories that emerge when fluorophores are categorized according to the functional features that they share.

The first group is responsible for staining individual components of the cell, such as nucleic acids, proteins, or lipids, whereas the second group is responsible for staining the entire cell. The second category of fluorochromes is vulnerable to fluorescent cell processes rather than fluorescing on their own (Tsuji et al. 1995; Riis et al. 1998). These fluorochromes do not fluoresce on their own. Studies on the spread of microorganisms do not usually focus on the activity of bacterial cells because it is not always a problem. There are many different fluorochromes that are based on different binding targets, such as acridine orang (DeLeo et al. 1997), ethidium bromide (Roser 1980), or DAPI for nucleic acid, FITC (Decho and Kawaguchi 1999), Mg-ANS (Mayfield 1975) for protein, and cellufluor (Hartmann et al. 1997) for polysaccharide. According to the findings of one study, the pathogenicity of resting spores of club root (*Plasmodiophora brassicae*) in soil was directly examined using a technique called fluorescence microscopy (Takahashi and Yamaguchi 1989). In addition, fluorescence microscopy is utilized in order to see nematophagous fungi in their natural habitats in soils (Saxena and Lysek 1993). Examination of the soil with a microscope has long been an essential part of the study of soil microbiology. The use of fluorescence microscopy allows for an ecological study of the many different kinds of microorganisms as well as the direct measurement of their population size. This direct insight into natural settings is made possible by the use of fluorescence microscopy.

18.7.2 DNA Measurement

It is essential to estimate the biomass of microorganisms in order to acquire an in-depth understanding of the roles that microbes play in the environments in which they are naturally found. Soil bacteria are crucially important to a number of processes, including the decomposition of organic matter, the mineralization of soil, and the creation of humus (Miltner et al. 2012; Semenov et al. 2018; Torsvik and Øvreås 2002; Van Den Hoogen et al. 2019; Veresoglou et al. 2015). Microorganisms in the soil are responsible for a number of important functions, including regulating the decomposition of organic matter and the cycling of nutrients. Consequently, this demonstrates the need for land management and soil fertility (Powlson et al. 1987). Agricultural soil management practices are responsible for a decrease in the fungal biomass, and the ratio of fungal biomass to bacterial biomass in agricultural soils is typically significantly lower than in natural soils (Bailey et al. 2002). Comparatively speaking, bacteria have a higher DNA concentration per unit of biomass compared to fungi. Because of this, ecophysiological indices such as qCO2 (microbial community respiration per biomass unit) and the Cmic:Corg ratio (microbial biomass C to soil organic C) are utilized frequently. Both the chloroform fumigation-extraction (CFE) and the substrate-induced respiration (SIR) methods are dependent on chloroform, however, and both of these methods have limitations when applied to soil in more severe conditions (Semenov et al. 2018).

The DNA content of soil microbes has become an increasingly significant metric for measuring the biomass of soil microbes (Semenov et al. 2018; Yokoyama et al. 2017), and the same can be said for the RNA content of soil microbes. The DNA ratio can be used as a measurement tool to determine the amount of bacteria in the soil (Loeppmann et al. 2016). In addition, a correlation between soil DNA concentration with Cmic (Fornasier et al. 2014; Gangneux et al. 2011; Marstorp and Witter 1999; Semenov et al. 2018) and Nmic (Yokoyama et al. 2017; Bouzaiane et al. 2007) has been reported in a number of research studies. This correlation has been found to be significant. However, there are studies that contradict the correlation between soil DNA concentration and Cmic (Griffiths et al. 1997; Leckie et al. 2004). These studies were conducted when the soil was contaminated with heavy metals over a prolonged period of time and in forest humus, which included pH and conductivity. As of today, DNA-based measurement of soil microbial biomass has been demonstrated to be constant even in extremely harsh soil conditions. This allows for the estimation of soil microbial biomass (Semenov et al. 2018).

The evaluation of microbial biomass in the quantification of microbial double-stranded DNA (dsDNA) is based on the amount of universal cell compound that is present in the sample. dsDNA quantitative analysis makes use of a fluorescent dye with a high level of sensitivity, such as PicoGreen® (Fornasier et al. 2014; Terrat et al. 2012). A wide variety of independent research proposed a conversion factor (FDNA) of μg dsDNA (g soil)$^{-1}$ to μg SIR-Cmic (g soil)$^{-1}$. This factor ranged in a remarkably small range, ranging from 5.0 (Anderson and Martens 2013) to 5.4 (Blagodatskaya et al. 2003) to 5.6 (Anderson and Martens 2013; Lloyd-Jones and Hunter 2001). It is possible to use DNA content to assess microbial growth dynamics after substrate addition to soil (Nannipieri et al. 2003; Anderson and Martens 2013), and in ecophysiological indexes, metabolic quotient, and activity parameters (Blagodatskaya et al. 2003, 2014), which are essential for accessing nutrient cycling and organic carbon decomposition in arid or semi-arid environments. This is an additional advantage of using DNA content (Vishnevetsky and Steinberger 1997). A fluctuation in DNA concentration is produced as a result of the non-uniform extraction procedures, which is one of the major constraints (Gong et al. 2021). The efficiency of DNA extraction may change depending on the conditions of the setting in which it is carried out (Torsvik et al. 1996). There is also the possibility that plant residues and recalcitrant extracellular DNA could change the DNA concentration, which would be an additional possible limitation. The DNA of dead plants can be discovered in the soil for a number of months after they have decomposed (Yokoyama et al. 2017) despite the fact that the dsDNA level of the plant never went above 2.6% of the total dsDNA content for a variety of soils (Gangneux et al. 2011).

18.7.3 Fluorescence In Situ Hybridization

The method of fluorescence in situ hybridization, or FISH, involves staining and counting the microorganisms that are found in the soil using oligonucleotide probes

that have been fluorescently labelled. Using this method, it is possible to do in-depth research on the microbial communities that are present in environmental samples (Amann et al. 1995; Daims et al. 2004; Stein et al. 2005; Eickhorst and Tippkötter 2008a, b). Visually detecting soilborne pathogens using FISH is another successful method that does not require the extraction of DNA from the sample (Milner et al. 2019). The FISH method relies on the detection of rRNA as its foundation; nevertheless, the number of phylogenetically diverse target organisms that can be recognized in a single experiment places limitations on the method's detection capabilities. FISH results are affected by the metabolic state of the organism or cell as well as the activity level of the organism or cell (Poulsen et al. 1993; Kemp et al. 1993; DeLong et al. 1989; Hahn et al. 1992; Moter and Göbel 2000; Amann et al. 1997; Pernthaler et al. 2001; Amann 1995; Christensen et al. 1999). It is feasible, through the use of FISH, to link geographical information with the metabolic capacities of microorganisms that have not been grown in the lab (Berry et al. 2013). When performing an analysis of FISH signals, it is generally agreed upon that the ability to detect active cells has a direct connection to the metabolic rates of the bacteria being studied (Bouvier and Del Giorgio 2003). A few thousand rRNA molecules must be present in order for a discernible FISH signal to be generated using monolabelled fluorescent oligonucleotide probes. This signal can be observed using fluorescence microscopy (Amann et al. 1995). The conventional technique is only applicable to bacteria that have a significant number of ribosomes. Because the FISH technique is incapable of detecting microorganisms that have a low ribosome concentration or that are dormant (Wagner et al. 2003; Daims et al. 2004).

