Rhizosphere Biology

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Anil Kumar Sharma *Editors*

Millet Rhizosphere

Springer

Rhizosphere Biology

Series Editor

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ISSN 2523-8442　ISSN 2523-8450 (electronic)
Rhizosphere Biology
ISBN 978-981-99-2165-2　ISBN 978-981-99-2166-9 (eBook)
https://doi.org/10.1007/978-981-99-2166-9

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Preface

Millets are the group of small-grained cereal crops that are cultivated for daily human carbohydrate needs. These are among the oldest cultivated crops mainly distributed into two groups—major and small millets based on seed size. Major millets are composed of sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*) while small millets are composed of finger millet (*Eleusine coracana* (L.)), little millet (*Panicum sumatrense*), kodo millet (*Paspalum scrobiculatum* (L.)), foxtail millet (*Setaria italica* (L.)), barnyard millet (*Echinochloa frumentacea* (L.)), proso millet (*Panicum miliaceum* (L.), etc. These crops were earlier considered orphan crops, but recently due to their nutritional values, it has gained importance. They are a significant staple in the semiarid tropics and guarantee food and nutritional security for farmers, who can't grow other food crops because of poor rainfall and soil fertility. Due to their dual-purpose application, they are used as both food and fodder and make the millets a good choice for farmers to achieve better profitability. Considering this, the U.N. General Assembly declared 2023 as the 'International Year of Millets'. The objective of all planned endeavors during this year on increasing public awareness of the health advantages of millets and their suitability for cultivation under harsh climatic conditions. The millets require very few Agri inputs and are highly tolerant to changes in temperatures, droughts, and floods. However, to fulfill the growing demands we need to increase their production *via* the utilization of natural and sustainable resources.

As we are aware, the interaction between microorganisms and plants has attracted increasing attention, and a community of microorganisms that inhabit the plant environment is called a different microbiome based on the location—above ground (phyllosphere), inside the plant tissues (endosphere), and below ground (rhizosphere). All these microbiomes play a vital role in maintaining the health of plants. The most diverse among them is rhizospheric microbiome that includes various microorganisms such as bacteria, fungi, actinomycetes, algae, protozoans, and nematodes. These microorganisms promote plant growth by nutrient acquisition, suppression of pathogens, and alleviation of abiotic stress.

This book reviews the role of the rhizospheric microbiome in millet plant health management. Various reports are published based on the role of the rhizosphere in the growth and health of these crops. The rhizosphere, being a dynamic interface among the plant roots and soil microbes, provides several advantages to the millets too. The soil properties in the rhizospheric region are also different as compared to the bulk soil. *Millet Rhizosphere*—a key to enhanced crop productivity—is the first book that explicitly establishes the links between the extraordinarily small-scale microbial processes and the growth and yield attributes of millet crops. This book has 16 chapters that are contributed by eminent subject specialists and emphasize the effects of rhizosphere biology on long-term millet crop management for achieving nutrition security. Considering all the aspects discussed in the book, we are confident that this compilation will help in promoting the use of beneficial microorganisms to enhance productivity of millets.

We are grateful to the authors and people who directly or indirectly helped to compile this book. We thank Ms. Akansha Tyagi and Ms. Muthuneela Muthukumar of Springer Nature for continuous support during the publication process. We would also like to thank our families, friends, and colleagues for the help in the course of activities of writing this book.

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Chapter 1
Plant–Microbe Interactions Promoting Millets Plant Growth and Health: Perspectives for Use of Microorganisms in Millets Production

Belur Satyan Kumudini and Savita Veeranagouda Patil

Abstract Soil nutrition and health is of utmost importance for the plants to survive and withstand the various stress factors (abiotic and biotic) posed in the present climatic change situation. Millet plant health and its productivity are in turn related to soil ecosystem. The soil microorganisms in specific plant growth-promoting microorganisms (PGPM) play major role in managing the stress factors and thereby augmenting the plant growth and health by different signal cascades. Studies have evidenced the role of different PGPMs in managing/regulating major biotic stress (bacterial, fungal, and viral diseases) and abiotic stress (drought, salinity, and heavy metal). Also, they can increase the carbon sink in the soil by different mechanisms. Hence, a concise review on the different PGPMs used to enhance millet plant health and growth in turn the productivity is given in this chapter.

Keywords Millets · PGPM · Nutrition · Plant growth and health

1.1 Introduction

Over the years, climatic variations have posed increase in temperature and undetermined pattern of rainfall. It has increased the necessity to increase the crop productivity by utilizing various strategies, for which it is important to understand the underlying mechanisms, signal cascades which have crafted the climate resilient smart adaptive features in some of the crop species. The best-studied cereal crops are millets which are semiarid tropical crops grown in minimal environments and used as food or livestock. The different types of millets are pearl millet (*Pennisetum glaucum*), kodo millet (*Paspalum scrobiculatum*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*) and barnyard millet

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_1

(*Echinochloa frumentacea*), and proso millet (*Panicum milliaceum*) which are considered to be rich in nutritional values (Dayakar et al. 2017; Patil and Kumudini 2019; Numan et al. 2021). In comparison to the other cereal crops, millets are nutritionally rich with high levels of essential amino acids, and micronutrients like vitamins and calcium (Konapur et al. 2014; Nithiyanantham et al. 2019; Sharma et al. 2021; Dey et al. 2022).

Millets are grown and produced in the semiarid and tropical regions. They are considered as underutilized or forgotten or orphan crops (Dey et al. 2022). To overcome this umbrella on millets, United Nations has declared 2023 as the *International Year of Millets*, to unleash the potential of millets for the well-being of people and the environment. Millets are largely produced in India accounting for more emphasis on pearl millet as it serves the food and fodder for many (Dayakar et al. 2017). Therefore, it is now important to have an insight in enhancing the growth and health of the millet crops which in turn is beneficial to the mankind. With this perspective, the current chapter highlighted the importance of plant–microbe interactions in promoting millet health and growth.

1.2 Plant–Microbe Interactions

The continuous, dynamic, and complex mechanism underneath existing since the colonization on earth is plant–microbe interactions (Dolatabadi 2021). This association has created a niche for the beneficial and damaging effects of host and nonhost, which is generally regarded as the battlefield. These interactions further signal the cascade of reactions to induce resistance against infection or biotic stress and to enhance the tolerance to the abiotic stress factors (Kumar and Verma 2018). The interactions are termed as plant–microbe interactions which are beneficial, symbiotic, benefiting both the host and the organism. Plants undergo different signal cascades to bring in the effect when there is an association with the microbes. These can be endophytes (microbes associated in the plant system), plant growth-promoting rhizobacteria (PGPR), or plant growth-promoting fungi (PGPF). These microbes play a vital role in dense, stress alleviation by producing various plant growth-promoting (PGP) metabolites (growth hormones, ammonia, siderophores, and hydrogen cyanide) and activities (phosphate solubilization, iron uptake, and biofilm formation). These bring in the importance of use of microbes for plant growth promotion under biotic or abiotic stress (Kumudini et al. 2017; Pal et al. 2021). Various studies have focused on the plant–microbe interactions and its impact; hence, this review pertains only on millets.

1.3 Role of Endophytes in Millets Health: The In-House Friends

The in-house microbes that colonize the plants, regarded as endosymbionts or endophytes, which result in the production of secondary metabolites possessing bioactive components known for anticancer and antimicrobial effects (Gouda et al.

2016). Bacterial endophytes are also known to alleviate the resistance against fungal pathogens in plants, by releasing pyrazines, chalconoids, and tryptophan derivatives that are attributed for this functioning (Garbeva and Weisskopf 2020). Interactions leading to the ROS signalling, PR protein enhancement, primary and secondary metabolite accumulation are the strategies developed by these endosymbionts for enhancing the plant health and growth (Morelli et al. 2020).

In this regard, Kumar et al. (2020) reported the impact of endophytic bacterial removal and induction in finger millet seeds. Isolated endophytes *Paenibacillus dendritiformis*, *Enterobacter hormaechei*, *Enterobacter cloacae*, *Bacillus safensis*, and *E. hormaechei* were positive for plant growth promoting parameters. These endophytes when inoculated for seedling protection assay showed significant protection against *Fusarium oxysporum* infection, suggesting the role of endophytic bacteria on seed and root colonization and their impact in plant protection. Similar results were observed when seed endophytes (bacteria) were inoculated onto pearl millet seeds by Kumar et al. (2021). Fluorescent microscopic results revealed the inter- and intracellular colonization of bacteria in root hair and parenchymal cells. The secondary metabolites like antifungals and lipopeptides showed leakage of protoplasmic substances of the invading fungi *Fusarium* due to the damage caused on the hyphae and fungal spores (Kumar et al. 2021).

Physiological and morphological changes are being rejuvenated for the enhancement of plant health. In this regard, plant root modifications, phytohormone levels, gene signals, and expressions will vary based on the stress prevailing, accordingly use of certain endophytes can alleviate this stress and modulate the manifestations. Studies by Manjunatha et al. (2022) revealed such a result when treated with the endophytes *Cronobacter dublinensis* strain of pearl millets thereby increasing the levels of abscisic acid, IAA (indole acetic acid) and antioxidants (proline) under field conditions, in turn alleviating the stress-responsive genes.

1.4 Role of PGPM in Millets Plant Health: The Neighbors

Genetic diversity analysis of fungal species adhering to the roots showed the role of different species, *Trichoderma asperellum* and *T. harzianum,* by RAPD (random amplified polymorphic DNA) and ISSR (inter simple sequence repeats) markers. *Trichoderma* spp. was observed to colonize the roots of pearl millet plants efficiently with varied levels of plant growth-promoting traits. Downy mildew pathogen suppression was observed by these species following the induction of systemic resistance, which can be the potential targets as biocontrol agents (Nandini et al., 2021).

Similarly, studies carried out by Mankar et al. (2022) by using *Burkholderia* sp. enhanced the biomass and yield of little millet under polyhouse conditions. This study was carried out to promote little millet production which required sustainable solutions. The study also used the nonnative *Azotobacter chroococcum* before its sowing as an inoculant, revealing the effectiveness of nonnative PGPM in reconditioning the soil. Also, this study validates the studies carried out previously using nonhost inoculants (Patil and Kumudini 2019; Mahadik and Kumudini 2020).

The isolates *Pseudomonas fluorescens, E. hormaechei*, and *P. migulae* stimulated seed germination and promoted growth of foxtail millets under severe drought stress conditions with increased PGP traits like increased ACC (1-aminocyclopropane-1-carboxylic acid) deaminase and exopolysaccharide production (Niu et al. 2018). *E. cloacae* strain showed increased PGP traits like production of ammonia, hydrogen cyanide, siderophore, ACC deaminase, IAA, and phosphate solubilization activity besides tolerance to heavy metals like aluminum, zinc, chromium, lead, and arsenic when treated on five millet cultivars. They showed increased seed germination, enhanced root and shoot elongation, when tested under pH 6.0–8.0 (Labhane 2020). A categorized list of the beneficial microbes in millet plant health enhancement has been enlisted in Table 1.1.

1.5 Microbes for Millet Health

Research on use and study of the mechanism of plant–microbe interactions is innumerable. However, the study on the role of these on millets plant improvement is short coming. Available bioformulations to millets are only a handful (Table 1.2). The novel approaches to ascertain the utilization of microbial formulations is the need of the hour to enhance millet health from the perspective of sustainable development.

From using omics technology, it is possible to tunnel the signalling mechanisms involved in millets under varying stress conditions, providing base for effective use of bio-inoculants to target the specified mechanism inducing plant resistance or tolerance. This will thereby enhance the millet plant health and growth. Also, appropriate guidelines on the use and manufacturing of the bioformulations must be congregated to minimize the repetitive data set accumulation, which can foster deeper systematic study in-depth on the efficacy of the same.

1.6 Conclusion and Future Prospects

Plant–microbe interactions, role of rhizosphere niche for plant growth and health, have been well established since decades with respect to different host systems. This suggests the behavioral changes pertaining to that host system with different manifestations (biotic or abiotic stress). In the wake of climate change, food security, it is now important to investigate the microbial interactions with millet crops. This can be well aided with usage of omics approaches—genomics, transcriptomics, proteomics, and metabolomics. A new approach will be to equip artificial intelligence in understanding the belowground interactions which suggests the new approaches to enhance millet plant growth and health as a holistic approach. This chapter and next chapters have highlighted the importance of using rhizospheric microorganism for the increased production on millets. These chapters will help the researchers to work to achieve nutrient security for global population through millets.

Table 1.1 Perspectives on millet plant health management by PGPM

Strain	Host	Effect on plant health	Reference
Acinetobacter calcoaceticus, *Penicillium* sp.	Foxtail millet	Enhanced accumulation of glycine betaine, proline, sugars, and decrease in lipid peroxidation by P-solubilizing microbes against drought stress	Kour et al. (2020)
Curtobacterium sp., *Microbacterium* sp., *Methylobacterium* sp., *Bacillus amyloliquefaciens*	Browntop millet	Protection against *Fusarium* infection was elucidated by antifungal lipopeptide genes for surfactin and iturin	Verma and White (2017)
Variovorax sp., *Achromobacter* sp., *Pseudomonas* spp., *Ochrobactrum* sp.	Finger millet	The treatments elevated the levels of antioxidants which scavenged the ROS under water stress and irrigated conditions	Chandra et al. (2020)
Fluorescent pseudomonad strains	Finger millet	Microbes showed accumulation of PR proteins in the primed plants which acted as antifungal agents against blast fungi	Patil et al. (2016)
Pseudomonas aeruginosa	Finger millet	Seed priming induced phenylpropanoid pathway signalling for host dense response against *Magnaporthe grisea*	Patil and Kumudini (2019)
		Primed plants increased levels of salicylic acid and other primary metabolites during induction of disease resistance	Patil et al. (2020)
Pseudomonas aeruginosa, Pseudomonas resinovorans	Finger millet	Treated plants showed enhanced growth promotion and plant health under high saline conditions	Mahadik and Kumudini (2020)
Pseudomonas spp.	Finger millet	ACC deaminase producing bacteria isolated from finger millet rhizosphere was able to improve the growth and nutritional parameter under drought stress under greenhouse conditions	Chandra et al. (2018)
Streptomyces griseus, Streptosporangium roseum	Pearl millet	Root colonization increased under greenhouse conditions along with disease-resistance capacity of the isolates against downy mildew	Jogaiah et al. (2016)
Pseudomonas extremorientalis, Bacillus subtilis, Bacillus amyloliquefaciens	Pearl millet	Single and in consortium these microbes enhanced plant growth by increased root and shoot length, chlorophyll, carotenoids, total soluble sugar content, phenolics, and flavonoids	Kaur et al. (2022)
Bacillus pumilus	Pearl millet	Associated with induction of disease resistance by hypersensitive reaction, accumulation proteins, antioxidants also demonstrated by histochemical studies on downy mildew pathogen	Raj et al. (2012)

(continued)

Table 1.1 (continued)

Strain	Host	Effect on plant health	Reference
Pseudomonas fluorescens, Trichoderma virens	Pearl millet	Defense-related enzymes accumulation was high in treated plants against *Magnaporthe grisea* which enhanced the growth	Basavaraj et al. (2019)
Bacillus amyloliquefaciens, Bacillus subtilis, Stenotrophomonas maltophilia	Pearl millet	ACC deaminase producing microbes when primed with pearl millet showed enhanced expression levels of antioxidant genes which thereby defend plants against drought stress	Murali et al. (2021)
Penicillium oxalicum	Pearl millet	Downy mildew disease resistance was achieved by treating the plants with fungi which showed increased resistance attributed to enhanced chitinase activity	Murali and Amruthesh (2015)
Dyadobacter sp.	Millets	Isolate showed enhanced plant growth with nitrogen fixation activity owing for their psychrotolerance isolated from Western Himalayas	Kumar et al. (2018)

Table 1.2 Prospective use of formulations for millets plant health

Strain	Host	Effect on plant health	Reference
Enterobacter cloacae	Millet cultivars	Abiotic stress	Labhane (2020)
Pseudomonas sp.	Finger millet	Blast disease reduction with liquid formulation	Sekar et al. (2018)
Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens	Finger millet	Liquid, alginate-based, fluid-bed dryer-based, lignite formulations when treated showed increased plant growth parameters; however, better results were observed on treatment liquid formulation	Gangaraddi et al. (2020)
Streptomyces nanhaiensis	Pearl millet	Liquid formulation of the strain enhanced plant growth with increased leaf biomass and pigment production. This significantly mediates mineralization and accumulation of minerals in rhizospheric region of the millet crop under pot trials	Patel and Thakker (2020)
Bacillus subtilis spp.	Finger millet	Talc-based formulation of the strain was used for seed treatment, seedling dip, and foliar spray. It increased defense enzymes (phenylalanine ammonia lyase, chitinase, and superoxide dismutase) in resistant plants. This isolate showed promising results against *Magnaporthe grisea* infection	Gnanasing and Ahila (2017)

Acknowledgments Authors acknowledge JAIN (Deemed-to-be University), Bengaluru for the financial support.

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Chapter 2
Diversity and Function of Microbes Associated with the Rhizosphere of Millets

Richa Agnihotri and Natarajan Mathimaran

Abstract Millets, also known as the “future food”, provide a viable option for combating malnutrition and mitigating climate change. The rhizosphere of millets accommodates numerous microbial communities that improve plant nutrient acquisition, growth, and productivity and also protect from abiotic and biotic stresses. Exploiting the millet rhizomicrobiome will improve soil health and plant nutrition, reduce the need for environmentally toxic synthetic fertilizers, and offer a more affordable alternative. The knowledge about the dominant microbial groups will also help in devising strategies for developing inoculum well-adapted to millets. The key microbial groups in the millet rhizosphere and their effects on plant growth, stress tolerance, and soil health have been described in this chapter. In addition, we have provided an overview of the methods used to examine rhizosphere microbial communities.