Despite the fact that fluorescent particles in the soil were the most important factor in determining whether or not FISH-stained cells were found (Hahn et al. 1993; Zarda et al. 1997), this impact could be prevented by employing laser scanning microscopy in conjunction with Nycodenz in order to eliminate bacteria prior to FISH (Bertaux et al. 2007). Using horseradish peroxidase (HRP)-labelled oligonucleotide probes during tyramide signal amplification (TSA) is a promising prospective technique to solving this problem. There are many more potential approaches as well. This improved approach is known as the CARD-FISH method (catalysed reporter deposition). According to Eickhorst and Tippkötter (2008a, b), the high signal intensities of the tyramides utilized in the CARD-FISH process and the utilization of fluorescein-dyes in a double filter excitation allowed stained cells to be easily differentiated from the background autofluorescence. It is ideally suited for identifying bacteria that have a low rRNA content, which is a result of low physiological activity (Ferrari et al. 2006). There is also a method known as multicolour DOPE-FISH, which makes use of oligonucleotide probes that are double-labelled with various fluorophores at their 5′- and 3′-ends in order to induce fluorescence signal intensities (Stoecker et al. 2010; Behnam et al. 2012).

CLASI-FISH is another method that uses dye combinatorial labelling and spectrum imaging to concurrently detect several different species for the purpose of phylogenetic research (Valm et al. 2012; Mark-Welsh et al. 2016). Each microbial taxon is tagged with a specific combination of two or more individually monolabelled probes. This allows the taxa to be differentiated from one another

based on the spectrum features of the combined fluorophores. Since it was developed, it has been used to successfully differentiate between as many as 15 separate target organisms (Valm et al. 2011; Mark-Welsh et al. 2016).

In a nutshell, fluorescence in situ hybridization (FISH) is a useful instrument for microbiologists working in all fields since it allows them to visualize, identify, count, and precisely locate individual microorganisms.

18.7.4 RNA Measurement

The molecular method known as 16S rRNA analysis makes it possible to do more in-depth research on the microorganisms that are found in the soil. The study of 16S rRNA genes has brought about a significant change in the field of bacterial systematics. This has resulted in greater comprehension of the microbial variety found in the natural world (Duineveld et al. 2001). Methods of biomass estimation that are generally acknowledged as competent are sufficient for high-biomass systems that have been thoroughly researched; nevertheless, these methods are typically insufficiently sensitive for systems with extremely low amounts of biomass. Even in low-biomass settings, one may produce physiologically relevant results by quantifying the biomass of prokaryotes by using the 16S rRNA gene and the biomass of fungi by utilizing the 28S rRNA gene (Knox et al. 2017; Mueller et al. 2016). In addition to this, a recent study sequenced the bacterial 16S rRNA gene as well as the fungal 28S rRNA gene in order to conduct an analysis of the taxonomic profile. In order to conduct an analysis of extremely low levels of microRNA (mRNA), the reverse transcriptase polymerase chain reaction (RT-PCR) technique, followed by the polymerase chain reaction technique, is required (PCR). Real-time RT-PCR with SYBR Green I detection as the method of choice for the detection step allows for the generation of data that are both prompt and accurate (Pfaffl and Hageleit 2001). The choice of RT-PCR quantification method is determined by a number of parameters, some of which are the target sequence, the predicted range of mRNA concentrations, the desired level of precision, and the question of whether or not absolute or relative quantification is required (Freeman et al. 1999).

It is now possible to analyse the transcriptional activity of various soil microbial communities in real time by employing a technique known as metatranscriptomics. This technique makes use of whole RNA sequencing. In addition, total RNA sequencing has been shown to be effective in determining the functional roles of active microbial communities in soil (Urich et al. 2008; Hultman et al. 2015; Epelde et al. 2015; Geisen et al. 2015; Schostag et al. 2019) because of its capacity to explore regulatory responses to changes in the surrounding environment (Carvalhais et al. 2012). RNA viruses, unlike bacteria and fungi, have the capability to affect the carbon cycle in soil. This ability distinguishes them from bacteria and fungi. Phylogenetic analysis of soil metatranscriptomes from a variety of soil environments and time points reveals that fungi are the most prevalent hosts for RNA viruses in the grassland soil that was investigated (Starr et al. 2019). We have a very limited understanding of the magnitude of the effects that viruses have on the carbon cycle in

the soil at this time. Fungal viruses in the soil can have small but important effects on a variety of physiological processes, including toxin generation, reproduction, mating success, symbiosis, and other physiological consequences (Márquez et al. 2007; Zhang et al. 1998; Rodríguez-Cousiño et al. 2011). Through RNA-viromics, researchers have found that soil RNA viruses have the capacity to influence grassland ecosystems at multiple trophic levels (Hillary et al. 2022). Studying the activity of soil microbes using a methodology that combines RNA biology with metagenomic and metatranscriptomic techniques has, on the whole, been shown to be a fruitful line of inquiry.

18.7.5 Stable Isotope Probing

The SIP method is a molecular strategy that involves treating bacteria with substrates that have been labelled with the heavier and more stable isotopes of common elements. Additionally, it is a way for understanding the variety of microbial communities in the soil and the role that they play in the soil (Dumont and Murrell 2005; Neufeld et al. 2007). It is possible to identify the individual molecular components that make up a complete cell, such as its DNA or RNA or its proteins or lipids, in order to specifically target one or several active bacteria. The nucleic acid isotopic marker that is most widely employed is 13C. It is possible to separate 13C-labelled molecules from unlabelled nucleic acid through the utilization of density gradient centrifugation. Because the rate of RNA synthesis is far higher than the rate of DNA synthesis, RNA is considered to be a superior biomarker for use in SIP investigations when compared to DNA. As a consequence of this, they are determined not by DNA replication but rather by copy number, which is a measurement of cell activity rather than a measurement of replication itself (Manefield et al. 2002). SIP of mRNA is more sensitive than that of DNA because the label can be rapidly incorporated into mRNA and does not depend on cellular replication (Franco Dias et al. 2013; Jakobs-Schönwandt et al. 2010). It may now be possible to include microorganisms in a sample that do not reproduce (Pratscher et al. 2011). In certain low-growth settings, such as marine silt and seawater, certain types of algae can be found. Because they do not replicate their DNA in significant amounts, the cells of microorganisms may actively turn over RNA (Frias-Lopez et al. 2009; Glaubitz et al. 2009; Vandieken et al. 2012). By utilizing mRNA-SIP, it is possible to identify the organisms and genes that are involved in the assimilation of a substrate. Although DNA-SIP combined with 18O-water has been used to investigate the development and death of microorganisms in soils from a wide variety of ecosystems, including terrestrial, marine, and aquatic systems, the results have been inconclusive. Not to mention the fact that 18O-water is also utilized in RNA-SIP research (Schwartz et al. 2016). In addition, phospholipid fatty acid (PLFA) SIP is utilized for the particular microbial activity, metabolic function, lipid biosynthesis, and carbon flow target (Hanson et al. 1999; Beulig et al. 2015).