Keywords Millets · Rhizosphere · Rhizomicrobiome · Soil health · Biofortification · Agricultural management · Environmental stress

2.1 Introduction

An agroecosystem is a dynamic self-organizing arrangement comprising numerous levels, chiefly soils, microbes, and plants networking with each other while remaining highly responsive to agricultural management practice (Ichihashi et al. 2020). Therefore, exploiting plant growth-promoting microorganisms (PGPMs) is a feasible strategy for augmenting crop production (Agnihotri et al. 2017). The recruitment of advantageous microbial communities in the rhizosphere of millets contributes to the development of high-yielding crops capable of growing in barren

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_2

soil and resisting climate change (Debenport et al. 2015; Xu et al. 2018). The rhizosphere harbors diverse microbes consisting of rhizobacteria, nitrogen-fixing, phosphate-solubilizing bacteria, arbuscular mycorrhizal fungi (AMF), and many others. Around the world, about 40% of the crop production area is extremely degraded and around 24% is still being subjected to constant degradation (Bai et al. 2008; Zhang et al. 2017). By regulating microbial communities and diversity, agricultural management practices such as reduced tillage, organic farming, crop rotations, and intercropping can improve soil health (Sharma et al. 2010). Hence, to sustain and boost the millet agroecosystem's stability and profitability, understanding microbial diversity associated with different millets is fundamental. This chapter aims to highlight the diverse millet rhizomicrobiome, its response to the various stresses and soil management practices, the use of organic and synthetic fertilizers, and its association with activities of enzyme and nutrient release. This will improve our concepts about the standing of microbial communities in influencing millet productivity, soil health, and fertility, and also help in selecting appropriate management practices for sustainable development of the agroecosystem under a climate-smarter agricultural approach.

2.2 Rhizosphere

Rhizosphere—the soil volume near the roots characterized by root exudation, strong microbial activity, and diversity—is a dynamic environment for plant–microbe communication (Kuzyakov and Razavi 2019; Liu et al. 2022; Maheshwari et al. 2020) (Table 2.1; Fig. 2.1). A vast exchange of signals taking place at the biochemical, and molecular stages, triggers a sequence of events that occur synchronously in the rhizosphere (Maheshwari et al. 2020). The chemical signalling in the rhizosphere alters the plant growth environment and soil characteristics and governs the success of plant survival, development, growth, and productivity (Hu et al. 2018a, b). The rhizosphere is influenced by bidirectional biotic and abiotic processes (Kuzyakov and Razavi 2019). Also, the quantity, composition, and quality of root exudates and residues from crops influence the rhizosphere microbiome by selecting specific microbes and generating plant-associated microbial community shifts (Hu et al. 2018a, b). Shortly after sowing, the microbes gather and microbial population keeps altering through plant growth stages depending on processes which could be deterministic (biological and abiotic factors-mediated selection) or stochastic (drift and diffusion related) (Ichihashi et al. 2020; Xiong et al. 2021). The plant growth-promoting microbes boost plant development and nutrient availability by altering the root system, producing siderophore, ligand-mediated chelation, acidification, or exuding phytohormones (Sirohi et al. 2015). The factors influencing microbial activities include (1) resident microflora, (2) host plant rhizo-exudations, and (3) soil physical and biochemical profile (Maheshwari et al. 2020; Raghavendra et al. 2020).

Table 2.1 Frequently used terms in rhizospheric studies

Term	Description	References
Rhizosphere	Nutrient-rich soil volume in the proximity of plants roots having intense microbial activity affected by root functioning. The area of root physical and biochemical influence created by root growth and exudation and the site for interaction between microbes and plants	Hakim et al. (2021); McNear (2013)
Endorhizosphere	The zone of tissue in roots comprising a part of endodermis, cortical cells, and also the apoplastic compartments present among the cells which might get colonized by root-specific microorganisms feeding upon root exudates	Kloepper et al. (1992b); McNear (2013)
Ectorhizosphere	The outer zone outspreading from the rhizoplane into the loose soil volume	McNear (2013)
Rhizomicrobiome/rhizobiome	Microbial communities inhabiting the rhizosphere	Maheshwari et al. (2020)
Rhizodeposits	Carbon (C)-sources derived from the roots of growing plants and released into the adjacent soil	Pinton et al. (2007)
Root exudates	Root exudates consist of actively released root secretions (as well as mucilage) and passively released diffusates (because of differences in osmotic pressure between soil solution and the cell), or lysates resulting from the self-digestion of root cells (epidermis and root cortex)	McNear (2013); Pinton et al. (2007)
Rhizozymes	Root-associated enzymes critical in the decomposition, nutrient cycling, and mineralization of soil organic matter (SOM)	Inamdar et al. (2022)
Quorum sensing	Complex bacterial density-dependent intracellular communication system operational in the rhizosphere between cells upon acquiring a certain density and controlled by chemical signals causing alteration in metabolic activity and gene expression	Everett and Rumbaugh (2014); Ghosh and Mandal (2022)
Chemoattractant	Compounds secreted by microorganisms or plants for attracting one another or conduct their movement frequently toward each other	Park et al. (2003)

(continued)

Table 2.1 (continued)

Term	Description	References
The chemotaxis index	The ratio of bacterial cell density gathered with the root exudates contained in the test microcapillaries to that of buffer control	Dheeman et al. (2020); Lopez-de-Victoria and Lovell (1993)
The relative chemotaxis index (RCI)	The chemotaxis index (treatment)/chemotaxis (positive control) × 10	Dheeman et al. (2020)
Microbe-associated molecular pattern (MAMP)/pathogen-associated molecular pattern (PAMP)	Microbial signature molecules activate the plant immune system upon interaction pattern recognition receptors (PPRs)	Maheshwari et al. (2020); Mhlongo et al. (2018)
Damage-associated molecular patterns (DAMPs)	Molecules, namely, small peptides, fragments of cell wall ascending from damaged or stressed cells	
Induced systemic resistance (ISR)	The defense response of the plant persuaded by an outside agent such as plant growth-promoting rhizobacteria (PGPR) acting against a pathogen applied to a localized area	Kloepper et al. (1992a); Maheshwari et al. (2020)
Common symbiotic pathway (CSP)	A pathway involving the signalling between AMF and rhizobia succeeded by a cascade of suitable responses toward either symbiotic partner	Genre and Russo (2016); Kafle et al. (2019)
Common symbiotic signalling pathway (CSSP)	The pathway critical in the establishment of the symbiotic association between AMF and plant which is initiated when AMF release signalling molecules (Myc factors) which are received by plant root receptors generating a series of molecular reactions	Kafle et al. (2019); Maclean et al. (2017)
Common mycelial networks/common mycorrhizal networks (CMNs)	AMF extraradical hyphal network extending beyond plant roots connecting adjacent plants, helping nutrient and water acquisition	Mathimaran et al. (2021); Smith and Read (2008); Walder et al. (2012)
Root colonization	A microorganism's ability to develop habitation and show rhizocompetitiveness	Dheeman et al. (2020); Ladygina and Hedlund (2010)
Allochthonous microbial communities	Non-indigenous microbial species	Dheeman et al. (2020)
Autochthonous microbial communities	Indigenous/native microbial species	

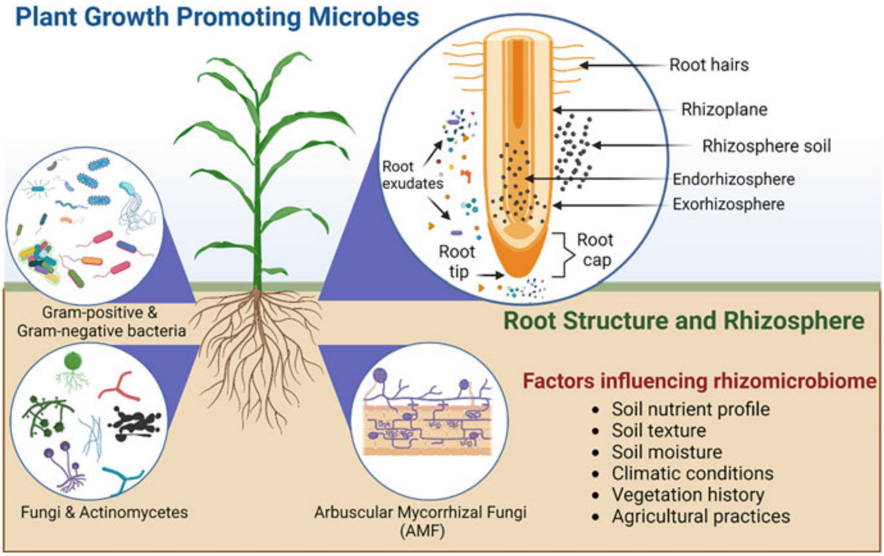

Fig. 2.1 An overview of the millet rhizosphere and associated microbial communities (Created with BioRender.com)

2.3 About Millets

Millets are "small-seeded grasses," and the principal categories classes of millets include major and minor millets. Pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) fall under major millets. "Small millets" or "minor millets" cover finger millet (*Eleusine coracana*), barnyard millet (*Echinochloa* spp.), foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), proso millet (*Panicum miliaceum* L.), teff (*Eragrostis tef*), fonio (*Digitaria exilis*), guinea millet (*Brachiaria deflexa*), job's tears (*Coix lacryma-jobi*), browntop millet (*Urochloa ramosa*) and guinea millet (*Brachiaria deflexa*). Representing nearly 80% of the Asia production of millets, India has emerged as the leading producer of millets (Bhagavatula et al. 2013). Only 2% of the cereal production across the globe is represented by millets, and Asia and Africa alone are responsible for 95% of the world's millet production (Bhagavatula et al. 2013). Owing to their nutritional content and health improvement traits, short maturity period, lesser water requirement, ability to grow in low-fertility soils, and climate resilience, millets have received vast recognition across the globe (Anbukkani et al. 2017; Gupta et al. 2017; Li and Siddique 2018; Muthamilarasan and Prasad 2021). Foxtail millet (*S. italica*) is a popular summer crop widely grown in the arid and semiarid regions of Asia and Europe. The soils of these regions are nutrient deficient, a trait that influences the production of millets (Xu et al. 2018). Finger millet (*E. coracona*) also known as "Ragi" is a popular minor millet cereal in the Asian and African semi-arid tropics (Gupta et al. 2017). Pearl millet is considered the most drought and high-temperature resistant of all millets and is ideal for cultivation in sandy loams or drained sands (Saleem et al. 2021).

Nearly half of the global population, particularly Asians and Africans have nutrition deficiency as they prefer cereal crops as their chief food. The limited production of millets is often credited to the preferred cultivation of economically valuable cash crops (Muthamilarasan and Prasad 2021; Vetriventhan et al. 2020). The dependence on cereals, more frequently known as "food monotony" is the main ground for micronutrient deficiency (Li and Siddique 2018). Micronutrients are a crucial constituent of biological systems, but are still lacking in soils. The formation of insoluble complexes, and chelation, makes micronutrients unavailable to plants in sufficient quantities (Noulas et al. 2018). The application of nutrient-based synthetic fertilizer is a viable strategy for micronutrient uptake by plants; however, only around 20–30% of the applied amount gets to the plant, and the rest is lost to the environment (Fageria 2014; Hakim et al. 2021). In this context, the soil microbe-mediated nutrient acquisition seems to suitably fulfil the nutrient requirement (Choudhary et al. 2020). Compared to bulk soil, the rhizosphere contains more available nutrients and rhizosphere microbes stimulate plant growth by improving the availability of nutrients such as N, P, and K (Liu et al. 2022; Maheshwari et al. 2020). Utilizing plant growth-promoting microbes isolated from millet rhizosphere as bio-inoculants is a viable method to improve micronutrient bioavailability for crop assimilation contributing to low-input agriculture (Kumar et al. 2012; Singh and Prasanna 2020; Smith and Read 2008). Based on their ability to colonize roots, produce biofilms, and rhizocompetitiveness, PGPR are chosen for inoculating millets (Dheeman et al. 2020). The chemotactic behavior and aggressive colonizing ability govern the rhizocompetitiveness (Chauhan et al. 2017). Another sustainable agricultural strategy to combat the phytochemical, micronutrient, and protein deficits in crops improving the grain's nutritional quality is biofortification (FAO, HarvestPlus 2019). Minor millets offer great potential for biofortification to upsurge the nutrient accumulation in grains (Vinoth and Ravindhran 2017). Besides, particularly in nutrient-deficient soils, the utilization of beneficial rhizosphere microbes would lessen the requirement for mineral fertilizer and chemical fungicides (Choudhary et al. 2020; Sekar et al. 2018). Therefore, identifying the core microbiome of the millet rhizosphere is a sustainable way to match the yield potential for cereal and millet crops, thereby assisting farmers in the production of higher-yielding and climate-resilient crops.

2.4 Distinct Bacterial Community Composition in Different Millets

The diversity and distribution of microbial communities in agroecosystems are ruled principally by the physicochemical characteristics of soil such as texture, N, P, organic C, pH, and agricultural practices (Sharma et al. 2010). The agronomic parameters, such as benefit–cost (BC) ratio, land–equivalent ratio, physiological features (C3 or C4), and nutrient requirements, and social aspects, such as labor availability, all play a role in determining the crop to be cultivated with millets (Agnihotri et al. 2017; Mathimaran et al. 2021). Utilizing mineral fertilizers (N, P, and K) and manure together increases soil microbial diversity, soil health, and crop yield (Xu et al. 2018). Intercropping can increase the yield, by assembling advantageous microorganisms in the pearl millet (*P. glaucum*) root zone (Debenport et al. 2015). Intercropping woody shrubs with pearl millet increased the number of *Chitinophaga* in the millet rhizosphere (Debenport et al. 2015). Under the combined application of mineral fertilizers (N, P, and K) and manure, the phyla *Actinobacteria* and *Bacteroidetes* flourished with the dominance of genera *Opitutus*, *Mycobacterium*, *Chitinophaga*, and *Devosia* in comparison to sole application or other combinations above fertilizers (Xu et al. 2018). A robust correlation observed between SOM and *Opitutus* and *Chitinophaga* revealed their possible role in improving soil health (Xu et al. 2018). Besides, soil enzyme activity strongly correlated with SOM and available P in the rhizosphere of foxtail millet (Xu et al. 2018). A significant correlation of bacterial communities with SOM and urease activity and available phosphorus in the foxtail millet rhizosphere was reported by Xu et al. (2018). Specifically, as revealed through Pearson's correlation, significant correlations observed for available N with *Devosia* and *Mycobacterium*, urease with *Devosia*, phosphatase with *Chitinophaga,* and *Luteimonas* revealed the role of these microbes in cycling and solubilization of the respective nutrients (Xu et al. 2018).

Rhizospheric microflora, that is, PGPR mediate millet growth and also help plants in coping with biotic and abiotic stressors by (1) raising the antioxidant potential by increasing the activities of the enzymes that scavenge reactive oxygen species, (2) cellular osmotic regulation through an increase in proline content, (3) micro- and macronutrient solubilization and acquisition, (4) siderophore production, (5) formation of endospores, (6) production of extracellular and hydrolytic enzymes, (7) production of peptide signal molecules and antifungal metabolites, and (8) accumulation of lignin and oxidative phenols (Chandra et al. 2020; Dheeman et al. 2017; Niranjan Raj et al. 2012; Sekar et al. 2018). Under drought stress, the alteration of root-associated characteristics namely root morphology (branching of lateral roots and growth of root hair) and functioning is an adaptive mechanism in plants. PGPR produce phytohormones by changing the root morphology and helping plants acquire more nutrients and water and influencing plant physiology (Vacheron et al. 2013). The rhizobacterial isolates conferring growth-stimulating and disease-suppressive effects on finger millets were characterized by their P-solubilizing ability, production of antifungal compounds, siderophore, indole acetic acid (IAA), hydrogen cyanide (HCN), ammonia β, 1–4 glucanase, and amylase (Kumari 2019). The ability of the *Pseudomonas* strain to protect finger millet from drought-induced oxidative damage and improve plant growth was attributed to increased antioxidant activity and aminocyclopropane-1-carboxylic acid (ACC) deaminase production (Chandra et al. 2018). By hydrolyzing the ACC (ethylene precursor) into -ketobutyrate and ammonia, the ACC deaminase decreases the ethylene concentration in plants and increases plant growth (Chandra et al. 2018). The inoculation of finger millet with a drought-resistant ACC deaminase-producing bacterial strain isolated from agricultural soil of the Himalayan region boosted antioxidant activity, root and shoot length, and biomass and leaf nutrient content under stressed and unstressed conditions compared to the control counterpart (Chandra et al. 2018). The strain later identified as *Pseudomonas* displayed the presence of the *acdS* gene (ACC deaminase structural gene) (Chandra et al. 2018). In one study, under water-stressed conditions, the sole inoculation of *Variovorax paradoxus* and a combined inoculation of *Ochrobactrum anthropi* + *Pseudomonas palleroniana* + *P. fluorescens* + *P. palleroniana* enhanced plant growth and proline, phenol, chlorophyll, and nutrient content in leaves and also reduced lipid peroxidation than control plants (Chandra et al. 2020). In addition to these, a higher activity of antioxidant enzymes, namely, ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and guaiacol peroxidase (GPX) were also observed in the aforementioned treatments than in their uninoculated counterparts (Chandra et al. 2020). In salt stress-sensitive finger millet, florescent *Pseudomonas* isolates SPF-33 and SPF-37 were effective in decreasing lipid peroxidation and H_2O_2 and improving physiological parameters such as height, germination, number of spikelet, total chlorophyll, proteins, phenolics, flavonoids, proline, and antioxidant activity, thus improving plant growth and alleviating salt stress (Mahadik and Kumudini 2020). *Enterobacter* sp. PR14, which is a halophilic rhizobacterium-conferred finger millet and rice with salinity stress tolerance by producing ACC deaminase-triggered growth promotion in terms of seed germination, plant biomass, and length (Sagar et al. 2020). *Sphingomonas faeni* expressing ACC deaminase genes when inoculated into finger and foxtail millet seeds, decreased proline content and increased activity of enzyme superoxide dismutase, catalase, peroxidase (POD), glutathione peroxidase, ascorbate peroxidase, and glutathione reductase (GR) involved in antioxidant defense and also enhanced plant biomass and root and shoot length, and therefore mitigated cold stress and augmented plant growth (Srinivasan et al. 2017).