Together, SIP and high-throughput sequencing are becoming an increasingly popular combination. The goal of this sequencing effort might be to create a big

amplicon database, or it might even be to sequence a full metagenome or metatranscriptome from soil (Dumont et al. 2006, 2013). mRNA molecules that have been tagged with stable isotopes can offer a powerful picture of the metabolic activity of the enzymes that are linked with them at any particular point in time. In addition to this, it can be utilized to acquire a specific metatranscriptome from a group of functional microorganisms (Dumont et al. 2013). In the subsequent stage, we are going to employ metaproteomics in concert with SIP labelling to determine which members of the community are involved in metabolically active processes. In addition, the accurate quantification of incorporation in protein-SIP enables us to recognize food webs within microbial communities throughout time-course investigations (Jehmlich et al. 2016). Interactions between rhizospheres and microorganisms are another aspect of microbial ecology that could benefit from the knowledge provided by SIP. It has been demonstrated that root exudation has a substantial impact on the microbial population dynamics surrounding plant roots (Das et al. 2021). This may be seen, for instance, in the transfer and cycling of carbon in the soil (Singh et al. 2004; Griffiths et al. 2004a, b). The future seems bright for SIP, particularly when combined with other cutting-edge technologies that are targeted at enhancing our understanding of microbiology and biogeochemistry.

18.8 Molecular Techniques to Measure Soil Health: Genetic and Functional Biodiversity

Soil health can be easily characterized by the genetic and functional biodiversity of various invertebrates and microorganisms organisms within the soil (Rutgers et al. 2016). The functional biodiversity of a soil microbial community is indicative of the extent to which it occupies a given niche space (Yin et al. 2020). However, the cumulative estimate of the inherent quantity of genes in a soil microbe and its corresponding physiological expression within a biological community represents its genetic diversity within that unique soil ecosystem (Carolina 2018).

A comprehensive investigation and evaluation of the impact of both functional and genetic diversity on the health status of the "living" soil are indisputable. Molecular-based tools have found their application in analysing how genetically and/or functionally diverse a sampled soil microbiome is and this does not seem to be novel. These tools have been used to monitor soil microbial diversity through environmental-controlled experiments that study species composition under stress conditions (Leflaive et al. 2008).

Various tools have been considered and will be extensively explained in this review. While certain tools such as Biolog EcoPlates™ probe into how functionally diverse a soil microbial community might be, others rely on the polymerase chain reaction (PCR) dependent to properly investigate the relative gene composition of specific microbial species within the soil such as terminal fragment length polymorphism**,** denaturing gradient gel electrophoresis, and temperature gradient gel electrophoresis (Shawy and Burns 2005). By encoding for 16S rRNA in prokaryotes (18S rRNA for eukaryotes), these tools serve as gene fingerprints (Arias et al. 2005).

18.9 Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE) is a molecular technique to works by the principle of DNA fragment separation from PCR products based on similar base-pair sizes but differing sequence patterns. To achieve such segmentation of DNA fragments, the polyacrylamide gel used is characterized by the presence of an increasing gradient of chemical denaturants. Urea and formamide are two denaturants that have been long considered and utilized (Strathdee and Free 2013; Zulfarina et al. 2018). This method dates back to the 1980s when it was primarily exploited in detecting mutations at specific points (e.g., single-nucleotide polymorphisms; SNPs) of the genes that were linked to very striking disease conditions. However, the first recorded application of the DGGE as an analytical tool to probe into microbial communities goes way back to the early 1990s (Valášková and Baldrian 2009). This was a remarkable milestone and consequently prompted its frequent application for explorative research into soil microbial community. However, it is quite important to note that the DDGE seems to face some limitations given how complicated a soil microbial community can be. Hence, researchers have had to intentionally select PCR primers only specific to the target microbe population. In many cases, the actinomycetes and NH_3-oxidizing beta proteobacteria have been easily studied in this regard (Nakatsu 2007). Despite its known constraints, the DGGE technique is credited with the ability to efficiently generate individual species profiles for a studied soil microbe (e.g., bacteria). This is observable as separate bands each representative of the individual species and confirmation of the species after each band have been excised, sequenced, and compared to existing databases (Arias et al. 2005).

Summarily, the application of DGGE for soil microbial community analysis typically involves five main steps. First, as expected, the soil samples have to be collected from specific study locations followed by the DNA or RNA extraction procedure. Second, following the PCR protocol, the target gene of the microbe is amplified leading to the generation of a composite mix of gene fragments. Third, the PCR products are subject to separation via gel electrophoresis where, specifically unique to the DDGE, a denaturing gradient is utilized. After separation, the gel profile is visualized, as the fourth step using precise equipment. Fifth, further analysis is carried out for proper interpretation of the derived data (Shawy and Burns 2005).

In the past years until recently, the DGGE has found its prioritized application in different research studies that focused on undermining how diverse microbial communities of the soil can be. Here are a few, among many, of such scientific studies. In Indonesia, research desired to know how diverse the community of nitrifying bacteria could be within the tropical rain forests of the Bukit DuaBelas National Park and oil palm plantations of Sumatera and the tool employed was the DGGE (Zulfarina et al. 2018). Another research took a completely different investigatory path and attempted to understand how bacteria communities lived and interacted in *Pseudomonas putida*/Cephalosporin antibiotics-treated soils. Again, at the discretion of the scientists, the DDGE was considered the best tool for this

study (Orlewska et al. 2018). Similarly, Gelsomino and Cacco (2006) evaluated the changes in soil bacterial community composition after solarization practice and incorporation of biodegradable amendments on already cultivated fields of the Mediterranean University of Reggio Calabria in Southern Italy. Diagnosis of the presence of certain microbes within soil biomes has not been left out as seen in research by Jousset et al. (2010) where the DGGE was used to identify ciliate populations inhabiting soils polluted with polycyclic aromatic hydrocarbons. Likewise, a comparative study on the utilization of different 18S rDNA primers in DGGE explored the broad communities of fungi within the cultivated soils in farming regions of Japan (Hoshino and Morimoto 2008). Using the PCR-dependent DDGE protocol, microbial communities of different heavily-polluted locations have been characterized and tracked for sudden or expected changes in community makeup and interaction (Chen et al. 2016; Li et al. 2006; Wakase et al. 2008). These and many more highlight the unique role of DGGE in microbial studies of the soil.

18.10 Temperature Gradient Gel Electrophoresis

Another interesting technique for molecular analysis of soil microbial communities is the temperature gradient gel electrophoresis (TGGE). Similar to the entire procedure of the DGGE, its unique feature is the application of a temperature gradient for the denaturation phase of the gel electrophoresis process. The soil sample with the target DNA or rRNA is subjected to a PCR process for amplification after which it is passed through the usual polyacrylamide gel for separation into defined bands (Nocker et al. 2007; Rastogi and Sani 2011). Amplification is executed by using primers with 50-base pairs GC clamp followed by the incorporation of a regulated Peltier-based heating/cooling system for the generation of temperature gradients (Valášková and Baldrian 2009). Although, if compared to the DGGE, the TGGE has found less application in protein analyses, it, however, has been recommended as an efficient tool for PCR amplification specific to Unlike DGGE is less commonly applied to proteins but can be very effective in PCR amplification of mutable regions of the 16S rRNA sequences (Bharagava 2019). Given the chemical homogeneity of TGGE, it is considered to have some advantage over the DGGE which stems from the fact that the analysis by TGGE is concluded in real time (6 h) in contrast to that of DGGE (14 h) (Valášková and Baldrian 2009).

Just like the DGGE, the TGGE has also been successfully applied in various scientific research sought to investigate microbial populations and compositional shifts in real time. It has been confirmed to offer proper detection of individual species within a complex bacteria population even at low levels in microbe communities (Fouratt et al. 2003; Likar and Regvar 2009). Given its highly reproducible ability, research studies carried out for a robust investigation into several bacteria species have employed the TGGE technique in developing in situ probes that are unique to individual species (Arias et al. 2005). Some research applied the temporary version of the TGGE method to characterize endophytic fungal populations that inhabit the soil microbial communities stressed by heavy metal

pollution in the presence of blooming *Salix caprea* L tress (Likar and Regvar 2009). Similarly, an attempt to do elaborate probation into the bacteria population that makes up a nitrifying bio-augmentation product was accomplished with the application of the TGGE (Fouratt et al. 2003). Hence, we conclusively say that the TGGE is a proven tool for the molecular evaluation of soil health.

18.11 Terminal Restriction Fragment Length Polymorphism (TRFLP)

Another very important molecular tool used to study microbial communities is the terminal restriction fragment length polymorphism. Specifically, it finds its use in profiling communities of microbes by considering the location of restriction sites that are closest in proximity to terminals of an amplified gene sequence labelled with fluorescent dye. As a result of being reproducible, the TRFLP effectively analyses genes that express polymorphic tendencies and consequently, it helps to unveil unique characteristic features of a specific microbial community (Arias et al. 2005; Bharagava 2019). The TRFLP works on the principle of creating T-RF patterns that result from the amplification of DNA fragments from bacteria assemblage. This is made possible by utilizing one or two primers marked with fluorescence for the PCR after which the products are digested by restriction enzymes (Zhang et al. 2008). The TRFLP technique is quite remarkable in its ability to assess very complicated microbial communities by exclusively spotting-out single ribo-types that indicate restriction fragments with fluorescently-branded terminals (Rastogi and Sani 2011).

Like the already mentioned DGGE and TGGE, the TRFLP has been applied in recent years to understudy various community structures of diverse soil microbes. Research studies that assessed the functional and genetic reaction of microbial communities, present in slightly contaminated sites, to lower concentrations of bioavailable anthracene, used the TRFLP to accurately achieve substantial results. Besides, the TRFLP was efficient to explore the complex mega communities of bacteria and archaea inhabiting composted soil ecosystems (Louati et al. 2013; Tiquia 2010). The dynamics of bacterial community interaction and their evolution, within soil ecosystems harbouring plants treated with industrial wastewater, have been extensively analysed using the TRFLP (Fredriksson et al. 2019). Similarly, another research that explored the impact of introducing novel microbes into an existing and dynamic community of actinobacterial endophytes in the roots of wheat had very amazing results by engaging the TRFLP tool (Conn and Franco 2004; Pavithra et al. 2020). Wu et al. (2015) exploited the power of the TRFLP in analysing how diverse and rich the bacterial communities were in soils of varying forms of vegetation (broad-leaf forest, coniferous forest, subalpine dwarf forest, and alpine meadow) within a mountainous national reserve location in China. In addition, the TRFLP found its relevant use in estimating microbial richness, abundance, and biodiversity in soils of forest regions already impacted by seasonal fires (Mabuhay et al. 2004).

Despite the extensive application of the TRFLP, it poses a great disadvantage—it generates a non-reliable underestimate of community diversity. This has been found to result from a limitation in the number of bands generated for each gel electrophoresis (up to 100) given that the diverse bacterial species being studied have similar T-RF lengths (Rastogi and Sani 2011). Regardless, the TRFLP has established its importance as a great molecular tool for estimating microbial abundance and biodiversity in different soil types.

18.12 BIOLOG™

The BIOLOG™ has become one of the most popular and fastest molecular tools uniquely suited for very extensive analysis of the functional biodiversity of whole microbial communities. Its primary use has been devoted to studying metabolic dynamics within very complex and mixed populations of microbes. By coupling with Ecoplates (Biolog Inc., Hayward, CA, USA), the BIOLOG™ works on the single principle of generating a real-time estimation of how much carbon substrates are used up to determine the dynamic constant interactions playing out in microbial communities of the soil (Checcucci et al. 2021). A proper analysis of the active metabolic reactions of microbes within different biological systems, such as soils and sediments (Anna et al. 2017), water plants and grains (Ge et al. 2018), has become simply realistic with the development of the BIOLOG™. The operation and utilization of the Biolog™ EcoPlates are considered basic yet elaborate enough to give a broad description of the physiological profiles of communities (Zheng et al. 2020). This is quite important because an understanding of the physiological profiles of microbial communities aids an adequate appreciation of the genetic and functional structure of soil (Gałązka et al. 2018).

Summarily, Biolog™ Ecoplates has found relevant application in also tracking time-space changes in the biochemical activity of microbial communities, labelling unique characteristic features of diverse communities as well as assessing carbon source utilization patterns (Ecoplate Brochure 2017).

A brief description of what makes up the Biolog™ EcoPlate is eccentric to understand how it works. Three recurring sets of 31 lyophilized carbon substrates make the 96-well microplates that form the entire component of the Biolog™ EcoPlate setup (Ecoplate Brochure 2017). Carbon substrates can be a mixture of different biomolecular sources such as carbohydrates, amino acids, and polymers. A blank well is left unfilled serving as a control. A tetrazolium redox dye plays a significant role as an indicator of metabolic activity (Sofo and Ricciuti 2019).

The Ecoplate Brochure (2017) gives a very detailed description of how the Biolog™ EcoPlate is used for assessing the functional diversity of a microbial community. First, soil samples are collected, suspended, diluted to a defined cell density, and directly pipetted into wells before incubation. Kinetic runs are made to generate specific patterns that indicate the metabolic activity of the microbial community under study (Checcucci et al. 2021). Certain key features are observed to assess the physiological profiles of soil microbial community and these include,

first, the rate of colour change in individual wells (activity), stability of the generated patterns, and richness of a positive reaction (diversity). The most striking indication of substrate utilization by the microbial community is a redox reduction of the tetrazolium violet dye resulting in a colour change in individual plate wells (Checcucci et al. 2021).