PGPR persuade disease resistance against many plant pathogens in millets. In response to pathogen invasion, PGPR trigger defense mechanisms at the histochemical and biochemical levels (Niranjan Raj et al. 2012). In the smut-resistant foxtail millet cultivars rhizosphere, *Bradyrhizobium* and *Streptomyces* were found to be negatively related to the prevalence of smut disease (Han et al. 2017). The rhizosphere of various smut-resistant foxtail millet cultivars harbored distinct bacterial communities, and a higher bacterial diversity could have contributed to the suppression of smut disease (Han et al. 2017). The sole and combined application of rhizospheric P-solubilizing bacterial strain and diazotrophic *Azospirillum lipoferum* of foxtail millet improved plant height and biomass in foxtail millet over control (Rafi et al. 2012).

Bacillus species produced secondary metabolites and had plant growth-promoting attributes and which protected finger millet plants and also enhanced yields (Dheeman et al. 2020). Rhizospheric bacillus isolates of finger millet have shown antagonistic activity toward foot rot disease and growth promotion traits (Dheeman et al. 2020). *Bacillus pumilus* (INR-7) caused callose apposition and acted as a biocontrol agent in pearl millet protecting against *Sclerospora graminicola* caused downy mildew by boosting the deposition of lignin in the epidermal tissues, and their possible role in suppression of disease in pathogen inhibition was predicted (Niranjan Raj et al. 2012). It was also noted that the vascular bundles were the location of the defense enzymes. Similar to this, the extracellular enzymes −1, 4-glucanase, and chitinase that broke down the fungal cell wall were considered to be responsible for the biocontrol mechanisms against *Sclerotium rolfsii* (Dheeman et al. 2020). It was observed that the native rhizospheric *Pseudomonas* strain MSSRFD41 improved plant growth metrics, germination, and the vigor index of finger millet seedlings and was effective against *Pyricularia grisea* caused blast disease (Sekar et al. 2018). Out of the 111 finger millet rhizobacterial isolates assessed by Kumari (2019), many showed antagonistic activity *P. grisea,* 38 were effective against *Rhizoctonia solani* causing the banded blight disease and 30 showed antagonistic activity against both. Each of these strains improved the nutrient uptake and growth of finger millet. Under sterilized and unsterile conditions, finger millet inoculated with *Chryseobacterium* sp. PSR 10 from the soybean rhizosphere improved plant chlorophyll content, nitrate reductase activity, plant biomass, and length of root and shoot (Veer and Goel 2015). The plant growth promotion by *Chryseobacterium* sp. PSR 10 was ascribed to its ability to solubilize P (Veer and Goel 2015).

2.5 Distinct AMF Community Composition in Different Millets

Arbuscular mycorrhizal fungi, the obligate symbionts associate well with millets and have gathered attention for the benefits of improving plant stress tolerance and nutrient status. The AMF glycoprotein glomalin or glomalin-related soil proteins (GRSP) have emerged as a robust indicator of soil carbon (C) sequestration and health (Agnihotri et al. 2022a, b). The survival of AMF in the soil is mostly reliant on the spread of a stable hyphal network and since GRSP are primarily produced on AMF mycelium, their production also depends on agricultural management (Agnihotri et al. 2022b; Driver et al. 2005). The factors regulating the maintenance of a stable hyphal network in soil include (1) undisturbed soil, (2) continuous presence of live roots of AMF supportive plants, (3) organic manure, and (4) presence of diverse plant species as crop rotation or intercropping (Agnihotri et al. 2017, 2021a). When soil remains undisturbed for prolonged periods the hyphal growth is continuous and the buildup of a stable hyphal network assures improved plant nutrient acquisition and boosts soil C-sequestration and health (Agnihotri et al. 2021a). Soil edaphic factors are also important determinants of AMF perpetuation. AMF spore numbers, glomalin content, and diversity indices were positively influenced by soil macronutrients N, P, and K in five minor millets grown in different soil types (Mythili and Ramalakshmi 2022). The community composition of AMF is influenced by soil pH, type, texture, C, N, as well as land management (Oehl et al.

2010; Xiang et al. 2014). Soil type and nutrient status can affect the performance of AMF (Kandhasamy et al. 2020). When plants are P-deficient, as a strategy to improve P and N uptake, the allocation of biomass into roots is higher than in shoots which are observed as the higher dry weight of shoots than roots (Marschner et al. 1996; Srivastava et al. 2014).

The rhizosphere of millets can harbor diverse AMF species. In the experiments of Mythili and Ramalakshmi (2022), a total of 24 AMF species belonging to 11 genera were recovered from the rhizosphere of five minor millets grown in three different soil types including sandy loam, clay loam, and sandy clay loam. The dominant genera isolated from the millet rhizosphere included *Acaulospora*, *Ambispora*, *Claroideoglomus*, *Funneliformis*, *Entrophosphora*, *Glomus*, *Gigaspora*, *Paraglomus*, *Rhizophagus*, *Scutellospora,* and *Septoglomus*. Among the five minor millets examined, little millet dominated in terms of mycorrhizal percentage. The millets could be ranked as follows in the order of highest to lowest mycorrhizal colonization: little millet > finger millet > foxtail millet > kodo millet > barnyard millet. Among the soil types, *F. mosseae*, *G. fascicultum*, and *R. intraradices* dominated with 83% isolation frequency, and *A. lepoticha* and *C. etunicatum* followed with 67% isolation frequency. Among crop types, the top three AMF genera found in soil types differed and *R. intraradices* (60%) dominated the other two, that is, *F. mosseae* (50%) and *G. fascicultum* (40%) in terms of isolation frequency. All 24 AMF species were detected in crop types albeit with a lower isolation frequency than soil types (Table 2.2).

In general, AMF interfere with plant physiological and cellular processes, and the effects are observed in the form of enhanced osmoregulation, a robust root system, superior photosynthetic efficiency, and an efficacious antioxidant system (Tyagi et al. 2018, 2021). The collective effect of processes taking place at the physiological, nutritional, and cellular levels during the plant-AMF symbiosis results in drought tolerance (Porcel et al. 2003). In response to AMF inoculation, plant tissues exhibit enhanced growth and biomass, P-solubilization and uptake, water and nutrient acquisition, the content of proline, soluble sugar, chlorophyll, flavonoid, and phenol, ascorbate levels, reduced lipid peroxidation, and glutathione levels (GSH) (Ramakrishnan and Bhuvaneswari 2014; Tyagi et al. 2017, 2018, 2021). The advancement of the millet genotype with higher AMF dependency could further facilitate the mycorrhiza-induced benefits to the plant (Srivastava et al. 2014). The ability to host AMF and mycorrhizal dependency could vary among host plant species and could also be influenced by genetic structure (Agnihotri et al. 2021a, 2022a, b, c; Kandhasamy et al. 2020). Accordingly, AMF species could also be put into high- and low-benefit categories based on their plant growth responses (Cope et al. 2022). Tewari and Tandon (1993) noted that although inoculation by six different AMF species stimulated growth and development in five different genotypes of finger millet, the efficacy of AMF species was contrasting. The best combination of AMF and finger millet genotype was *Glomus caledonicum* with HR-374 yielding a higher root, shoot, and whole plant biomass, substantially developed infectious propagules (arbuscules, vesicles, and hyphae) inside the root cortex and extraradical hyphal connected with colonized roots (Tewari and Tandon

Table 2.2 AMF species recovered from different soil and crop types (Mythili and Ramalakshmi 2022)

Soil type[a]	Crop type[b]	References
Glomus species (*G. fasciculatum*, *G. macrocarpum*, *G. aggregatum*, *G. clarum, G. microcarpum*, *G. albidum*); *Gigaspora* species (*G. margarita*, *G. gigantea); Claroideoglomus* species (*C. etunicatum*, *C. claroideum); Rhizophagus intraradices; Funneliformiss* species (*F. mosseae*, *F. geosporum*); *Paraglomus brasilianum; Entrophosphora colombiana; Acaulospora* species (*A. denticulata*, *A. delicate); Ambispora lepoticha*: and *Septoglomus constrictum*	*Glomus* species (*G. fasciculatum*, *G. macrocarpum*, *G. aggregatum*, *G. clarum, G. microcarpum*, *G. hoi*, *G. albidum*); *Gigaspora* species (*G. margarita*, *G. gigantea*); *Claroideoglomus* species (*C. etunicatum*, *C. claroideum); Rhizophagus intraradices*; *Funneliformiss* species (*F. mosseae*, *F. geosporum*); *Paraglomus brasilianum; Entrophosphora* species (*E. infrequens*, *E. colombiana*); *Acaulospora* species (*A. scrobiculata*, *A. denticulata*, *A. delicate*); *Ambispora lepoticha*: *Scutellospora erythropha:* and *Septoglomus constrictum*	Mythili and Ramalakshmi (2022)

[a] Sandy loam, sandy clay loam, and clay loam

[b] Barnyard millet, finger millet, foxtail millet, little millet, and kodo millet

1993). The uptake of Zn^{2+} increased by 2.55 and P by 2.20 times in plants inoculated with *G. caledonicum* than in control (Tewari and Tandon 1993). AMF may also show ecological specificity and there can be preferential association and plants may select particular AMF species for colonization (Gollotte et al. 2004; McGonigle and Fitter 1990).

Plants can also respond differently to mixed or single species inoculum (Agnihotri et al. 2022a, b, c). The differential ability of AMF species to support growth and nutrient uptake could be associated with the increased expression of genes associated with photosynthesis, strigolactone, lipid biosynthesis, sugar and nutrient transporters, and environmental stress responses (Cope et al. 2022). On the contrary, in some cases, the colonization by AMF such as *G. aggregatum* could elicit plant defense and also be perceived as pathogen invasion (Cope et al. 2022). Despite delivering similar growth benefits, AMF species could differ in terms of colonization and it has been noted that the more aggressive colonizer, that is, *G. intraradices* possessed higher mycorrhizal responsiveness and could improve finger millet growth more than *G. etunicatum* (Srivastava et al. 2014). In general, the finger millet rhizospheric isolates of *Glomus* species, that is, *G. intraradices* and *G. etunicatum* improved P uptake in most of the finger millet genotypes examined (Srivastava et al. 2014). Even though *G. intraradices* and *G. etunicatum* boosted the P-status of the plant, the inoculation by the former improved shoot biomass and P status in all the examined finger millet genotypes to a slightly higher extent than the latter (Srivastava et al. 2014). However, AMF-induced plant growth in any situation may not necessarily stimulate root growth. In the experiments of Srivastava et al. (2014), the control plants registered higher root biomass and root/shoot ratio. Since the AMF hyphae extend the root zone to acquire nutrients for the plants, roots may not develop after attaining a certain level of growth (Srivastava et al. 2014) (Table 2.3).

To improve plant survival, C-allocation to AMF tends to increase under stressful situations (Treseder and Turner 2007). Upon closer investigation using compartmented microcosm, it was observed that the plant that invests a little C into the common mycorrhizal network (CMNs) connecting the two plants gets more benefits than the plant that makes a huge C-investment into the same (Walder et al. 2012). In the case of CMN-facilitated bioirrigation, though shallow-rooted finger millet benefited from CMN-promoted bioirrigation by the deep-rooted pigeon pea, the extent of the spatial connection between roots played a critical role in the course (Singh et al. 2019). Another study confirmed that under field conditions, the selection of AMF species and crop spacing might affect AMF functioning (Schütz et al. 2022).

Mathimaran et al. (2020), while working on AMF + PGPR consortia found out co-inoculation was way better than sole inoculation for boosting crop productivity and the effectiveness of biofertilizers was inversely proportional to soil fertility. In one study, the inoculation of *R. intraradices* and endophyte *Piriformospora indica* in finger millet boosted drought stress tolerance (Tyagi et al. 2017). Though distinct genetic and biochemical processes could be involved in their functioning, the inoculation improved chlorophyll content, the antioxidant defense, and osmoregulatory mechanism and reduced lipid peroxidation, electrolyte leakage, and also the levels of malondialdehyde and hydrogen peroxide (Tyagi et al. 2017). The sole inoculation of AMF in finger millet improved P uptake, whereas the co-inoculation of AMF with *Azospirillium brasilense* and *Bacillus polymyxa* improved growth parameters, N and P uptake, and AMF biomass in terms of root infection, and spores (Ramakrishnan and Bhuvaneswari 2014). The co-inoculation of AMF and *Azospirillum* promoted plant growth and increased the finger millet yield by 36.8% over control (Bama and Ramakrishnan 2010). Precisely, AMF helped in P mobilization, whereas *Azospirillium* contributed to N uptake and improved the straw biomass and grain yield (Bama and Ramakrishnan 2010). Similarly, the co-inoculation of P-solubilizing bacteria *Azotobacter chroococcum* and AMF species *Glomus mosseae* improved finger millet productivity and reduced the fertilizer cost by 25–50% without compromising productivity. In this case, the individual effects of *A. chroococcum* were observed in the form of hormone production and N-fixation and *G. mosseae* in the form of P-acquisition (Chandana and Venkataramana 2018). *Glomus intraradices* performs well with *Pseudomonas* and *Streptomyces* strains in terms of plant growth, and biomass in finger millet under irrigated and moisture deficit conditions (Kamal et al. 2015). The combination of *Pseudomonas poae* and *G. intraradices* accumulated the proline substantially and also recorded a superior activity of SOD in leaves under the conditions of water stress (Kamal et al. 2015).

Table 2.3 The effect of various PGPMs and their consortia on millets

Microbial species	Millet	Benefits to the plant	References
Piriformospora indica	Proso millet	Drought stress tolerance and plant growth	Ahmadvand and Hajinia (2018)
Acinetobacter calcoaceticus (EU-LRNA-72) and *Penicillium sp.* (EU-FTF-6 e)	Foxtail millet		Kour et al. (2020)
Pseudomonas sp. with ACC deaminase-producing capacity	Finger millet (variety: VL-149)		Chandra et al. (2018)
Pseudomonas sp. (AKM-P6)	Finger millet		Ali et al. (2009)
V. paradoxus (RAA3)	Finger millet		Chandra et al. (2020)
O. anthropi + *P. palleroniana* + *P. fluorescens* + *P. palleroniana*	Finger millet		
Psychrotolerant *S. faeni* with ACC deaminase-expressing plasmid	Finger millet and foxtail millet	Cold stress tolerance and plant growth	Srinivasan et al. (2017)
Aspergillus terreus	Pearl millet	Salinity stress tolerance and plant growth	Khushdil et al. (2019)
Fluorescent *Pseudomonads* (strains: SPF-33 and SPF-37)	Finger millet (variety: Indaf-9, salinity-sensitive)		Mahadik and Kumudini (2020)
Enterobacter sp. PR14 with halophilic ACC deaminase-producing capacity	Finger millet (variety: CO 14) and sorghum (variety: SCV 20)		Sagar et al. (2020)
B. pumilus (INR-7)	Pearl millet	Resistance against downy mildew	Niranjan Raj et al. (2012)
Pseudomonas sp. (MSSRFD41)	Finger millet	Plant growth promotion and management of Blast disease caused by *Pyricularia grisea*	Sekar et al. (2018)
B. pumilus (MSTA8) and *Bacillus amyloliquefaciens* (MSTD26)	Finger millet	Management of foot rot disease caused by *Sclerotium rolfsii*	Dheeman et al. (2020)
Glomus caledonicum	Finger millet (genotype: HR-374)	Improved shoot nutrient content, plant biomass, and AMF root colonization	Tewari and Tandon (1993)

(continued)

Table 2.3 (continued)

Microbial species	Millet	Benefits to the plant	References
R. intraradices	Finger millet (cv. Ragi Korchara)	Plant growth promotion and drought stress tolerance	Tyagi et al. (2018)
Mixed AMF inoculum dominant in *R. irregularis, F. mosseae,* and *F. geosporum* multiplied in a mixture of soil-sand, soybean hulls, and vermicompost	Sorghum	Higher soil glomalin indicated improved C-allocation to plant and soil C-sequestration and health	Agnihotri et al. (2021b)
Native *R. irregularis, F. mosseae,* and *F. geosporum* multiplied in raised beds	Sorghum	Higher soil and root glomalin, and increased soil C-sequestration and health	Agnihotri et al. (2021a)
Azospirillum and AMF	Finger millet	Growth, yield, and nutrient status	Bama and Ramakrishnan (2010)
AMF (*Rhizophagus fasciculatus and Ambispora leptoticha*) + *Pseudomonas* (strain MSSRFD41)	Finger millet and pigeon pea	Yield and reduced fertilizer dependence	Mathimaran et al. (2020)
R. irregularis (strain BEG-75) + *Bradyrhizobium* (DSMZ-5969), *P. fluorescens* (strain R62 and R81) or *G. fasciculatum* and *G. leptotichum* + *Bradyrhizobium* (DSMZ-5969), *P. fluorescens* (strain R62 and R81)	Pigeon pea (BRG-2) and Finger millet (GPU-28)	Improved bioirrigation in finger millet	Singh et al. (2019)
G. intraradices and *G. etunicatum*	Finger millet (47 genotypes)	Improved P and plant growth promotion	Srivastava et al. (2014)
Azospirillium + *B. polymyxa* + *G. mosseae*	Finger millet	Plant growth promotion and nutrient uptake	Ramakrishnan and Bhuvaneswari (2014)
R. intraradices and *P. indica*	Finger millet	Drought stress tolerance	Tyagi et al. (2017)
G. intraradices + *P. poae* (KA-5)	Finger millet (cv. Korchara)	Drought stress tolerance	Kamal et al. (2015)
The common mycorrhizal network formed by *G. intraradices* or *G. mosseae*	Flax (*Linum usitatissimum*) and Sorghum (*S. bicolor*)	Improved nutrient uptake by flax and higher biomass in mixed cultures	Walder et al. (2012)

2.6 Techniques to Study Soil Microbial Diversity

There are many methods available to characterize microbial community structure and assess the functional and metabolic diversity (Table 2.4). The developments in analytical tools and microbiological techniques help in soil and plant microbiome research and the advancement of innovative microbial bioinoculants (Debenport et al. 2015). Structural profiling includes techniques such as phospholipid fatty acid analysis, which provides the quantitative and qualitative estimation of the total microbial biomass as well as the dominant soil microbial groups (Neha et al. 2022). The fluctuations in metabolomic profiles also throw light on the association between plants and microbes (Mythili and Ramalakshmi 2022). Metabolomic analysis revealed the presence of alkane and fatty acyls as the main compound classes detected in the exudates of maize roots inoculated with the AMF spores recovered from the millet rhizosphere (Mythili and Ramalakshmi 2022). Precisely, a sum of 117 compounds belonging to 26 different classes was recovered from the maize roots colonized by *C. etunicatum*, *F. mosseae*, *G. fascicultum*, *G. margarita*, and *R. intraradices* (Mythili and Ramalakshmi 2022). Principally, omics strategies reveal the nonculturable soil microbial community (Meena et al. 2017). High-throughput illumina sequencing is another method to study and compare the rhizospheric bacterial community structure in different crop species having specific attributes such as disease resistance (Han et al. 2017). The obligate symbiotic nature of AMF complicates their quantification, and therefore their study requires distinct techniques (Table 2.5). The quantification of AMF, however, also requires expertise and results warrant careful interpretation (Agnihotri et al. 2022a, b, c).

2.7 Need of the Hour

Action steps for utilizing PGPMs to enhance growth and productivity of millets cover the following: (1) the assessment of microbial species for their differential growth responses and patterns with diverse millet varieties in controlled experiments followed by field trials prior to inoculum development, (2) preparation of inoculum from nutrient-poor or stressed sites to get an ecologically-adapted microbial inoculum, (3) development of mycotrophic millet genotypes, and (4) the identification of best plant-AMF/microbial combination for a particular agroecosystem. The identification and assessment of soil microbiomes associated with millets will assist in (1) formulating biofortification strategies to cut the synthetic fertilization-induced losses in soil health and quality and also reduce its application cost, (2) the selection of agricultural practices capable of supporting the growth of beneficial microbes, (3) the enrichment of native microbes, (4) the adjustment of agricultural management to utilize rhizosphere microbiome as a natural source of nutrients, (5) bioinoculant inoculation and commercialization, and (6) for generating agriculturally significant germplasms. Specific requirements for AMF studies and inoculum

Table 2.4 Techniques to study soil microbial diversity

Metabolic profiling and functional assessment	
Substrate utilization pattern/C-source utilization	Dheeman et al. (2017)
Community-level physiological profiling (CLPP)	Söderberg et al. (2004)
Substrate-induced respiration	Anderson and Domsch (1978)
Microbial biomass carbon	Vance et al. (1987)
Production of indole acetic acid (IAA), phosphate solubilization, hydrogen cyanide (HCN), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophore production, and enzymatic activities	Chandra et al. (2018); Dheeman et al. (2017)
Fatty acid methyl ester (FAME) analysis/phospholipid fatty acid (PLFA) profiling	Chandra et al. (2018); Neha et al. (2022)
Root metabolomics	Mythili and Ramalakshmi (2022)
Genetic profiling	
Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) PCR-temperature gradient gel electrophoresis (PCR-TGGE) Restriction fragment length polymorphism (RFLP) Terminal-restriction fragment length polymorphism (T-RFLP) Single-strand conformation poly-morphism (SSCP) Amplified ribosomal DNA restriction analysis (ARDRA)	Sharma et al. (2010)
Microbiome sequencing (expression profiling)	
16S rRNA sequencing	Chandra et al. (2018); Dheeman et al. (2020)
18S rRNA sequencing	Banos et al. (2018)
High-throughput illumina sequencing	Debenport et al. (2015); Han et al. (2017); Tveit et al. (2014); Xu et al. (2018)
ABI solid sequencing	Lata et al. (2010)
Microbial diagnostic microarrays (MDMs)	Sessitsch et al. (2006)
Macroarray analysis	Puranik et al. (2011)
Microbial Diversity Index	
Simpson Diversity Index	Simpson (1949)
Shannon Diversity Index	Shannon and Weaver (1949)

development to improve the growth and yield of millets include (1) the identification of the most potent plant-AMF combination to derive maximum benefit from symbiotic association (Srivastava et al. 2014); (2) the development of microbial inoculum from the rhizosphere of native soil would also help overcome the hostpreference phenomenon (Agnihotri et al. 2022a, b, c; Torrecillas et al. 2012); (3) the development of centralized biobanks maintaining native AMF strains with a complete set of information regarding their mycotrophic capacity, host compatibility, responsiveness and growth-promoting capacity, competition with other microbial species, and adaptability to stressed situations (Agnihotri et al. 2018; Peterson et al. 1984). The

Table 2.5 Techniques to study AMF biomass

Assessment	References
Spore density	Gerdemann and Nicolson (1963)
Mycorrhizal colonization percentage	Phillips and Hayman (1970)
16:1ω5c is phospholipid fatty acids	Agnihotri et al. (2021a); Qin et al. (2015)
16:1ω5c is neutral lipid fatty acids	
Glomalin-related protein (soil/root)	Agnihotri et al. (2022a, b, c)
Small ribosomal subunit (SSU) rDNA gene sequence	Öpik et al. (2006, 2010, 2013)
Large ribosomal subunit (LSU) rDNA gene sequence	Jansa et al. (2014)
Internal transcribed spacer (ITS) region	Stockinger et al. (2010)

plant growth-promoting traits of the rhizosphere microbiome can boost the growth and nutrient status of millets under moisture and nutrient-deficit conditions and could serve as the basis for selecting better candidates for inoculation in agroecosystems, particularly drought-affected agricultural fields. Owing to the abovementioned factors, the inoculum should be carefully tailored to cater to the requirements of growing plants. As the awareness about microbial bioinoculants continues to rise, techniques to identify microbes residing in the millet rhizosphere will support the introduction of novel microbial biofertilizers. Practices such as intercropping, and the application of organic residues offer a practical alternative for small farmers to boost crop yield, and plant and soil health (Debenport et al. 2015). In conclusion, using millet-specific PGPMs will offer a practical, long-lasting, and environmentally safe substitute for synthetic fertilizers. It will also help to improve soil health, prevent environmental deterioration, and alleviate nutrient deficiencies to meet the needs of the growing human population.

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Chapter 3
Biodiversity of Arbuscular Mycorrhizal Fungi and Its Impact on Millets Growth

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Abstract Millets are popular for their nutrient richness and environmental smartness. Millets are often grown on marginal lands under rainfed conditions and are favored crop species in intercropping systems. Roots of millets are colonized by a wide range of fungi including the arbuscular mycorrhizal (AM) fungi. Diverse AM fungi associate with millets as revealed by the diversity of AM spores in the soils and through the examination of millet roots using molecular techniques. *Glomus* is the dominant taxa associated with millets followed by *Acaulospora*, *Funneliformis*, and *Rhizophagus*. AM fungal symbiosis enhance the growth and nutrient uptake of millets in a wide range of soil and environmental conditions. The response of millets to AM symbiosis happens despite the presence of an elaborate root system. However, the responsiveness of millets to AM fungal symbiosis tends to vary with species and genotypes of the same species. Nevertheless, the yield response of millets to AM fungal presence is not well explored when compared to other popular cereal crops. AM symbiosis also imparts tolerance in millet against abiotic stresses like drought and salinity and induces changes in the structure and diversity of microorganisms in the soil. Agronomic and cultivation practices like the application of fungicides and synthetic fertilizers, crop rotation, and intercropping are known to affect AM fungal symbiosis in millets. The development of appropriate strategies for efficient use of millet-AM symbioses would increase millet production under limited inputs in resource-poor regions.

Keywords Agronomic practices · Drought · Salinity · Intercropping · Crop rotation · Sorghum · Finger millet · Pearl millet

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_3

3.1 Introduction

Millets are grown worldwide for food and fodder. The adaptability to thrive under low soil moisture and fertility, high soil pH, salinity, and temperature has rendered millets more popular in arid and semiarid regions where other cereals like rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), or maize (*Zea mays* L.) often fail. Millets are an important source of nutrients like calcium (Ca), iron (Fe), and zinc (Zn) for humans (Passot et al. 2016). In addition to these, some millets also have a short maturation time. For instance, Indian barnyard millet (*Echinochloa frumentacea* Link) has a maturation period of 45–70 days against 120–140 days for popular cereals like rice (Kumar et al. 2018).

The role of plant root architecture in the acquisition of water and nutrients is well documented in different crop species. Root characters are important targets in the genetic improvement of crops to enhance the efficiency of nutrient uptake (Lynch 2019). Millets like other cereal crops possess a fibrous root system. The root system of millets contains various groups of roots that vary in their extent of growth, tropism, and branching patterns in addition to water acquisition and transport. Notably, great variations in root traits were observed in pearl millet (*Pennisetum glaucum* (L.) Morrone) grown in different soils (Brück et al. 2003; Passot et al. 2016). Nevertheless, information is limited pertaining to the structure and function of root systems for millets compared to other widely cultivated cereal crops.

The root of millets like other crop species is colonized by a wide range of fungi belonging to different groups. For instance, Mofini et al. (2022) have shown that the roots of cultivated and wild pearl millet growing in three different agroecological regions of Senegal were associated with a wide range of fungi belonging to Ascomycota, Basidiomycota, Chytridiomycota, and Glomeromycota. Nevertheless, the root fungal communities of the wild and cultivated pearl millets are dissimilar with cultivated varieties harboring more diverse root fungal taxa than the wild varieties (Mofini et al. 2022). The nature of the relationship between these endorrhizal fungi-colonizing roots and millets can range from mutualism to parasitism. In addition, mutually beneficial fungi-colonizing millet roots perform a wide range of functions ranging from nutrient absorption to modification of the soil environment. Among the different types of soil fungi-colonizing millet roots, arbuscular mycorrhizal (AM) fungi belonging to the subphylum Glomeromycotina of the phylum Mucoromycota (Spatafora et al. 2016) are one of the most habitual and ubiquitous ones. Nevertheless, studies examining the role of AM fungi on millet growth or imparting tolerance against various stresses are limited when compared to cereal crops like wheat or maize. Moreover, millets like the finger millet [*Eleusine coracana* (L.) Gaertn.] and sorghum [*Sorghum bicolor* (L.) Moench] are the major experimental plants used in examining the role of AM fungi on millet growth, physiology, or biochemical changes in millets. In this chapter, we first discuss the general aspects of AM symbiosis followed by the diversity of AM fungi associated with millets, the influence of AM fungi on millet growth, mechanisms involved in AM-mediated plant growth improvement, and factors influencing AM symbiosis.

3.2 Arbuscular Mycorrhizal Fungi

One of the most common and widespread types of symbiosis in the plant kingdom is the AM fungal association established between the endorrhizic AM fungi and plant roots. Roots of millets like other field-grown crops are colonized by AM fungi. The fungal symbiosis is established from the different types of AM fungal propagules that persist in the soil. The infective propagules of AM fungi include the spores, the soil extramatrical hyphal network, and the mycorrhizal roots. The proportion of different types of AM fungal propagules tends to vary with vegetation types. As mycorrhizal roots and extraradical hyphae are the chief propagules in vegetations like the forests where new roots development happens throughout the year. Contrarily, spores are the chief perennating propagules for AM fungi in seasonal vegetations like the agroecosystems where the soil is barren for some part of the year. Similarly, the type of propagules initiating mycorrhization can also vary with AM fungal taxa. For example, as mycorrhization is initiated from the spores in Gigasporales, it is chiefly from the soil hyphae in Glomeraceae or Acaulosporaceae (Abdelhalim et al. 2014). The mycorrhization of the roots is initiated after the exchange of a wide range of molecular signals between the symbionts and the stimulation of the plant-signalling pathway (Park et al. 2015).

The AM fungus forms an appressorium on the root surface before entering the root. Once entered within the root, the fungal hyphae coil in the first few cells and then spread through the root cortex intercellularly and/or intracellularly. Based on the spread of the intraradical hyphae, the AM morphology is characterized by *Arum*-type and *Paris*-type. In the *Arum*-type, the hyphae spread intercellularly, whereas in *Paris*-type the hyphal spread intracellular. However, there are a wide range of intermediate types sharing the characteristic of both *Arum*- and *Paris*-types (Dickson 2004). The fungi also form different structures for the exchange of resources and storage in the endorhizosphere. The structures involved in the exchange of nutrients are the finely branched hyphal structures called arbuscules, which are essential for the functioning of the symbiosis. The development of arbuscules is determined by the nutrient demand of the host plant and these structures have a limited life span (Kobae et al. 2016). The arbuscules are either rudimentary or may be absent in typical *Paris*-type AM. Nevertheless, the function of arbuscules is replaced by the hyphal coils or arbusculate coils with different extent of arbuscule development in the *Paris*- or intermediate-type AM morphology. Many plant genes are involved in the development and functioning of the arbuscules (Park et al. 2015). Unlike arbuscules, vesicles (the fungal storage structures) are not found in all taxa of AM fungi. Members of Gigasporales do not form vesicles inside plant roots; instead, they produce special extraradical structures known as auxiliary cells. Although the function of auxiliary cells is obscure, they are suggested to function as storage structures. The proportion of different AM fungal structures in plant roots tends to vary with the growth stages of the plants. As arbuscular abundance peaks during the early stages of plant growth, the development of vesicles happens during later stages of plant growth. The different types of AM fungal structures frequently observed in millet roots are presented in Fig. 3.1.

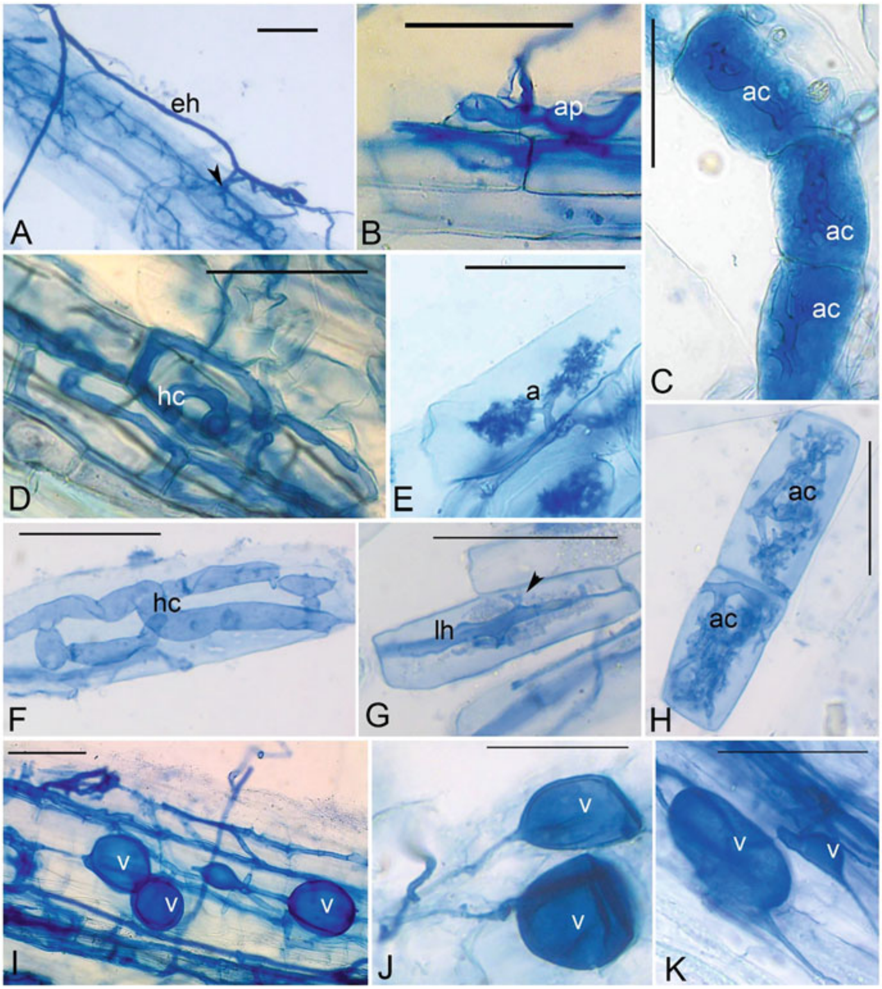

Fig. 3.1 (**a–k**): Arbuscular mycorrhizal (AM) fungal structures associated with roots of millets. (**a**) Extraradical hyphae (eh) on the root surface and entry of roots (black arrowhead); (**b**) Appressorium (ap) on the root surface; (**c, h**) Arbusculate coils (ac) in cortical cells; (**d**, **f**) Hyphal coils (hc) in cortical cells; (**e**) *Arum*-type arbuscule (a); (**g**) Arbuscule (black arrowhead) formed on intracellular linear hyphae (ih); (**i**–**k**) Intercellular vesicles (v) in the cortex. (**a**, **g**, **i–k**) Finger millet; (**b**, **c**, **d**, **e**, **g**) Foxtail millet; (**f**, **h**) Pearl millet. Scale bars = 50 μm

3.3 Presence of AM in Millets

The AM symbiosis is prevalent in all the millet species that have been examined for their mycorrhizal status (Table 3.1). Nevertheless, a few millet species like Sonoran millet (*Panicum hirticaule* J.Presl), black fonio millet (*Digitaria iburua* Stapf), Raishan millet [*Digitaria compacta* (Roth ex Roem. & Schult.) Veldkamp], and early

Table 3.1 Millet species, their common names, and arbuscular mycorrhizal (AM) status