The application of the Biolog™ EcoPlate technology in various research studies has predominantly focused on an assessment of the metabolic dynamics of diverse bacterial communities. This is quite significant given that free-living, predatory, and parasitic bacteria account for an extremely large portion of whole microbial communities. Hence, single research was devoted to analysing contaminated soils of the Riyadh community for bacterial strains and their active utilization of biomolecules by using the Biolog™ EcoPlate tool (Al-Dhabaan and Bakhali 2017). Similarly, communities of heterotrophic bacteria, inhabiting various soils in the Netherlands, were investigated and profiled for their physiological components (Rutgers et al. 2016).

Very recent research studies reveal that sustainable olive orchards in Southern Italy were examined by the Biolog™ EcoPlate technology to determine the functional diversity of their resident soil bacterial community (Sofo and Ricciuti 2019). Besides, soils stressed by long-term pollution with petroleum hydrocarbons were analysed with the aid of Biolog™ EcoPlate to assess their bacterial microbiome in terms of genetic and functional biodiversity (Gałązka et al. 2018). Specifically, in East China, the Biolog™ tool found its eccentric use in tracking the physiological profiles of soil microbes in the Chaohu Lakeside Wetland (Zhang et al. 2014). This tool remains effective today in estimating the health status of different soil groups around the world despite the evolving climate change events.

18.13 Microbial Resilience

A soil's resilience capacity is directly related to its microbial biodiversity and hence microbial resilience can be thought to be an estimation of the population of a given microorganism living within a stressed soil (Mehta et al. 2022). Such stress could be drought, water logging, ground fires, and even contamination with non-biodegradable substances (Arias et al. 2005). The ability of the population of a defined soil microbial species to withstand adverse soil environmental conditions and still bounce back to its full physiological potential post-stress represents a significant component of soil stability (Griffiths et al. 2008). There are concrete assumptions that a microbe's physiology and the composition of its highly diverse community play peculiar roles in estimating its innate resilient capacity which is also a function of the soil's physicochemical characteristics (Griffiths et al. 2004a, b).

Unlike other tools that have been highlighted as indicators of soil health, the adoption of microbial resilience is unique yet quite unpopular. Its uniqueness stems from the fact that it focuses on providing a reliable estimate of how hardy a soil-residing microbial population can be under stress conditions. Therefore, several research studies have found microbial resilience applicable in investigating

microbial communities. To understand the behaviour of a soil-dwelling decomposer to differing conditions of the soil, research studies investigated how much time it took for *P. fluorescens* when grown first in sterile sandy or clay-loam soil and then in soil stress with copper and heat (Griffiths et al. 2008). Similarly, sites across Kadi, India, with oil-contaminated soils were sampled and examined using microbial resilience analysis to quantify the functional diversity of the communities of microbes present in them (Patel et al. 2016).

In some cases, examining the changes in extracellular enzyme activity (EAs) of soil microbes subjected to abiotic stress has been used as a strong indicator of microbial resilience. A scientific study observed a significant decrease in the inherent SOM content of the soils exposed to heat waves in the high plains of Texas. Such a conclusive finding was based on changes to the makeup of the evaluated communities of microbes stemming from a sharp increase in EAs (Acosta-Martínez et al. 2014). However, variations were found across different soils in Scotland in terms of how resilient their microbial communities were when exposed to stress conditions (Kuan et al. 2007). This is certainly indicative of the health status of such soils. Another research applied the concept of microbial resilience to understand the microbial dynamics of different cultivated soils under drought conditions (Pérez-Guzmán et al. 2020). Nevertheless, the use of microbial resilience as a tool in determining soil health is highly dependent on the application of previously mentioned techniques.

18.14 Omics and Soil Microbial Diversity

18.14.1 Soil Nucleic Acid High-Throughput Sequencing Technologies

The quality, speed, and cost of high-throughput sequencing technologies are all rising quickly. As a result, it is increasingly being utilized to research entire communities of prokaryotes in a variety of fields (Di Bella et al. 2013). Prokaryotes are dominant in our globe. Estimates place the total number of microbial cells on Earth at 10^{30} (Turnbaugh and Gordon 2008). There are up to 100 trillion creatures in the human body, which is roughly ten times the amount of our own human cells (Savage 1977). There are literally millions of prokaryotic species, though most have not yet been cultivated (Jordan 2017). There are likely to be numerous enzymes and metabolic capacities encoded by these species' genes that have yet to be discovered. Bacteria have a vital part in the control of digestive, endocrine, and immunological systems in the human body. The makeup and diversity of the human microbiome are being discovered thanks to the development of more recent culture-independent sequencing-based technologies (Di Bella et al. 2013). The earliest direct cloning of environmental microbial DNA was proposed by Lane et al. (1985), while the term "metagenome" was proposed by Handelsman et al. (1998) to refer to "the genomes of the whole microbiota found in nature," which refers to the entire collection of genetic information for all bacteria in a given environment. Microbiomes, especially

those linked to human health and disease, have gained great insight thanks to advancements in technologies such as sequence- and function-based gene screening, high-throughput sequencing, and metatranscriptomics (Hess et al. 2011; Qin et al. 2010). Soil is a very complex environment containing huge microbial diversity (Torsvik and Øvreås 2002; Cameron et al. 2018). Its characteristics depend on physical and chemical but also biological factors (Marcote et al. 2001). The biotic component makes up about 0.2% of the soil, with microorganisms accounting for 20–40% of the total and influencing 80–90% of soil processes (Gregorich et al. 1997). Although there is a tremendous amount of micro- and meso-organism variety in soil, little is understood about the mechanisms that these creatures are engaged in (Geisen et al. 2019). A deeper understanding of soil biodiversity and its functions is urgently required given our limited understanding of the involvement of the biotic fraction in soil biochemical pathways. Since successful downstream analyses mainly depend on good-quality DNA, this is the approach's goal. There are a lot of inhibitory chemicals in soil, according to Bessetti (2007) and Huang et al. (2016), which stop or obstruct DNA amplification. The main challenge for the PCR amplification processes is these chemicals. Inhibitors can co-precipitate with DNA, adversely affecting the extract's quantity and quality (Demeke and Jenkins 2010). The most frequent inhibitors are humic chemicals, followed by heavy metals and aromatic compounds (Fornasier et al. 2014).