Millet species	Common names	Mycorrhizal status[a]	References
Brachiaria deflexa (Schumach.) C.E.Hubb. ex Robyns	Guinea millet	AM	Utaile et al. (2021)
Brachiaria ramosa (L.) Stapf	Browntop millet	AM	Duponnois et al. (2001)
Coix lacryma-jobi L.	Adlay millet	AM	Bei et al. (2021); Ndoye et al. (2016)
Digitaria compacta (Roth ex Roem. & Schult.) Veldkamp	Raishan	NA	–
Digitaria exilis (Kippist) Stapf	Fonio, fonio millet, hungry rice, acha rice	AM	Ndoye et al. (2016)
Digitaria iburua Stapf	Black fonio	NA	–
Digitaria sanguinalis (L.) Scop.	Polish millet	AM	Rashidi et al. (2020); Rashidi et al. (2022)
Echinochloa crus-galli (L.) P.Beauv.	Common barnyard grass	AM	Hossler (2010); Htay et al. (2021); Veiga et al. (2011)
Echinochloa esculenta (A. Braun) H. Scholz	Japanese barnyard millet	NA	–
Echinochloa frumentacea Link	Indian barnyard millet	AM	Ragupathy et al. (1990)
Echinochloa oryzoides (Ard.) Fritsch	Early barnyard grass	NA	–
Echinochloa stagnina (Retz.) P.Beauv.	Burgu millet	AM	Rodríguez-Echeverría et al. (2017)
Eleusine coracana (L.) Gaertn.	Finger millet	AM	Krishna et al. (1982); Mythili et al. (2021); Kandhasamy et al. (2020)
Panicum hirticaule J.Presl (=*P. sonorum* Beal)	Sonoran millet	NA	–
Panicum miliaceum L.	Broomcorn millet, Common millet, hog millet, proso millet, white millet	AM	Caruso et al. (2018); Channabasava and Lakshman (2012); Channabasava and Lakshman (2015); Channabasava et al. (2015a)
Panicum sumatrense Roth (=*Panicum miliare* Lam.)	Little millet	AM	Channabasava and Lakshman (2015)
Paspalum scrobiculatum L.	Kodo millet	AM	Channabasava and Lakshman (2011); Channabasava and Lakshman (2015)
Pennisetum glaucum (L.) R.Br.	Pearl millet	AM	Bielders et al. (2010); Pal (2017); Pal and Pandey (2017)

(continued)

Table 3.1 (continued)

Millet species	Common names	Mycorrhizal status[a]	References
Setaria italica (L.) P. Beauv.	Foxtail millet, Italian millet, panic	AM	Channabasava and Lakshman (2015); Shen et al. (2022); Suharno et al. (2021)
Sorghum bicolor (L.) Moench	Great millet	AM	Symanczik et al. (2018)
Spodiopogon formosanus Rendle	Taiwan oil millet	NA	–

[a] *NA* not assessed

barnyard grass [*Echinochola oryzoides* (Ard.) Fritsch] are yet to be assessed for their mycorrhizal status. Likewise, the AM morphology of millets is also largely unknown. Sorghum is shown to possess both typical *Arum*- and intermediate-type AM morphologies (Dickson 2004). The AM morphology of sorghum is shown to vary with the colonizing AM fungi. As *Funneliformis coronatus*, *Rhizoglomus intraradices*, and *Funneliformis mosseae* formed typical *Arum*-type morphology in sorghum roots, *Scutellospora calospora*, *Gigaspora margarita*, and *Gigaspora rosea* established a varying type of intermediate morphologies (Dickson 2004). Observations of AM morphology of field-grown millets also suggest the predominance of intermediate-type AM morphologies.

3.4 Diversity of AM Fungi Associated with Millets

Information on the AM fungal communities associated with millet roots and soils is limited compared to those on other field crops. The AM fungal spores isolated from the root zone soils of millets are shown in Fig. 3.2. Most studies examining the diversity of AM fungi with millets involve the morphological identification of spores, and others have utilized molecular techniques.

3.4.1 Morphological Diversity of AM Fungal Spores in Millets

An examination of the diversity of AM fungi associated with finger millet growing in the Hassan district of India indicated the presence of 11 spore morphotypes belonging to *Acaulospora*, *Glomus*, *Rhizophagus*, and *Scutellospora* (Sunilkumar and Garampalli 2010). The spore density in the soil was 320 spores/100 g of dry soil, and the percentage of root length colonized by AM fungi was 40% (Sunilkumar and

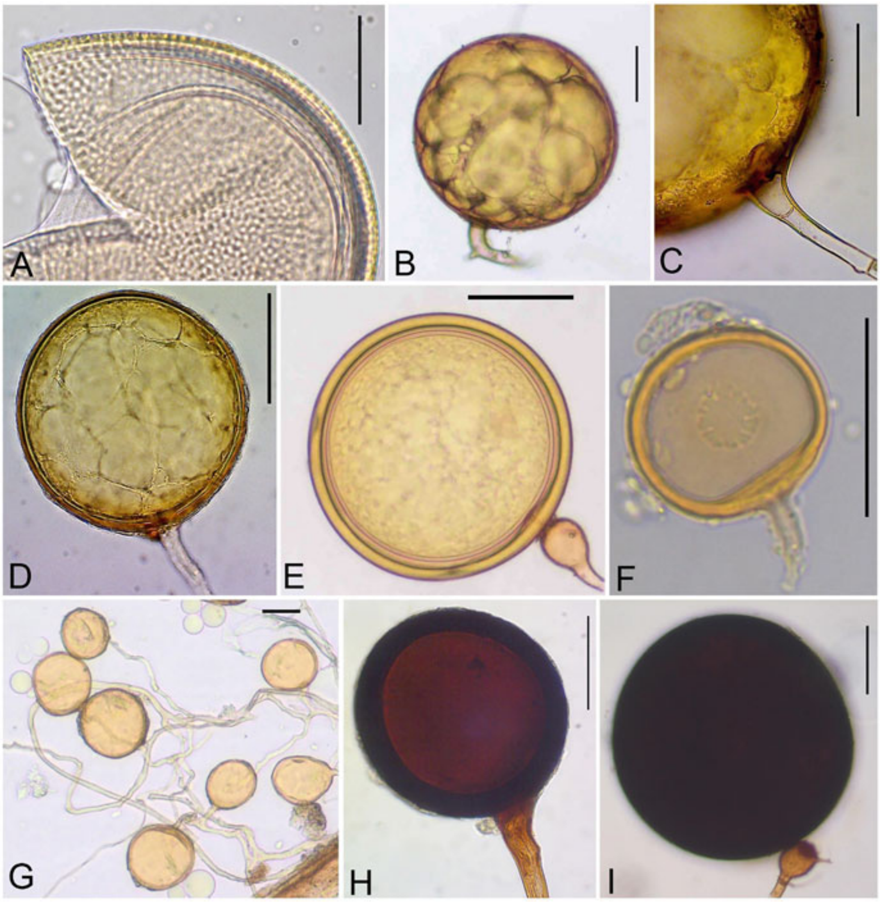

Fig. 3.2 (**a-i**) Spores of arbuscular mycorrhizal fungi reported from the root zone soils of millets. (**a**) Fractured spore of *Acaulospora scrobiculata*; (**b**) *Funneliformis mosseae*; (**c**) Funnel-shaped hyphal attachment and the curved septum of *F. mosseae*; (**d**) *Claroideoglomus etunicatum*; (**e**) *Scutellospora calospora*; (**f**) *Rhizoglomus microaggregatus*; (**g**) *Rhizophagus aggregatus*; (**h**) *Funneliformis geosporus*; (**i**) *Dentiscutata heterogama*. Scale bars = 50 μm

Garampalli 2010). An assessment of AM fungal communities associated with sorghum through trap culture technique in the Sikasso region of Mali indicated the association of 24 spore morphotypes belonging to *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora* (Kone and Kante 2021). Nevertheless, most of the spore morphotypes in the study were not characterized beyond the genus level. Among the spore morphotypes recorded in the six AM fungal genera, 65% belonged to *Glomus*, 10% each to *Gigaspora* and *Scutellospora*, and 5% each in the *Acaulospora*, *Entrophospora*, and *Sclerocystis* (Kone and Kante 2021). Similarly, an examination of AM fungal communities associated with different genotypes of sorghum (CSV-8R, E 36-1, M 35-1, NSH-27, and RS-29) cultivated in different regions of Andhra Pradesh, India during Kharif and Rabi seasons indicated the occurrence of 19 AM fungal morphotypes of *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis* (Hindumathi and Reddy 2011). In addition, *Glomus* was the dominant genera represented by 11 spore morphotypes and *Rhizophagus fasciculatus* was the most prevalent species during both the growing seasons (Hindumathi and Reddy 2011).

A summary of the diversity of AM fungal species in the millet soils in line with the current nomenclature indicates the association of spores of 63 taxa with the rhizospheres of millets (Table 3.2). The AM fungal diversity in millets is dominated by spore morphotypes belonging to *Glomus* followed by *Acaulospora*, *Funneliformis*, and *Rhizophagus* (Fig. 3.3). All the other AM fungal genera are represented by less than five taxa (Fig. 3.3). Of the different AM fungal species, *Rhizophagus clarus* frequently associates with the soils of most millet than the other species (Table 3.2). However, this general diversity of AM fungi in millets can vary with individual studies as the diversity of AM fungi is determined by the tripartite interaction of the host plant, AM fungi, and the soil environment.

3.4.2 Molecular Diversity of AM Fungi in Millets

A few studies have examined the diversity of AM fungi associated with millets using molecular techniques. An investigation of AM fungal community structure in roots of 19 sorghum genotypes in a semiarid region of Sudan using next-generation sequencing revealed the presence of 102 operational taxonomic units (OTUs) belonging to Claroideoglomeraceae, Diversisporaceae, Glomeraceae, and Paraglomeraceae (Badi et al. 2019). Of these, the family Glomeraceae was represented by 87 OTUs and was the dominant family followed by Claroideoglomeraceae (11 OTUs), Paraglomeraceae, and Diversisporaceae (3 OTUs each). Moreover, the presence of three OTUs of *Rhizoglomus* in more than 75% of the sorghum root samples examined suggested that the AM fungal community in sorghum roots was represented by a few genera list taxa and a large number of rare taxa (Badi et al. 2019).

Meta genomics has revealed that Glomeromycota appears to be less represented in the root mycobiome of millets compared to other root endophytic mycoflora. For instance, DNA meta barcoding studies of root mycobiome of wild and pearl millet from different agroecological regions of Senegal through a rainfall gradient revealed the less occurrence of OTUs belonging to Glomeromycota in the core root mycobiome (0.09–1.28%) of wild and cultivated pearl millets that was dominated by fungi belonging to Ascomycota and Basidiomycota (Mofini et al. 2022). The low occurrence of AM fungi associated with millets is also confirmed by the metagenomics of the soil. Ascomycota followed by Basidiomycota were the most dominant phylum in the soils that were under continuous monocropping of proso millet [*Pennisetum glaucum* (L.) R.Br.] in the Guan-Zhong Plain of China (Yang et al. 2020). Though the relative abundance of Glomeromycota along with other

Table 3.2 Diversity of arbuscular mycorrhizal (AM) fungal taxa associated with different millet species

AM fungal species[a]	Millet species	References
Acaulospora bireticulata F.M. Rothwell & Trappe	*Eleusine corocana*	Sunilkumar and Garampalli (2010)
Acaulospora colombiana (Spain & N.C. Schenck) Kaonongbua, J.B. Morton & Bever	*E. corocana*, *Paspalum scrobiculatum*, *Sorghum bicolor*	Carrenho et al. (2002); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Acaulospora delicata C. Walker, C.M. Pfeiff. & Bloss	*Setaria italica*	Mythili and Ramalakshmi (2022)
Acaulospora denticulata Sieverd. & S. Toro	*Echinochloa frumantacea*, *E. corocana*, *Panicum sumatrense*	Mythili and Ramalakshmi (2022); Mythili et al. (2021); Sunilkumar and Garampalli (2010)
Acaulospora foveata Trappe & Janos	*Panicum miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Acaulospora laevis Gerd. & Trappe	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, b)
Acaulospora longula Spain & N.C. Schenck	*S. bicolor*	Carrenho et al. (2002)
Acaulospora morrowiae Spain & N.C. Schenck	*S. bicolor*	Friberg (2001)
Acaulospora scrobiculata Trappe	*S. italica*, *S. bicolor*	Carrenho et al. (2002); Mythili and Ramalakshmi (2022)
Acaulospora spinosa C. Walker & Trappe	*P. miliaceum*	(Channabasava et al. 2015b)
Ambispora leptoticha (N.C. Schenck & G.S. Sm.) C. Walker, Vestberg & A. Schüßler	*E. corocana*, *E. frumantacea*, *P. scrobiculatum*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Archaeospora trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker	*P. miliaceum*	Channabasava et al. (2015a, b)
Archaeospora undulata (Sieverd.) Sieverd., G.A. Silva, B.T. Goto & Oehl	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Claroideoglomus claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler	*E. frumantacea*, *E. corocana*, *P. scrobiculatum*, *P. miliaceum*, *P. sumatrense*, *S. italica*, *S. bicolor*	Channabasava et al. (2015a), Friberg (2001); Mythili and Ramalakshmi (2022); Mythili et al. (2021); Carrenho et al. (2002)
Claroideoglomus etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßler	*E. corocana*, *S. italica*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Claroideoglomus luteum (L.J. Kenn., J.C. Stutz & J.B. Morton) C. Walker & A. Schüßler	*E. corocana*	Sunilkumar and Garampalli (2010)

(continued)

Table 3.2 (continued)

AM fungal species[a]	Millet species	References
Corymbiglomus globiferum (Koske & C. Walker) Błaszk. & Chwat	*P. miliaceum*, *P. sumatrense, S. italica*, *S. bicolor*	Channabasava et al. (2015a, b); Carrenho et al. (2002)
Dentiscutata erythropus (Koske & C. Walker) C. Walker & D. Redecker	*E. frumantacea*, *E. corocana*, *P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Dentiscutata heterogama (T.H. Nicolson & Gerd.) Sieverd., F.A. Souza & Oehl	*E. corocana*, *S. bicolor*	Carrenho et al. (2002); Kone and Kante (2021); Sunilkumar and Garampalli (2010)
Dentiscutata nigra (J.F. Redhead) Sieverd., F.A. Souza & Oehl	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Diversispora epigaea (B.A. Daniels & Trappe) C. Walker & A. Schüßler	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Entrophosphora sp.1	*P. sumatrense*	Mythili and Ramalakshmi (2022)
Entrophosphora sp.2	*S. italica*	Mythili and Ramalakshmi (2022)
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid.	*P. scrobiculatum*, *S. italica*	Mythili and Ramalakshmi (2022)
Funneliformis caledonius (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	*P. miliaceum*	Channabasava et al. (2015b)
Funneliformis coronatus (Giovann.) C. Walker & A. Schüßler	*S. bicolor*	Kone and Kante (2021)
Funneliformis dimorphicus (Boyetchko & J.P. Tewari) Oehl, G.A. Silva & Sieverd.	*E. frumantacea*, *E. corocana*, *P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a); Mythili and Ramalakshmi (2022); Sunilkumar and Garampalli (2010)
Funneliformis geosporus (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	*E. corocana*, *P. miliaceum*, *P. sumatrense, S. italica, S. bicolor*	Carrenho et al. (2002); Channabasava et al. (2015a); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Funneliformis monosporus (Gerd. & Trappe) Oehl, G.A. Silva & Sieverd.	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler	*E. corocana*, *P. miliaceum*, *P. scrobiculatum*, *P. sumatrense, S. italica, S. bicolor*	Carrenho et al. (2002); Channabasava et al. (2015a); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Gigaspora decipiens I.R. Hall & L.K. Abbott	*S. bicolor*	Carrenho et al. (2002)
Gigaspora gigantea (T.H. Nicolson & Gerd.) Gerd. & Trappe	*E. corocana*, *P. sumatrense*, *P. scrobiculatum*, *S. bicolor*	Friberg (2001); Mythili and Ramalakshmi (2022)

(continued)

Table 3.2 (continued)

AM fungal species^a	Millet species	References
Gigaspora margarita W.N. Becker & I.R. Hall	*E. corocana*, *P. miliaceum*, *P. scrobiculatum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Gigaspora rosea T.H. Nicolson & N.C. Schenck	*P. miliaceum*, *P. sumatrense, S. italica*, *S. bicolor*	Channabasava et al. (2015a, b); Kone and Kante (2021)
Glomus ambisporum G.S. Sm. & N.C. Schenck	*E. corocana*	Sunilkumar and Garampalli (2010)
Glomus bagyarajii V.S. Mehrotra	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Glomus citricola D.Z. Tang & M. Zang	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Glomus delhiense Mukerji, Bhattacharjee & J.P. Tewari	*P. miliaceum*	Channabasava et al. (2015b)
Glomus flavisporum (M. Lange & E.M. Lund) Trappe & Gerd.	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Glomus heterosporum G.S. Sm. & N.C. Schenck	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Glomus hoi S.M. Berch & Trappe	*E. frumantacea*, *E. corocana*, *P. scrobiculatum*, *P. sumatrense*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Glomus macrocarpum Tul. & C. Tul.	*E. corocana*, *P. miliaceum*, *P. scrobiculatum*, *P. sumatrense, S. italica*, *S. bicolor*	Carrenho et al. (2002); Channabasava et al. (2015a, b); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Glomus microcarpum Tul. & C. Tul.	*E. corocana*, *E. frumantacea*, *P. sumatrense*, *S. italica*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Glomus pubescens (Sacc. & Ellis) Trappe & Gerd.	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, 2015b)
Glomus reticulatum Bhattacharjee & Mukerji	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Glomus versiforme (P. Karst.) S.M. Berch	*E. corocana*, *P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a); Sunilkumar and Garampalli (2010)
Paraglomus albidum (C. Walker & L.H. Rhodes) Oehl, G.A. Silva & Sieverd.	*E. corocana*, *P. miliaceum*, *E. frumantacea*, *P. scrobiculatum*	Channabasava et al. (2015b); Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Paraglomus albidum (C. Walker & L.H. Rhodes) Oehl, G.A. Silva & Sieverd.	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Paraglomus brasilianum (Spain & J. Miranda) J.B. Morton & D. Redecker	*E. frumantacea*, *E. corocana*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)

(continued)

Table 3.2 (continued)

AM fungal species[a]	Millet species	References
Paraglomus occultum (C. Walker) J.B. Morton & D. Redecker	*S. bicolor*	Friberg (2001)
Redeckera fulva (Berk. & Broome) C. Walker & A. Schüßler	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, b)
Rhizoglomus microaggregatum (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl	*P. miliaceum*, *P. sumatrense, S. italica*, *S. bicolor*	Carrenho et al. (2002); Channabasava et al. (2015a)
Rhizophagus aggregatus (N.C. Schenck & G.S. Sm.) C. Walker	*E. frumantacea*, *E. corocana*, *P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, b), Mythili and Ramalakshmi (2022) Mythili et al. (2021)
Rhizophagus clarus (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüßler	*E. frumantacea*, *E. corocana*, *P. miliaceum*, *P. sumatrense, P. scrobiculatum, S. italica*, *S. bicolor*	Carrenho et al. (2002) Channabasava et al. (2015a, b), Friberg (2001), Mythili and Ramalakshmi (2022), Mythili et al. (2021), Sunilkumar and Garampalli (2010)
Rhizophagus fasciculatus (Thaxt.) C. Walker & A. Schüßler	*E. corocana*, *P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, b), Mythili et al. (2021)
Rhizophagus intraradices (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler	*E. corocana*, *E. frumantacea*, *P. scrobiculatum*, *S. italica*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Rhizophagus manihotis (R.H. Howeler, Sieverd. & N.C. Schenck) C. Walker & A. Schüßler	*E. corocana*	Sunilkumar and Garampalli (2010)
Sclerocystis dussii (Pat.) Höhn.	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, b)
Scutellospora biornata Spain, Sieverd. & S. Toro	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a)
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	*P. miliaceum*, *P. sumatrense, S. italica*	Channabasava et al. (2015a, b)
Scutellospora gregaria (N.C. Schenck & T.H. Nicolson) C. Walker & F.E. Sanders	*S. bicolor*	Kone and Kante (2021)
Septoglomus constrictum (Trappe) Sieverd., G.A. Silva & Oehl	*E. frumantacea*, *E. corocana*, *P. scrobiculatum*	Mythili and Ramalakshmi (2022); Mythili et al. (2021)
Sieverdingia tortuosa (N.C. Schenck & G.S. Sm.) Błaszk., Niezgoda & B.T. Goto	*E. corocana*	Sunilkumar and Garampalli (2010)

[a] Names of AM fungal species are presented in line with the current nomenclature

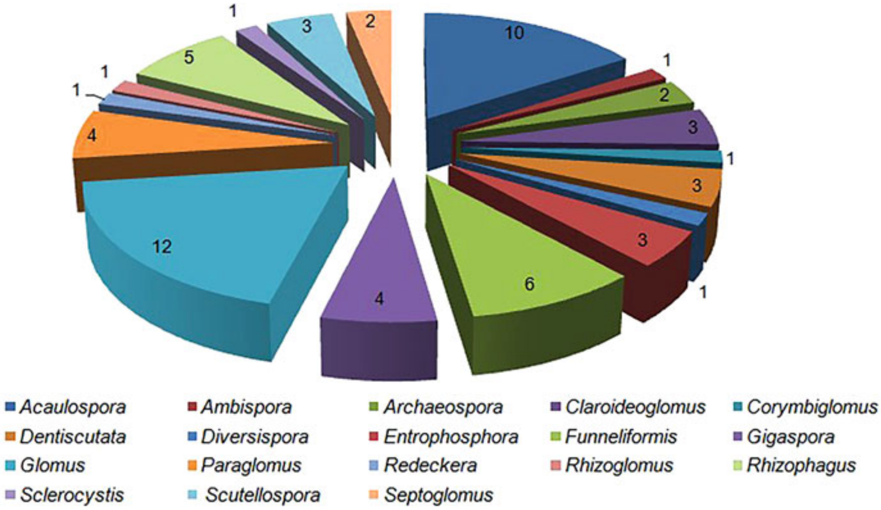

Fig. 3.3 Diversity of species in different arbuscular mycorrhizal fungal genera reported from millets

zygomycetous fungi was less than 2% of the total OTUs, it was significantly different from soils that were under continuous monocropping of common bean (*Phaseolus vulgaris* L.) and common buckwheat (*Fagopyrum esculentum* Moench) (Yang et al. 2020). The rhizosphere of proso millet is also dominated by Ascomycota followed by Mortierellomycota and Basidiomycota (Tian et al. 2022). An analysis of the rhizosphere soils of 13 pearl millet lines from an experimental field at Centre National de Recherches Agronomiques, Senegal revealed the dominance of Ascomycota with a relative abundance of 38–62% followed by Basidiomycota with 1.6–19% and Glomeromycota with 0.1–2.8% (Ndour et al. 2021).

Land use could also elicit changes in the diversity of AM fungal communities. In a recent study, Balami et al. (2021) have shown a shift in AM fungal communities when fields under cultivation of millet or other crops were transformed into different land usage. As OTUs of Acaulosporaceae, Claroideoglomeraceae, Gigasporaceae, and Paraglomeraceae were significantly more abundant in soils that were under agriculture, OTUs of Glomeraceae were least abundant in agricultural soils. However, OTUs of Glomeraceae dominated other land-use types like abandoned agricultural fields and regenerated forests (Balami et al. 2021).

3.5 Influence of AM Fungi on Millet Growth and Yield

A large number of studies have shown that AM fungi either individually or along with other plant growth-promoting microorganisms increase the growth of millets (Table 3.3). The growth parameters that are normally influenced by AM fungi in

Table 3.3 Effect of arbuscular mycorrhizal (AM) fungi and plant growth-promoting microorganisms on growth and yield of millets

Millet species	Type of study	Conditions	AM fungal species	Co-inoculated organism	Enhanced parameters			References
					Growth	Yield	Nutrients[a] uptake/ content	
Digitaria sanguinalis (L). Scop.	Greenhouse	Sterilized soil	*Funneliformis mosseae*, *Rhizophagus fasciculatus*, *Rhizoglomus intraradices*		PH, LN, tPDW		P_C, K_C, Fe_C, Cu_C	Rashidi et al. (2022)
Eleusine coracana (L.) Gaertn.	Greenhouse	Sterilized soil	*F. mosseae*	*Azospirillium brasilense*, *Bacillus polymyxa*	PH, SDW, RDW		P_U, N_U	Ramakrishnan and Bhuvaneswari (2014)
E. coracana	Greenhouse	Sterilized soil	*Funneliformis caledonius*, *F. mosseae*, *R. fasciculatus*, *Diversispora epigaea*, *Scutellospora calospora*, *Gigaspora margarita*		PH, LA, absolute growth rate, SDW, RDW, R/S ratio		N_C, P_C, Zn_C, Cu_C	Tewari et al. (1993)
E. coracana	Glasshouse	Sterilized soil	*R. fasciculatus*	*Streptomyces cinnamomeous*	SDW, RDW		Shoot and root P_C	Krishna et al. (1982)
E. coracana	Greenhouse	Sterilized soil	*R. intraradices*	*Streptomyces*, *Pseudomonas* spp.	PH, RL, SDW, RDW			Kamal et al. (2015)
E. coracana	Glasshouse	Steam sterilized soil andsand mixture	*R. intraradices*, *Claroideoglomus etunicatum*		SDW, RDW		P_C	Srivastava et al. (2014)

E. coracana	Greenhouse	Sterilized and unsterilized soil	*R. aggregatus*, *Acaulospora scrobiculata*, *R. intraradices*, *Funneliformis geosporum*		PH, LN, SDW, RDW, R/S ratio		P_C, N_C	Kandhasamy et al. (2020)
Panicum miliaceum L.	Experimental field	Natural soils	*R. intraradices*, *F. mosseae*			100-grain weight, grain yield, harvest index		Shishehbor et al. (2013)
Paspalum scrobiculatum L.	Greenhouse	Sterilized soil	*R. fasciculatus* with *G. margarita* and *Sclerocystis dussi*, *R. fasciculatus* with *G. margirata* and *S. dussi*		PH, SFW, RFW, SDW, RDW	Grains per plant	P_U	Channabasava and Lakshman (2011)
P. scrobiculatum, P. miliaceum, Panicum sumatrense Roth and *Setaria italica* (L.) Beauv.	Greenhouse	Unsterilized soil	*R. fasciculatus*, *Glomus macrocarpum Glomus bagyarajii*, *S. dussii*		PH, RL, SFW, RFW, SDW, RDW		P_C	Channabasava and Lakshman (2012)
Pennisetum glaucum (L.) R. Br.	Experimental field	Field soil	*R. intraradices*		PH, LA, StDW, LDW	PaDW, seed yield, hectoliter weight, 1000 seed weight,		López-Ortega et al. (2018)

(continued)

Table 3.3 (continued)

Millet species	Type of study	Conditions	AM fungal species	Co-inoculated organism	Enhanced parameters			References
					Growth	Yield	Nutrients[a] uptake/ content	
						Seed number		
P. glaucum	Natural conditions	Sterilized soil	Consortium of *R. fasciculatus*, *R. intraradices*, *F. mosseae*, *R. aggregatus*, *Acaulospora mellea*, *Sclerocystis*		SDW, RDW		$\mathrm{N_C}$, $\mathrm{P_C}$, $\mathrm{Zn_C}$, $\mathrm{Fe_C}$, $\mathrm{Cu_C}$, $\mathrm{Mn_C}$	Sharif et al. (2011)
P. glaucum	Open air conditions	Sterilized soil	*F. mosseae*, *R. fasciculatus*, *Gigaspora decipiens*		PH, RL, EL, PFW, PDW			Pal and Pandey (2017)
P. glaucum, *Sorghum bicolor* (L.) Moench	Growth chamber	Sterilized soil	*F. mosseae*		SDW, RDW, RL, RD		$\mathrm{P_C}$, $\mathrm{K_C}$, $\mathrm{Mg_C}$, $\mathrm{Ca_C}$, $\mathrm{Zn_C}$ (*S. bicolor*) $\mathrm{Ca_C}$, $\mathrm{Zn_C}$ (*P. glaucum*)	Bagayoko et al. (2000)
P. glaucum	Greenhouse	Sterilized soil	*Glomus* spp.		PH, LN, LL, RL, SRN, PDW			Burni et al. (2017)
S. italica	Greenhouse	Commercial potting mix soil	*R. intraradices*, *F. mosseae*, *R. aggregatus*, *C. etunicatum*		PH, SFW, RFW, SDW, RDW			Dhawi et al. (2018)

S. italica	Greenhouse	Sterilized soil	*C. etunicatum, Claroideoglomus lamellosum*		PH, LA, PDW, PFW, R/S ratio		N_U, K_U, shoot P_C	Suharno et al. (2021)
S. italica	Greenhouse	Sterilized soil	*R. fasciculatus*		PH, RL, Rootlet number, LN, SDW, RDW		P_U	Kadam (2022)
S. italica	Greenhouse	Soil + iron ore mine spoils	*R. fasciculatus*		PH, RL, SFW, RFW, SDW, RDW		P_U	Lakshman and Channabasava (2013)
S. bicolor	Greenhouse	Unsterilized soil	*R. aggregatus*, *R. fasciculatus*, *Paraglomus occultum*, *Acaulospora longula*, *A. scrobiculata*, *Acaulospora spinosa*, *Glomus* spp., *Scutellospora* sp.		PH, LN, SDW, RDW	Grain dry weight	P_U, N_U, K_U	Nakmee et al. (2016)

Ca calcium, *Cu* copper, *E* ear length, *Fe* iron, *K* potassium, *LA* leaf area, *LDW* leaf dry weight, *LL* leaf length, *LN* leaf number, *Mg* magnesium, *Mn* manganese, *N* nitrogen, *P* phosphorous, *PaDW* panicle dry weight, *PDW* plant dry weight, *PFW* plant fresh weight, *PH* plant height, *R/S ratio* root shoot ratio, *RD* root density, *RDW* root dry weight, *RL* root length, *RWF* root fresh weight, *SDW* shoot dry weight, *SFW* shoot fresh weight, *SRN* seminal root number, *StDW* stem dry weight, *tPDW* total plant dry weight, *Zn* zinc

[a] Symbols for elements followed by the subscript “C” indicate content and “U” indicates uptake

millets include plant height, leaf number, and area, root architecture, and plant fresh and dry weights (Watts-Williams et al. 2022). However, most of these studies are conducted under controlled conditions using sterilized substrate and selected AM fungal species. Sorghum plants when used as trap plants for isolating AM fungi in soils containing different AM fungal spore densities were 3–52% taller, had 13–26% more leaves, and accumulated 3–37% more average biomass when compared to plants growing in sterilized soils (Husein et al. 2022). Different AM fungal species affect varied growth responses, in terms of biomass production and growth patterns with plant species and their genotypes leading to the concept of AM fungi host preference (Torrecillas et al. 2012).

Millet growth response tends to vary with host genotypes and AM fungal species. In glasshouse conditions, most of the finger millet genotypes except VHC 4170 and Khairna 24 inoculated with the AM fungi *R. intraradices* or *Claroideoglomus etunicatum* accumulated more biomass and had reduced root/shoot ratios than uninoculated control (Srivastava et al. 2014). Nevertheless, higher shoot dry weight was found in all the finger millet genotypes inoculated with *R. intraradices* when compared to *C. etunicatum*. This variation in response of genotypes to different AM fungi was attributed to the varied dependency of the finger millet genotypes on specific AM fungi (Srivastava et al. 2014). The responsiveness of millets to AM fungi is also determined by the host plant-fungal genotype combinations in addition to the fungal genotype. This is evidenced in a study by Watts-Williams et al. (2019), where 15 diverse sorghum accessions were inoculated individually with four AM fungal species (*Rhizophagus irregularis*, *Diversispora versiformis*, *Claroideoglomus claroideum*, and *Gigaspora gigantea*) or a combination of 2 AM fungi (*D. versiformis* and *C. claroideum*). The sorghum lines exhibited a range of growth responses to the different AM fungi with some genotypes responding better than others to a particular fungus. However, this response was reversed with a different fungus (Watts-Williams et al. 2019).

The dependency of finger millet on AM fungal symbiosis also differs with cultivars. Kandhasamy et al. (2020) examined the mycorrhizal dependency of 10 genotypes of finger millet in a greenhouse experiment using two soil types. The results of the study indicated that the presence of AM fungi positively influenced plant growth and nutrient uptake. Further, the experiments also revealed the existence of an interactive influence of plant genotypes and soil types on mycorrhizal dependency in finger millet (Kandhasamy et al. 2020). Cobb et al. (2016) examined the responsiveness of three landraces (Ajabsid, Macia, and Sureno) and three hybrids (Dekalb, Pioneer, and Seneca) of sorghum to the presence of indigenous AM fungi. The results of the study indicated that the biomass of sorghum landraces increased by 206% compared to hybrids in the presence of AM fungi (Cobb et al. 2016). Overall, it is evident that AM fungal inoculations generally resulted in a positive response to plant growth in the millet species and their genotypes.

The influence of AM symbiosis on the yield of millets is limited compared to other cereal crops. Inoculation of specific AM fungi is shown to increase yield parameters in foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), and proso millet under greenhouse conditions (Channabasava and Lakshman 2011; Shishehbor et al. 2013). In their meta-analysis, Zhang et al. (2019a, b) indicated that the influence of AM fungi on grain yield was less prominent under field and noninoculation conditions. The grain yield was shown to increase by 16% in field studies when crops like sorghum were inoculated with AM fungi. The presence of AM fungi increased the grain yield of sorghum landraces by 285% compared to hybrid sorghums grown in unfertilized soils under greenhouse conditions (Cobb et al. 2016). Moreover, the total grain protein content of mycorrhizal sorghum landraces was also 320% higher compared to hybrid sorghum cultivars. However, sufficient data are unavailable to validate the AM response pattern on grain yield in millets (Zhang et al. 2019a, b).

3.6 Mechanisms of Plant Growth Improvement

3.6.1 Changes in Root Architecture

The modifications in the root architecture are primarily an essential strategy to improve nutrient uptake in plants. The alterations in the root morphology caused due to soil environment decrease the metabolic cost for maintenance of the root system and increase the nutrient uptake (Fernandez and Rubio 2015). Plants usually enhance root growth characteristics such as specific root length, number, and diameter of root hairs, or root/shoot ratio particularly, in nutrient deficient conditions. The AM fungi are long known to induce changes in the root morphology and development of the root system to increase plant growth and development under normal and stressed conditions. The AM fungal colonization in the host plant impacts the structure and function of the root system (Smith and Read 2008). One of the important mechanisms involved in the modifications of the root system by AM fungi includes the nutritional status of the plants (Yao et al. 2009; Nagaraj et al. 2021).

The AM fungi promote the formation of lateral roots and enhance root length and diameter which can increase surface area and promote water and nutrient absorption in plants (Shao et al. 2018). For instance, inoculation of *F. mosseae* or *R. intraradices* in sorghum increased the root traits including total root length and specific root length when compared to uninoculated plants (Sun and Tang 2013). Alterations in root architecture include an increase in root/shoot ratio, modifications in distribution of metabolites and root morphology (Iqbal et al. 2020). Finger millet plants raised in the presence of indigenous AM fungi increased the root/shoot ratio than those raised in without AM fungi (Kandhasamy et al. 2020). Increased lateral root formation influenced by AM fungal colonization has been associated with enhanced plant nutrition. The AM fungi supply phosphate and N to the root cortex directly from which nutrients are absorbed by particular plant ion transporters restricted in the peri-arbuscular membrane (Yang et al. 2012).

One of the commonly cited hypotheses states that the plants with coarse root architecture possessing larger root diameter and fewer root hairs and density receive more growth benefits from AM fungal colonization (Newsham et al. 1995; Smith and Read 2008). This results from the capability of coarse roots to uptake only limited nutrients, and thus depends on AM fungal hyphae to enhance their surface area for nutrient absorption (Bates and Lynch 2001). Nevertheless, a meta-analysis by Maherali (2014) suggested that having coarse root architecture does not essentially predict the growth response of the plant host to mycorrhization. However, several other studies have reported improved root growth such as enhanced root branching and volume in response to AM colonization (Sukumar et al. 2013; Ramírez-Flores et al. 2019).