18.14.2 Soil Metaproteomics

Soil is a complex and dynamic network of biological processes that are intricately linked to allow ecosystems to function properly. Microbial diversity and function are essential for the proper functioning of ecosystems and their long-term survival. To date, metagenomic investigations have revealed the enormous diversity of both culturable and unculturable microbiomes in distinct ecosystems, but their precise significance in ecosystem functioning remains unknown. This can be done by looking at the ecosystem’s protein repertoire, which are the direct and undeviating key participants in metabolic processes. Metaproteomics is a new discipline that attempts to capture all of the proteins present in a given environment at a defined time interval. Profiling microbial enzymes may be a sensitive indication of the soil ecosystem because it connects the phylogeny and functionality of soil microorganisms, describing not only at the level of the individual dominant organism, but also at the community level. The method to mining these functional complex soil microbiomes became viable with the advent of high-performance mass spectrometry; nevertheless, it is hampered by the presence of several interfering compounds in the soil samples (Abiraami et al. 2020). Environmental meta-omics in situ is quickly growing, offering a snapshot/profile of both cultivable and non-cultivable microbial populations present, as well as their functional functions in the environment. Soil is a huge, heterogeneous, and dynamic environment, and understanding its microbial life is crucial for biogeochemical cycling, restoration, and bioremediation. Although the active microbial community in the soil

is small, only about 1.8–2% (Bastida et al. 2009) the dead microbial biomass and dormant microflora, as well as their metagenome, can overture the relative number of phyla present. Metatranscriptome can be more useful in capturing community function and tracking active microbial diversity. Metatranscriptome approaches recurrently misrepresent community functioning because of the regulatory control of translation in alternative splicing of mRNA, codon bias, mRNA degradation along with post-translational modification of proteins, protein turnover rate, and low quality of transcript assembly obtained (Lau et al. 2018). These constraints can be circumvented by examining the proteins that are the ultimate functional participants in the cells that perform the function (Gutleben et al. 2018). The advent of high-throughput mass spectrometry, advances in protein identification platforms and separation techniques, concentrated efforts in extraction standardization, and the availability of a plethora of genomic databases have all resulted in significant advancements in the metaproteomics domain. Metaproteomics, by definition, is the study of aiding the identification of the protein repertoire at the community level in order to gain a better knowledge of how ecosystems work. It also assists in identifying the most active enzymes in the community as well as the phyla responsible for the function (Abiraami et al. 2020).

18.14.3 Soil Metabolomics

Metabolomics, the large-scale study of low molecular weight organic compounds in soil, offers one potential approach to characterize soils and evaluate the metabolic status of the soil biological community (Withers et al. 2020). Soils are central to a wide range of ecosystem services that are essential to earth system functioning (Bünemann et al. 2018a, b). As a result, it is critical that we keep an eye on our soils' health so that ecosystem services can continue to be provided (e.g., nutrient cycling, water purification, food provisioning, climate regulation). While a variety of soil quality indicators have been developed, the majority of them are focused on measuring conventional chemical features of the soil (e.g., pH, accessible P and K, organic matter content) as well as physical qualities of the soil (e.g., texture, structure, aggregate stability, bulk density) (Schloter et al. 2018). The creation of reliable markers of soil biological quality that may be widely used has eluded researchers despite numerous attempts (Schloter et al. 2018). Measurements of biological activity, such as baseline and substrate-induced respiration, enzyme activity, and the size and composition of the microbial population, such as $CHCl_3$ fumigation-extraction and fatty acid biomarkers, are a few examples of classic indicators (Bending et al. 2004). However, new approaches to evaluating soil biological function have been made possible by the advent of "omic"-based technologies for the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). While metagenomics and metabarcoding are becoming more common (Cameron et al. 2018; George et al. 2019) metabolomic analysis of soil microbial populations has received far less attention, untargeted metabolomics permits a worldwide

examination of the low molecular weight (1000 Da) metabolites present in a sample (Untargeted Metabolomics_Enhanced Reader.pdf n.d.). Recent advances in spectroscopy have made it possible to identify and quantify the relative abundance of thousands of metabolites present in biological samples (Patti et al. 2012). Metabolomics and its use in ecology (Enhanced Reader.pdf n.d.) and is not constrained by unknown levels of epigenetic control and post-translational modifications, respectively. A metabolomic technique is comparable to genomics and proteomics in terms of cost (Wilson et al. 2005) and allows for rapid sample processing (Patti et al. 2012). Furthermore, the approach can detect biochemical intermediates in interconnected metabolic pathways, potentially boosting our overall understanding of biological processes in soil and our ability to anticipate results (Withers et al. 2020).

18.15 Targeted and Untargeted Approaches to Soil Microbial Diversity Management

Soils act as a main habitat for many living organisms which includes fungi, bacteria, insects, and plants; however, soil microbes play an indispensable role in the ecosystem services such as nutrient cycling and waste recycling, maintaining soil structure, detoxification of harmful chemicals, reducing soil erosion, and reducing greenhouse gas emissions by carbon storage (Aislabie et al. 2013). It is believed that 1g of soil may accommodate billion of bacteria, and furthermore, higher diversity and population of microbes are found near the rhizosphere as microbes require water and nutrients for their survival and such requirement is met at rhizosphere (Terrence et al. 2021). Presence of functioning microbial community in the soil is an invaluable asset as it helps in achieving soil quality, fertility, and sustainable agriculture (Sun et al. 2016). Soil microbes play a significant role in regulating plant diversity and productivity; for instance, plant growth is promoted by rhizobium bacteria through nitrogen fixation, and likewise, diversity and productivity of plant community is improved with increased Arbuscular mycorrhizal fungi (AMF) richness and this is due to the reason that AMF improves plant uptake of resources from the soil and also protects the plants from disease-causing soil microorganisms (Schnitzer et al. 2011; Gray and Smith 2005; Vogelsang et al. 2006).

Plant roots emit exudates into the rhizosphere which provides nourishment to soil bacteria and as a result much higher population will be found in the rhizosphere when compared to other regions of the soil (Gray and Smith 2005). Crop plants which require sulphur for vitamin and protein synthesis are completely dependent upon soil for the sulphur uptake and on the other hand immobilization and mobilization of sulphur in the soil (which is organically bound) are believed to be carried out by soil microbes (Kertesz and Mirleau 2004). Activities related to management of soil microbes in the agricultural field can have beneficial impacts; however, farmers have limited tools/no tools to measure the impacts of implemented management practices and sometimes such management practices can also cause negative/unwanted impact (Terrence et al. 2021). There are two approaches (Targeted &

Untargeted) for soil microbial diversity management. Targeted approach includes zero tillage/conservation tillage and biofertilizer application. Untargeted approach includes organic farming and conservation agriculture.

18.16 Targeted Approach

18.16.1 Zero Tillage/Conservation Tillage

Tillage is performed for the following reasons: to prepare a seedbed suitable for sowing/planting; to minimize the soil compaction; to control the growth and spread of weeds; to incorporate crop residues, fertilizers; to follow the tradition learnt from ancestors (Gebhardt et al. 1985; Feng et al. 2003). Soils based on their structure, moisture level, and organic matter respond distinctively to the tillage, and as a matter of fact, soils are prone to erosion under conventional tillage (Gebhardt et al. 1985). Conventional tillage disrupts the basic structure of soil and lowers not only the crop residues on the surface of the soil but also the soil quality (Das et al. 2014). Zero tillage or conservation tillage helps the soil to preserve moisture and build organic matter, and thereby, it creates suitable habitat for soil microbial community, and moreover, many studies show that soils with zero tillage contain highest soil microbes when compared with soils which are conventionally tilled (Lauren Quinn 2016).