The root architecture of plants is managed by genes, and most recent studies have shown the molecular mechanisms of AM fungi involved in the root modification through the RNA-sequence method. Moreover, Fusconi (2014) suggested that the AM fungi influence the formation of lateral roots by regulating the metabolism of P, carbohydrate, and plant hormones in the host plants. For instance, inoculation of *R. irregularis* enhanced the formation of lateral roots through the P metabolism pathway in plants. In addition, 909 differentially expressed genes related to plant hormone, sugar, and P metabolisms were identified through RNA sequence analyses indicating that AM fungi regulate the formation of lateral roots (Chen et al. 2017). The modifications of gene expression in AM-inoculated plant roots have revealed the molecular mechanism involved in the root architecture alterations stimulated by the AM fungi (Chen et al. 2021). Although increased production of the lateral root through stimulation of proliferation of lateral roots by RNA sequence method has been reported in millets (Ahmad et al. 2018), the molecular mechanism of AM-mediated root alterations in millets is yet to be explored.

The AM fungal colonization alters the concentrations of various plant hormones like ethylene, cytokinins, some auxins, jasmonic acids, and strigolactones in roots (Zhang et al. 2019a, b; Mishev et al. 2021). These plant hormones take place in the modulation of plant root architecture (Zou et al. 2017; Sharma et al. 2021). Auxin biosynthesis was stimulated in plants by AM fungal colonization which consequently improved the root morphology (Liu et al. 2018). In addition, polyamines, a type of plant hormone are involved in the lateral and adventitious root formation (Bose and Howlader 2020). The root characteristics of plants are improved by AM fungi by modifying the metabolism of endogenous polyamines (Wu et al. 2012; Yousefi et al. 2019). However, studies pertaining to changes in root characteristics induced by AM fungal colonization through alterations in phytohormones is lacking in millets.

3.6.2 Increased Nutrient Uptake

3.6.2.1 Macronutrients

It is a well-known fact that AM fungi improve nutrient acquisition particularly, phosphorus (P) by plants. The AM fungi significantly promote nutrient uptake especially macronutrients including P and nitrogen (N) by increasing the surface area and by solubilizing sparsely accessible nutrients. In general, plants derive the required nutrients from the soil through two pathways: (1) plant pathway by root hairs and root epidermis directly or (2) by mycorrhizal or symbiotic pathway by developing AM fungal structures including intaradical and extraradical hyphae, hyphal coils, and arbuscules in the root cortex indirectly (Smith and Smith 2011). The direct pathway of nutrient uptake takes place at the soil–root interface in which plants uptake P, N, or potassium (K) directly from the soil. In the symbiotic pathway, P is captured from surrounding soil through fungal phosphate importers by extraradical mycelium to intraradical mycelium (Wang et al. 2017). Likewise, nitrate and ammonium are absorbed by extraradical mycelium of AM fungi which is converted into glutamine and then into arginine (Smith and Read 2008). The phosphate and ammonium importers existing in the perifungal membrane are expressed in mycorrhizal plants (Smith and Smith 2011). The arbuscules and hyphal coils present around the perifungal membrane are activated by H^+-ATPase (Rosewarne et al. 2007).

The role of AM fungi in phosphate uptake is mostly ascribed to (1) extension of soil surface or volume by extraradical mycelium which scavenges phosphate and transports it to the root cortex (Jakobsen et al. 2001); (2) the diameter of AM fungal hyphae which permits the penetration of fungus to small soil cores to scavenge P and increase the level of P influx per surface area (Sharif and Claassen 2011); (3) ability of AM fungi to accumulate or store P in other forms like, polyphosphates, that permits the fungus to maintain a low concentration of internal phosphate and effective P transport to intraradical mycelium from extraradical mycelium (Hijikata et al. 2010); and (4) production of organic and acids phosphatase enzymes that promotes the liberation of P from insoluble organic phosphate sources (Sato et al. 2019). In addition, AM fungi express P transporters with high affinity in the extraradical mycelium for uptake of P from the soil. The availability of external P concentration and requirement of P by AM fungi can modulate the expression of P transporters (Calabrese et al. 2019). The genes of the phosphate transporter1 (PHT1) family have a crucial role in the uptake of inorganic phosphate from soil (Liu et al. 2016). For example, Ceasar et al. (2014) analyzed the effect of AM fungal colonization (*F. mosseae*) and different concentrations of inorganic P on foxtail millet growth. Around 12 genes of the PHT1 family were identified in foxtail millet through bioinformatic analysis and these genes showed different expression patterns depending on P level, plant tissue, and AM colonization. The study indicated that these transporters could play an important role in increasing P uptake in foxtail millet (Ceasar et al. 2014). Likewise, Pudake et al. (2017) cloned four phosphate transporter genes from finger millet for analyzing role of AM fungi (*G. intraradices*) in P uptake and revealed that *EcPT4* gene was specific to AM fungi and expression of *EcPT4* gene was greater in finger millet roots where the AM colonization levels was high. In addition, the gene expression level of *EcPT1-4* was constant with P acquisition in finger millet seedlings under P stress (Pudake et al. 2017).

Nitrogen is an important macronutrient for plants that are limited in soils. Like P, AM fungi also contribute to the uptake of N in plants. In addition, Reynolds et al.

(2005) indicated that increased P uptake can improve the N acquisition in plants. Several studies have shown that colonization by AM fungi has enhanced N uptake in host plants using ^{15}N-labelled substrates (Barrett et al. 2011; Zhu et al. 2016). The extraradical mycelium of AM fungi absorbs inorganic N and incorporates into amino acids through glutamine synthetase, glutamine oxoglutarate aminotransferase pathway, and convert into arginine (Jin et al. 2005). Arginine is transferred to intraradical mycelium via fungal hyphae and converted into urea and ornithine in the intraradical mycelium. Later, urea undergoes hydrolyzation and forms ammonia that is liberated to the mutualistic interface and thus acquired by the plants (Govindarajulu et al. 2005; Cruz et al. 2007). In addition, some of the ammonium transporters such as *GintAMT1* and *GintAMT3* are involved in the ammonium uptake in host plants that are expressed in both extraradical and intraradical mycelium of AM fungi (Pérez-Tienda et al. 2011; Calabrese et al. 2016). Inoculation of *C. etunicatum* and *Claroideoglomus lamellosum* in foxtail millet plants increased N and K uptake when compared to noninoculated plants (Suharno et al. 2021).

The uptake of P and N by AM symbiosis is largely explored when compared to symbiotic K acquisition in plants (Liu et al. 2018). However, AM fungi increase K uptake and tissue K content in several plant species (Zhao et al. 2015; Zhang et al. 2017) which indicates symbiotic K uptake in plants. For instance, inoculation of AM fungi increased the uptake of K in the shoot and roots of sorghum (Nakmee et al. 2016). The K transporters of AM fungi participating in the uptake of K from soil to plants are scarce. Casieri et al. (2013) recognized four K^+ transporters from sequences of *R. irregularis*. Moreover, LjHAK transporter (KT/KUP/HAK), a plant K^+ transporter was upregulated in AM-inoculated *Lotus corniculatus* L. roots when compared to uninoculated roots (Guether et al. 2009). The whole genome RNA sequencing of AM plants raised under K-deficient conditions was shown to upregulate putative transporter gene K^+/H^+ (Garcia et al. 2017). Due to insufficient data, the mechanism underlying symbiotic K uptake in plants has not been explored well.

3.6.2.2 Micronutrients

Besides macronutrients, micronutrients such as manganese (Mn), magnesium (Mg), Fe, Zn, and copper (Cu) are essential for plant growth. The AM fungi can increase the availability of these micronutrients and transport them to plants from nutrient-limited soils (Briccoli Bati et al. 2015; Watts-Williams et al. 2022). The extraradical mycelium of AM fungi explores the rhizosphere and helps in the uptake of immobile or low-mobility micronutrients (Saboor and Ali 2021). In a recent study, upregulation of *MtZIP14*, a putative Zn transporter gene has been identified through RNA sequence analysis in *R. irregularis*-inoculated plant roots which revealed the role of AM fungi in Zn transport to the host plant through the peri-arbuscular membrane (Watts-Williams et al. 2020). The AM fungal inoculation enhanced the Zn and Cu uptake in finger millet when compared to uninoculated plants (Tewari et al. 1993). In another study, the application of AM fungal consortia consisting of

R. fasciculatus, R. intraradices, F. mosseae, R. aggregatus, Acaulospora mellea, and *Sclerocystis* in pearl millet-enhanced soil micronutrients such as Zn, Cu, Fe, and Mn essential for growth (Sharif et al. 2011). Similarly, in a pot experiment, pearl millet and sorghum treated with P and AM fungi enhanced the Zn, Ca, and Mg uptake by 2.5 folds (Bagayoko et al. 2000).

3.6.3 Higher Nutrient-Use Efficiencies

Maintenance of higher crop production along with higher nutrient-use efficiency (NUE) has become more challenging worldwide with the growing population, reduction of natural resources, and environmental conditions. The NUE is the capability of crops to acquire and utilize nutrients for attaining maximum grain yields (Baligar et al. 2001). Nutrient-use efficiency is a crucial approach for monitoring crop production which is mostly influenced by plant and soil–water relations and fertilizer management (Baligar et al. 2001). Millets are important crops rich in nutrients and also help in increasing food and nutritional security. However, the production of millets is affected by environmental stress conditions and deficient soil nutrients subsequently resulting in decreased grain yield. Therefore, increasing NUE in plants could enhance crop yields; improve soil and water relations and decrease the cost of nutrient input (Gupta et al. 2012). Numerous studies have reported the improvement in NUE in various millets under different environmental conditions. For example, pearl millet treated under different available soil water levels (40%, 60%, 80%, and 100%) with different concentrations of N fertilizers (0, 75, 150, and 225 kg/ha) increased N-use efficiency and N utilization under full irrigation (100%) and maximum concentration of N fertilizer at 225 kg/ha (Rostamza et al. 2011).

The application of beneficial microorganisms including AM fungi is one of the approaches to enhance NUE in addition to nutrient uptake in crops. For example, in a greenhouse study, Channabasava et al. (2015a) carried out an experiment using kodo millet to examine the mycorrhizoremediation of different rates of fly ash (2%, 4%, and 6%) with or without *R. fasciculatus* inoculation. The study revealed that the NUE of P, K, Ca, Mg, and sodium (Na) was significantly affected by AM fungus and various levels of fly ash. Application of AM fungus decreased (9–43%) the P-use efficiency of kodo millet when compared to plants treated with fly ash. Nevertheless, the efficiency of other nutrients such as K, Ca, Mg, and Na was improved with AM fungus inoculation than noninoculated plants treated with 4% fly ash. Also, AM-inoculated kodo millet treated with or without 2% fly ash also recorded higher K and Mg-use efficiency than uninoculated plants. Overall, kodo millet plants amended with the highest rate of fly ash in the absence of AM fungi increased the NUE (Channabasava et al. 2015a). Besides NUE, the plant growth, nutrient uptake, and grain yield were increased with AM fungus inoculation in kodo millet-plants with or without fly ash treatments (Channabasava et al. 2015a). The reduced NUE in AM-inoculated plants could be attributed to enhanced nutrient concentration in plants with ample application of nutrients (Koide 1991). The response of NUE to

AM fungi in millets is still obscure due to the lack of studies. Therefore, research should be focused on the application of AM fungi on NUE in millets in the future.

3.6.4 Improved Water Relations

The AM fungi improve water uptake by plants under both stressed and normal conditions. Similar to nutrient uptake, the AM fungal hyphae extend beyond the rhizosphere and transfer water from the soil to plants (Augé 2004). Nevertheless, water uptake through AM fungi has been reported to be low or negligible than plant transpiration demands (Püschel et al. 2020). Therefore, passive water transport is considered to be more significant than active water acquisition by fungal hyphae (Smith et al. 2010). In the passive transport, the AM fungi increase the water uptake by enhancing the soil hydraulic conductivity (Bitterlich et al. 2018). The extent of water distribution through AM fungi reflects both soil hydraulic properties and concentration of soil water, depending upon the nature of soil pores (Zhu et al. 2015). In addition, AM fungi can directly improve water relations by modulating transpiration and photosynthetic rates, promoting root water uptake and leaf water potential (Augé 2004). The water uptake through AM symbiosis are related to plant morphology and the soil environment. The AM fungal hyphae form a dense network by branching dichotomously and these absorbing networks could explore the soil away from the roots (Jansa et al. 2003). These branched fungal hyphae enhance the surface area for absorption and promote plant water uptake over certain distances through which hyphae could capture water from small soil pores that are unreachable to the roots (Püschel et al. 2020). In addition to branched fungal hyphae, runner hyphae could serve as a pathway for the transport of water by potentially extending the plant roots to reach remote inaccessible water resources (Bitterlich and Franken 2016). Under water-deficit conditions, the AM fungi improve the host plant growth by increasing the stomatal conductance, transpiration rates, and production of antioxidant enzymes to counteract oxidative stress. Some of the millets such as pearl millet, finger millet, foxtail millet, and sorghum plants inoculated with AM fungi have shown to mitigate drought stress by increasing the relative water content, chlorophyll, enhanced production of antioxidants, and non-antioxidant enzymes such as proline, catalase (CAT), guiacol peroxidase, and peroxidase (POD) (Fabbrin et al. 2015; Caruso et al. 2018; Thangaraj et al. 2022; Tyagi et al. 2017a, b, 2021).

3.6.5 Mitigation of Heavy Metal Stress

Arbuscular mycorrhizal symbiosis also alleviates heavy metal toxicity in plants. An investigation of the effects of AM fungal (consortium of 10 species belonging to Glomeraceae and Acaulosporaceae) inoculation on pearl millet growth and yield in soils spiked with different concentrations of cadmium (Cd) indicated an increased growth and yield of pearl millet in response to AM fungal inoculation (Mohamad et al. 2011). In addition, AM fungal inoculation also reduced the Cd accumulation in shoots and the bioconcentration factor in pearl millet (Mohamad et al. 2011). Inoculation of foxtail millet with the AM fungus *R. fasciculatus* in soils amended with different proportions of Fe ore mine soils under greenhouse conditions indicated that inoculation of *R. fasciculatus* along with 25% mine spoil amendment enhanced the growth and grain yield of foxtail millet and the soil microbial density than those raised in 50% and 75% of mine spoil amendment (Lakshman and Channabasava 2013).

Similarly, in a greenhouse study inoculation of finger millets with isolates of *Gigaspora*, *Glomus,* and *Scutellospora* in soils spiked with different concentrations of Zn (0–450 mg/kg) showed that the isolate of *Glomus* tolerated the different levels of Zn in the soil and improved the growth of finger millet during various stages of plant growth (Krishna et al. 2013). In a later study (Krishna and Sachan 2017), the same *Glomus* isolate was also shown to tolerate different levels of Cd in the soil and improved finger millet growth in soils spiked with different concentrations of Cd under greenhouse conditions. In a recent study, Shen et al. (2022) examined the influence of AM fungi and soil waste-based soil conditioner on foxtail millet production and the safety of the millet grains under field conditions. The results of the study indicated that AM symbiosis increased the millet yield by 19.5% compared to control and this was further increased to 28% in the presence of a water-based soil conditioner. Besides AM fungal association also reduced the calculated pollution indices and translocation factor to safer levels. The hazard quotient and hazard index for the millet grains were less than one suggesting the absence of any potential health risk for human consumption (Shen et al. 2022). These studies clearly show that AM fungi can reduce the negative influence of heavy metals on millet growth and yield. The AM-mediated heavy metal tolerance in plants involves several direct and indirect mechanisms as recently reviewed by Janeeshma and Puthur (2020) and Riaz et al. (2021).

3.6.6 Changes in Soil Structure

The AM fungi contribute to increased plant productivity by improving soil fertility and inducing beneficial changes for maintaining soil stability and structure. The dense external AM hyphal network forms a three-dimensional matrix that traps and connects soil particles without compaction (Leifheit et al. 2014). The AM fungi influence the soil structure by binding soil particles to external AM hyphae, entangling microaggregates to macroaggregates through fungal hyphae, and supplying carbon sources for microbes and plants (Zhang et al. 2020). The AM fungal hyphae are regarded as primary soil aggregators and a linear relationship is known to exist between AM hyphal length and stability of soil aggregates. Moreover, AM fungi stabilize soil aggregates and enhance the growth of other organisms in the soil by supplying photosynthetically fixed carbon into the rhizosphere (Rillig et al.

2015). The AM fungi may influence soil aggregation by forming skeletal structures to retain the minerals in the soil.

The AM fungi produce an important soil-related glycoprotein known as glomalin which is hydrophobic and serves as a binding agent in soil. Glomalin is usually accumulated in the outer walls of fungal hyphae and nearby soil particles (Rillig 2004). This glycoprotein enhances water-holding capacity, and plant production, maintains soil aggregation; improves soil fertility and soil aeration (Fokom et al. 2013; Rillig et al. 2015). For instance, individual or combined inoculation of *F. mosseae*, *R. fasciculatus*, and *Gigaspora decipiens* in pearl millets raised in barren soil conditions revealed increased plant growth and total glomalin and easily extractable glomalin content with dual inoculation of *F. mosseae* and *R. fasciculatus* when compared to other AM-inoculated and uninoculated treatments (Pal and Pandey 2017). Similarly, in a recent study, Mythili and Ramalakshmi (2022) examined the AM fungal communities and metabolites including total glomalin associated with five minor millets [finger millet, little millet (*Panicum miliare*), foxtail millet, kodo millet, and barnyard millet] collected prior to cultivation (soil type) and during the cultivation of millets (crop type) in three different soil types. The results of the study revealed a greater concentration of glomalin in the sandy loam soils when compared to sandy clay soils and the highest total glomalin content was reported in kodo millet and the lowest in little millet. Also, the glomalin content was linearly correlated to organic matter and spore density in crop type (Mythili and Ramalakshmi 2022). Therefore, AM fungi could enhance soil fertility and improve plant productivity through increased production of glomalin in the soil.