This may be due to the reason that tillage has an impact on soil microbes through altering the microclimate of the soil and organic matter content, and meanwhile, in the global perspective, zero tillage is being implemented only on 11% of total arable land (Zuber and Villamil 2016). In conservation tillage, chisel ploughs are used to cause least possible disturbance to the soil, and as a result, higher microbial community is associated with such soils than the soils which are tilled with disc ploughs or mouldboard ploughs (Lauren Quinn 2016). When the soil is less disturbed, fungal hyphae are not affected and play a vital role in nitrogen and carbon cycling (Zuber and Villamil 2016). Governments in the developing countries as far as concerned are likely to support conventional tillage and this may be due to lack of expertise to support them in switching from conventional system to conservational agricultural systems (Kassam et al. 2014). Soils which are not tilled for longer period of time showed increased levels of nitrogen, soil carbon, phosphatase activities, and total phospholipid fatty acids when compared to conventionally tilled soils, and on the other hand, crop residues which get accumulated on the soil surface in zero tillage systems are transformed into soil organic matter, and as a result, density of soil microorganisms gets increased (Mathew et al. 2012).

18.16.2 Biofertilizer Application

Biofertilizers are composed of living cells of microorganisms which promotes nutrient uptake by plants and improves the quality of the soil (Fasusi et al. 2021).

Especially in the last two decades, there seems to be a lot of growing interest in the research of microbial populations colonizing various habitats and their combined contribution to several parameters such as plant growth and health (Kumar et al. 2021). Biofertilizers have always had the capability to directly expand the beneficial soil microbes. For testing microbes as biofertilizers, they are first examined for higher colonization capacity and characteristics that promote plant development (Kumar et al. 2021; Pandey et al. 2019). Carbon requirement of soil microbes is met through the exudates emitted by the plant roots (Kumar et al. 2017). Crop yields are expected to rise by 10–40% with the usage of biofertilizers (Mahanty et al. 2017). Plants tend to absorb more phosphorus from the soil when the AMF are introduced into it (Duponnois et al. 2005). Mixed inoculation of *Azospirillum brasilense* and arbuscular mycorrhizal consortia resulted in higher quantity of Azospirillum colony-forming units (CFU) and arbuscular mycorrhizal (AM) spores in the soil (Mishra et al. 2008).

When *Pseudomonas monteilii* strain HR13 is inoculated in the soil where Australian *Acacia* species is grown, ectomycorrhizal colonization with the plant roots was greatly improved (Duponnois and Plenchette 2003). When *Glomus intraradices* was introduced into the soil where *Acacia holosericea* is cultivated, the fluorescent pseudomonads community has increased dramatically (Duponnois et al. 2005). Few microorganisms which naturally occur in the rhizosphere are found to arrest the development of disease-causing microbes in the soil and also have the potential to influence the immune response of the plants (Sahu and Sindhu 2011; Wang et al. 2022). When *Glomus mosseae* was introduced into the soil, the biomass of the soil microbial community continued to rise (Zarea et al. 2009). Cytochemical and plating tests could be a good way to investigate the impact of bacterial fertilizers on the soil microbial community (Sharma et al. 2012).

The application of *Bacillus amyloliquefaciens* strain NJN-6 as a biofertilizer has decreased the prevalence of fusarium wilt disease in banana plantations by modifying the soil microbial community in a certain manner that *Bacillus, Cantharellus, Synchytrium* biomass has improved and has acted negatively with fusarium populations by significantly reducing their growth (Shen et al. 2015). Soils treated with two biofertilizers (one containing *Bacillus amyloliquefaciens* W19 and the other containing *Trichoderma guizhouense* NJAU4742) has shown greater phylogenetic diversity of microbes and showcased far more fungal and bacterial abundance than in the soils treated with chemical fertilizers and these biofertilizers has encouraged the growth of microorganisms in the soil which shows antifungal activity and suppressed the growth of *Fusarium oxysporum* which causes serious disease known as fusarium wilt (Xiong et al. 2017).

18.17 Untargeted Approach

18.17.1 Organic Farming and Conservation Agriculture

Long-term organic farming significantly enhanced microbial heterogeneity and richness under the plastic tunnel cultivation system (Liao et al. 2018) and also in the general agricultural fields and this may be due to the availability of organic carbon through organic manures and also the presence of few weed species creates suitable habitat for soil microbes (Lupatini et al. 2017). Presence of higher microbial diversity in the organic soils depends upon the type of management followed, for instance, organic fertilizer application and controlling pests using biological control agents (Chaudhry et al. 2012; Lupatini et al. 2017). Higher microbial diversity and evenness are seen in the soils of organic farms than conventional farms and this is due to the usage of plant protection products in conventional agriculture which creates unfavourable conditions and eventually results in death of few microbial groups present in the soil (Sugiyama et al. 2010; Lupatini et al. 2017). Organic fertilizers, crop rotation, cover cropping, and non-chemical pest and disease management are the main components of organic farming whereas permanent soil cover, crop rotation, and intercropping are the main components of conservation agriculture.

18.17.2 Organic Fertilizers/Manures

When compared to chemical fertilizer treatments, the densities of bacteria and fungi are relatively high in organic fertilizer treatments (Xiong et al. 2017). Farmyard manure application has altered the soil microbial community in a positive manner by increasing richness and lowering dispersion (Liao et al. 2018). Frequent organic manure application in agricultural fields has resulted in elevated concentrations of soil microbial biomass when compared to mineral (chemical) fertilizer application (Esperschütz et al. 2007). Compost made from sewage sludge has accelerated soil microbial activity (Bastida et al. 2008). Organic fertilizers not only promote the multiplication of soil microbes through their carbon and nitrogen but also lowers the microbial diversity in the soil if excess phosphorus is released through such manures/fertilizers (Ren et al. 2018; Wu et al. 2021). In rice fields, the richness of soil microorganisms is comparatively higher in the field treated with organic fertilizer than in chicken manure-treated field, and moreover, population of pathogenic *Pseudomonas* has expanded in the chicken manure-treated field (Li et al. 2020). Bacterial community richness and activity of few enzymes such as saccharase and urease have declined with increasing manure application (Sun et al. 2014). Vermicompost application to the soils increases iprodione concentration in the soils and which in turn causes reduction in microbial density and diversity in the soil (Verdenelli et al. 2012).

18.17.3 Crop Rotation

Soil microorganisms react differently to the root exudates released by different crops, and in addition, greater diversity of crop residues which are deposited on the farm as a result of crop rotation serves as a source of organic matter to the soil microbes and eventually their diversity gets increased (Costa et al. 2006; Venter et al. 2016). Crop rotations can lower the densities of soilborne plant pathogens and minimize the outbreak of soilborne diseases (Larkin et al. 2012). The infection percentage of *Rhizoctonia solani* (soilborne plant pathogenic fungus) was reduced more than half in potato when it is grown in rotation with *Vicia villosa* Roth, *Lupinus albus* L.ultra, *Avena sativa* Astro, and *Medicago sativa* L. Nitro (Honeycutt et al. 1996; Chosdon et al. 2021). When *Brassica napus* L. is used in crop rotation programme, not only microbial activity has improved but also culturable bacterial populations in soil has improved to a certain level (Bernard et al. 2012; Larkin et al. 2010).

Total microbial activity and culturable bacteria were improved when potato crop is grown under crop rotation programme with barley and canola when compared with potato monocropping, and moreover, the fluorescent pseudomonads and actinomycetes population is higher under potato–barley rotation than with canola and sweet corn rotations (Larkin 2003). The type of plant species also influences the soil microbial population as different plants release different kinds of organic compounds into the soil (Grayston et al. 1998; Larkin 2003). The bacterial population and diversity remained constant in soil whether it is maize monoculture or wheat-maize crop rotation (Navarro-Noya et al. 2013). When pulses are cultivated as rotation crop in wheat cropping system, they favoured the growth of plant pathogenic microbes (especially fungal populations) in the soil indicating the possibility of outbreak of soilborne diseases (Yang et al. 2021).

18.17.4 Cover Cropping/Permanent Soil Cover

Soil microbial richness is also determined by the type of cover crop grown as the crop residue which will be returned to the soil varies and therefore when Oat is used as a cover crop where organic farming practices are followed, the quantity of saprotrophic fungi was increased when compared with rye as a cover crop leading to a greater fungal: bacterial proportion (Martínez-García et al. 2018). Cover crops help in the stable development of beneficial microorganisms in the soil (Mercado-Blanco et al. 2018). When spring wheat, hairy vetch, and forage oat are cultivated as cover crops, more even population of AMF are linked with vetch and oat, and on the other hand, greater fungal richness is associated with wheat (Benitez et al. 2016).

The type of management practices followed in the farm and the cover crop together has the capacity to influence the catabolic nature of the soil microbiota (Martínez-García et al. 2018). Cover crops which yield high-quality residues support more bacterial growth, and accordingly, the ones which yield lower quality residues encourages fungal growth (Kramer et al. 2012; Muhammad et al. 2021). In organic

Zea mays L. fields, the colonization capacity of arbuscular mycorrhizal fungi has been improved by cover cropping (Njeru et al. 2014). When winter rye is cultivated as a cover crop in a potato-rapeseed/canola crop rotation, common scab and black scurf (soil-borne diseases) are decreased by 25–41% (Larkin et al. 2012). Planting *Flemingia macrophylla* as a cover crop for a period of 10 years enhanced bacterial populations and diversity in rubber plantations up to a depth of 60 cm inside the soil (Liu et al. 2019).

18.18 Future Prospects

Given the environmental challenges, current and future generations of scientists are and will be facing (e.g. climate change, soil erosion, water and soil pollution, salinization, loss of soil nutrients) in combination with the need to secure enough food production for a growing human population, it is fair to say that soil health measurement will continue to be a recurring topic among researchers and policymakers in the near future. Therefore, a more systematic approach to the exploration of soil health indicators and measurements is desirable. Some improvement areas are listed below.

- Making a distinction between general (that may be considered as universal) and specific (that depend on the geographic location, climate, soil type and history) biological indicators (Van Bruggen and Semenov 2000).
- Putting chemical and physical soil health indicators in relation with the newest biological indicators. In order to achieve this objective, a shared effort from scientists and researchers to fill knowledge gaps on the biochemical properties of soil is needed (Gil-Sotres et al. 2005). The fast development and validation of high-throughput -Omic technologies could speed up this process.
- Integrating different measurements to compose a complete picture of soil health (i.e. a soil health index) that can be used consistently across (or easily adaptable to) different environments and geographical areas. Rinot et al. (2019) suggested a multivariate-complex soil health approach with the aim of developing a new soil health index which could consider the connections between soil attributes and the Ecosystem Services provided (Fig. 18.1).
- Bridging the gap between scientific research and the agricultural sector (Doran 2002), following examples of best practices such as the Indian Soil Health Cards (Patel et al. 2017).

With regard to microbes as bioindicators, Fierer and Schimel (2002) gave some perspectives on how microbial data should be considered and microbial indicators adopted. Among those, they suggest to define microbial indices oriented to measure specific soil health outcomes rather than broadly profiling the microbial community, provide clear interpretation and guidance on how to interpret microbial measures also in specific geographical contexts, use microbial data to track how soils change over time (also as a consequence of agricultural management practices) rather than to

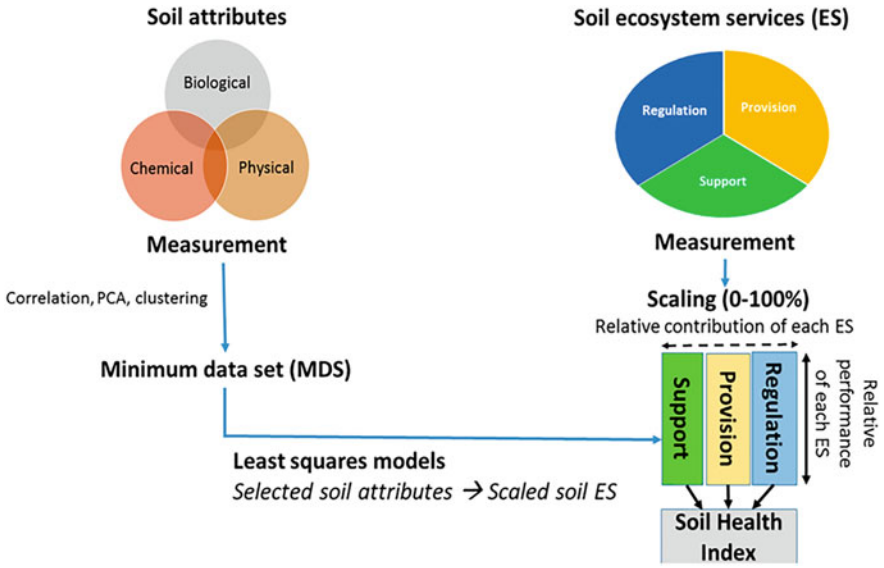

Fig. 18.1 The approach to soil health assessment proposed by Rinot et al. is depicted. By means of measurement and selection of specific soil attributes through monitoring and scaling of ecosystem services, a Soil Health Index is calculated. (From Rinot et al. 2019)

expect healthy soils to have a particular soil microbial community and use microbial measurements to infer soil characteristics only when other pre-existing methods are not sufficient, especially when the first ones are cheaper or easier to obtain.

18.19 Conclusion

Soil health is evidently an important component of sustainable development, especially with regard to the agricultural sector. In this review, we showed how soil health measurement evolved over time, following the discovery of its main biological drivers. We reviewed the most recent advancements on those measurement techniques and delineated targeted and untargeted approaches for a sustainable microbial community management of agricultural soils. We showed the importance traditional soil measurement techniques can still have, and the powerful information and progress the newest -Omics techniques can bring, despite the ongoing discussion on a shared definition of soil health. Overall, this article provided an overview on the currently used soil health measurement techniques accompanied by the most recent advancements on this topic, with the aim of giving a complete framework on the state of the art of this discipline. Soil health will surely benefit from soil scientists' efforts towards a more systematic, clearly interpretable set of traditional and biological indicators. It is clear that there are still knowledge gaps to be filled and methodological details to be discussed and to agree on among the scientific

community. Nevertheless, it is also evident that important advancements in this field of study will unravel the full potential of soil health measurement in securing a sustainable soil health management approach that will benefit the Earth's ecosystems.

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