3.6.7 Interactions with Other Microorganisms

The AM fungi are regarded as natural bioinoculants in the agricultural crop production owing to its ability to enhance plant productivity and synergetic interaction with other beneficial soil microorganisms. Interactions between AM fungi and plant growth-promoting microbes increased plant growth and nutrient uptake in millets. The combined inoculation of N-fixing bacteria (*Azospirillium brasilense*), phosphate-solubilizing bacteria (*Bacillus polymyxa*), and AM fungi (*F. mosseae*) increased plant growth, plant height, shoot and root dry weights, and P and N uptake in finger millet when compared to uninoculated, individual, and dual inoculated plants thus exhibiting synergistic effect among these organisms (Ramakrishnan and Bhuvaneswari 2014). Similarly, Dhawi et al. (2018) reported enhanced shoot length, plant biomass, chlorophyll content, and total sugars in foxtail millet inoculated with plant growth-promoting bacteria (*Pseudomonas*), endomycorrhiza (*R. intraradices*, *F. mosseae*, *R. aggregatus* and *C. etunicatum*), and combination of ectoendomycorrhizal species (*Rhizopogon villosullus*, *Rhizopogon luteolus*, *Rhizopogon amylogpogon*, *Rhizopogon fulvigleba*, *Pisolithus tinctorius*, *Scleroderma cepa*, and *Scleroderma citrinum*) when compared to noninoculated plants. In addition, the co-inoculation of plant growth-promoting bacteria with ecto-/

endomycorrhizal species increased 28 metabolites in shoots of foxtail millet (Dhawi et al. 2018). The AM fungi benefit the plants under abiotic stresses through interactive effect with other soil organisms. For example, combined inoculation of *R. intraradices* with *Streptomyces* spp. and *Pseudomonas* spp. in finger millet significantly improved shoot length, root length, shoot and root biomass under both well-watered and water-deficit conditions over uninoculated plants. Further, proline and superoxide dismutase (SOD) contents were increased under water stress in finger millet upon combined inoculation when compared to noninoculated plants (Kamal et al. 2015).

3.7 Growth Improvement in Stressed Environment

Soil salinity and drought are the two important factors that affect crop growth and yield worldwide. Symbiosis with AM fungi is shown to improve plants tolerance to different types of stresses. The role of AM fungi in improving plants tolerance to various abiotic stresses is more pertinent to millets as many of these are grown under rainfed conditions on marginal lands (Table 3.4).

3.7.1 Salinity Stress

Sweet sorghum cultivars (Liaotian5 and Yajin2) inoculated with the AM fungus *A. mellea* and grown under different concentrations of NaCl (0–3 g NaCl/kg soil) exhibited more biomass and nutrient uptake compared to noninoculated plants (Wang et al. 2019). Moreover, *A. mellea* symbiosis increased the soluble sugar concentrations and activities of CAT, POD, and SOD activities in leaves. Among the two cultivars, Yajin2 with low salt tolerance exhibited a higher response to *A. mellea* inoculation than Liaotian5 (Wang et al. 2019). Inoculation of *F. mosseae* and *Funneliformis geosporus* isolated from sodic soils improved the colonization and arbuscular abundance in sorghum in sodic and plant growth parameters like height and fresh and dry biomasses and root/shoot ratio in sodic and saline soils (Chandra et al. 2022). In addition, the uptake of P, tissue nutrient content, and K^+/Na^+ ratio was more in AM fungi-inoculated soils. In addition, AM fungal inoculation also increased the soil P levels, glomalin, and soil enzymes like dehydrogenase and alkaline phosphatase (Chandra et al. 2022).

Although AM fungal symbiosis is generally known to positively influence millet growth under salinity, some field studies also indicate the lack of response of millets to AM fungal inoculation under salinity stress. For instance, field inoculation of proso millet genotypes (Unikum and Kinelskoje) with a commercial AM fungal inoculum consisting of *F. mosseae*, *R. intraradices*, and *Glomus* spp. revealed that though AM fungal inoculation increased fresh biomass yield of proso millet under normal conditions, it failed to influence fresh biomass under salt stress (Caruso et al.

Table 3.4 Effect of arbuscular mycorrhizal (AM) fungi on growth, yield, and physiological characteristics of millets under different abiotic stress conditions

Millet species	Type of study	Conditions	Stress type	AM fungal species	Co-inoculated organism	Enhanced growth/ nutrient/yield parameters	Physiological parameters increased (↑) or decreased (↓)	References
E. coracana (L.) Gaertn.	Greenhouse	Sterilized soil	Drought	*Rhizoglomus intraradices*	*Piriformospora indica*	PH, LN, RL, SFW, RFW, SDW, RDW	↑Chlorophyll content, ↑Relative water content, ↓Electrolytic leakage, ↓MDA ↓H_2O_2, ↑Proline, ↑TSP, ↑ASA, ↑GSH, ↑Total phenol and flavonoid contents; ↑SOD, ↑PPO, ↑CAT, ↑GPX activities	Tyagi et al. (2017a)
E. coracana	Glasshouse	Autoclaved soil	Drought	*R. intraradices*		PH, RL, SFW, RFW, SDW, RDW, P content	↑Chlorophyll content, ↓MDA in roots, ↑ Root ASA, ↑Leaf and root GSH, ↑Root phenol and flavonoid contents	Tyagi et al. (2021)
Pennisetum glaucum (L.) R.Br.	Greenhouse & growth chamber	Sterilized soil	Heat stress	*R. aggregatus, Funneliformis mosseae*		RDW, SDW	↑Chlorophyll content	Ndeko et al. (2022)
P. glaucum	Greenhouse	Sterilized soil	Salinity	*Rhizophagus fasciculatus*		PH, RL, PFW, PDW	↑Chlorophyll content, ↑Proline, ↑CAT, ↑POD, ↑SOD	Borde et al. (2011)

P. glaucum	Greenhouse	Mixture of vermiculite–siliceous sand–light peat	Elevated carbon dioxide and reduced water availability	*R. intraradices*		SFW, SDW, RFW, RDW, LN, TN	↑ Shoot and root RWC; ↓Raffinose, ↓Sucrose, ↓Glucose, Xylose, ↓Fructose, ↓Sorbitol, ↓TSS, ↓Starch, ↑TSP, ↑Proline	Fabbrin et al. (2015)
Panicum miliaceum L.	Field conditions	Field soil	Salinity and sodicity	*F. mosseae*		Grain yield, straw yield and panicle per square meter, P content, K/Na ratio		Ahmadi et al. (2021)
P. miliaceum	Field conditions	Field soil	Salinity and drought	*R. intraradices*, *F. mosseae*, *Glomus* spp.		FBY, SD, LN, LFW, PL, PaFW	↑ Chlorophyll content	Caruso et al. (2018)
Pennisetum spicatum L.	Growth chamber	Sterilized soil	Salinity	*F. mosseae*		PH, LN, SFW, RFW	↑ Chlorophyll content, ↑Proline	Alshoaibi (2021)
S. italica (L.) Beauv.	Greenhouse	Sterilized soil	Drought	*R. intraradices*		PH, RL, CD, PL, single panicle weight, grain weight	↑SOD, ↑CAT, ↑POD, ↑GR, ↓MDA, ↓ H_2O_2	Gong et al. (2015)
Sorghum bicolor (L.) Moench	Greenhouse	Sterilized soil	Drought	*F. mosseae*		Grain yield	↑ SOD, ↑ POD, ↑CAT, ↑PPO, ↑Proline, ↑GSH, ↓MDA, ↓H_2O_2	Thangaraj et al. (2022)

(continued)

Table 3.4 (continued)

Millet species	Type of study	Conditions	Stress type	AM fungal species	Co-inoculated organism	Enhanced growth/ nutrient/yield parameters	Physiological parameters increased (↑) or decreased (↓)	References
S. bicolor	Field conditions	Field soil	Drought	*F. mosseae*	*Azospirillum*, *Azotobacter*	Grain yield, PL, number of panicles per plant, grain number per panicle, 1000-grain weight	↑Chlorophyll content, ↑TSP, ↑WUE, ↑RWC, ↑N content, ↑Proline, ↓Electrolyte leakage, ↓Water saturation deficit	Kamali and Mehraban (2020)

ASA ascorbate, *CAT* catalase, *CD* collar diameter, *FBY* fresh biomass yield, *GPX* guiacol peroxidase, *GR* glutathione reductase, *GSH* glutathione, H_2O_2 hydrogen peroxide, *LFW* leaf fresh weight, *LN* leaf number, *MDA* malondialdehyde, *N* nitrogen, *P* phosphorous, *PaFW* panicle fresh weight, *PDW* plant dry weight, *PFW* plant fresh weight, *PH* plant height, *PL* panicle length, *POD* peroxidase, *PPO* polyphenol oxidase, *RDW* root dry weight, *RFW* root fresh weight, *RL* root length, *RWC* relative water content, *SD* shoot density, *SDW* shoot dry weight, *SFW* shoot fresh weight, *SOD* superoxide dismutase, *TN* tiller number, *TSP* total soluble protein, *TSS* total soluble sugars, *WUE* water-use efficiency

2018). These studies along with those summarized in the Table 3.4 clearly show that AM fungi can play an important role in the growth of millets in salt-affected soil. Nevertheless, more field studies are necessary to determine if these works under field conditions as crops growing in fields are already colonized by indigenous AM fungi.

3.7.2 Drought Stress

Inoculation of forage sorghum with *F. mosseae* had improved plant growth and accumulated more dry matter and more specific leaf area and life span under simulated drought conditions (Sun et al. 2017). Foxtail millet inoculated with *R. intraradices* and subjected to water stress (-0.68 MPa) under greenhouse conditions were taller, and had greater collar diameter, panicle height, panicle weight, and grain weight (Gong et al. 2015). In addition, the SOD, CAT, POD, and glutathione reductase (GR) activities of mycorrhizal foxtail millet plants were higher. Moreover, the mycorrhization of foxtail millet also reduced the concentrations of H_2O_2, O_2^-, and malondialdehyde (MDA), when compared with non-mycorrhizal foxtail millet under both well-watered and water-stressed conditions. The results demonstrated that *R. intraradices*-inoculated foxtail millet showed higher drought tolerance by improving their growth and productivity, reactive oxygen species (ROS), and antioxidant enzyme activities when compared with non-mycorrhizal foxtail millet plants under drought stress (Gong et al. 2015).

Symbiosis with *R. intraradices* increased the drought tolerance in finger millet (Tyagi et al. 2021). The positive influence of *R. intraradices* on finger millet under severe drought stress was exemplified by increased plant growth, P uptake, and chlorophyll content in leaves. Moreover, mycorrhizal finger millet plants under drought stress had increased levels of osmolytes like soluble sugars and proline. The leaves of finger millet accumulated more phenols, and the root flavonoid content of mycorrhizal plants was 16% more than non-AM plants. Contrarily, the leaf lipid peroxidation of AM finger millet seedlings was 29% less compared to non-mycorrhizal seedlings. The leaf ascorbate level was 25% higher and the glutathione (GSH) levels increased to a maximum of 182% in AM plants under severe stress (Tyagi et al. 2021). The influence of AM fungi-improving plant growth and stress tolerance under drought conditions was reported to be almost similar or superior to other plant growth-promoting endophytic fungi. For example, in a comparative study, Tyagi et al. (2017a) showed that finger millet plants inoculated with either *R. intraradices* or *Piriformospora indica* exhibited better drought tolerance through increased chlorophyll content, enriched osmoregulation activity, and strong antioxidant defense system. However, *R. intraradices* colonized finger millet plants accumulated more biomass, total soluble sugars, phenols, and proline compared to plants colonized by *P. indica*. Moreover, *R. intraradices* effectively reduced the electrolyte leakages as well as the MDA and H_2O_2 content in finger millet plants than *P. indica* under drought stress.

In addition to the direct influence of AM fungi on millet growth under drought stress conditions, millets also benefit from AM symbiosis indirectly. In a compartmented microcosm study, the uptake of deuterium-enriched water by pigeon pea [*Cajanus cajan* (L.) Millsp.] plants from the deeper profile of the soil was shown to be transferred to roots of finger millet lying in the drier profile of the soil through the common mycelial network (CMN) between pigeon pea and finger millet formed by the AM fungi comprising of *R. irregularis* (strain BEG-75) or *R. fasciculatus* and *Ambispora leptoticha* (Singh et al. 2019). A subsequent study (Singh et al. 2020) also revealed that pigeon pea can promote water relations of finger millet during drought. The improved water relations in finger millet through CMN resulted in maintenance of the stomatal conductance; reduce foliar damage and subsequently the mortality of finger millet plants under drought. Nevertheless, pigeon pea exerted strong competition with finger millet when connected by the CMN before the onset of drought. The results suggest that the role of CMN in plant benefit in an intercropping system involving CMN can vary under sufficient moisture and drought conditions (Singh et al. 2020). These studies do indicate that AM fungal symbiosis is a potential method for improving the performance of millets under drought conditions and AM fungi modulate the drought tolerance in millets through improved photosynthetic efficiency, enhanced osmoregulation, and an efficient antioxidant system (Fig. 3.4).

3.8 Millets in AM Fungal Inoculum Production

Millets like sorghum and finger millet are often used as the host for trapping AM fungi. For example, sorghum when used as the host plant in trap culture increased the AM fungal propagules by 3.43 folds (222 spores to 761 spores/100 g trap medium) to 30.31 folds (13 spores to 394 spores/100 g trap medium) (Husein et al. 2022). The AM fungal hyphal density was 61.8 ± 1.7 cm g^{-1} dry substrate with a spore number of 1149 ± 41 kg^{-1} dry substrates. Further, sorghum aided the sporulation of eight AM fungal taxa in the rhizosphere (Husein et al. 2022). Similarly, sorghum was able to support the sporulation of 86% of the 14 AM fugal taxa present in the rhizosphere of maize. Of the different species of AM fungi that sporulated with sorghum as host plant, *Funneliformis geosporum*, *Rhizoglomus microaggregatum*, and *C. claroideum* constituted 71% of the total spore numbers (Carrenho et al. 2002). Harinikumar and Bagyaraj (1988) found that the production of infective propagules and spores of AM fungi in finger millet was next to that of groundnut (*Arachis hypogea* L.).

On-farm production of inoculum of *F. mosseae* using finger millet for fruit crops was developed by Mohandas et al. (2002). Raising finger millet in formaldehyde pre-fumigated soil resulted in an inoculum with a spore count of 40–70 spores/g soil. Likewise, solarization of soil before growing finger millet resulted in spore numbers of 50–60 spores/g soil. These methods resulted in the production of around 4 tons of AM fungal inoculum in an area of 25 m^2 in 12 weeks (Mohandas et al. 2002). The cost of inoculum production in India was calculated as Rs. 3/kg using soil and as

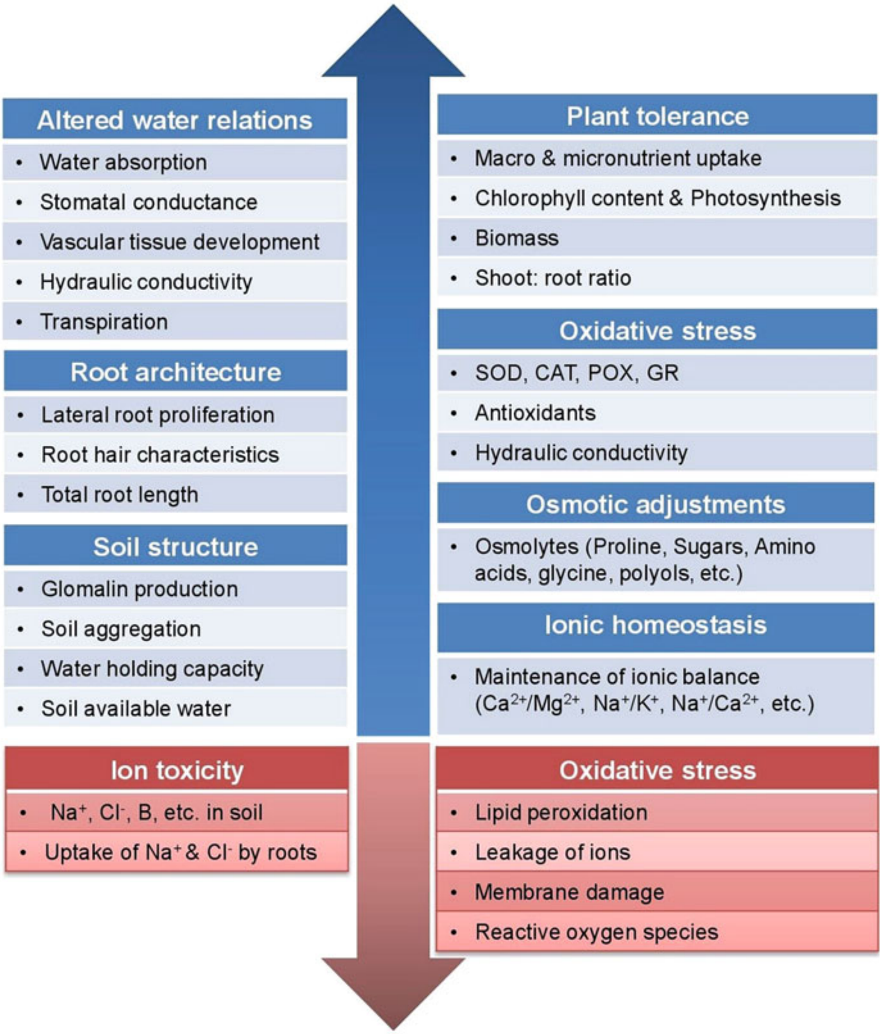

Fig. 3.4 Mechanisms involved in arbuscular mycorrhizal symbiosis-mediated alleviation of salt and drought stress in plants. Blue arrow is indicative of the plant, biochemical, and soil parameters that are increased and those indicated by brick red arrow that is decreased. *SOD* superoxide dismutase, *CAT* catalase, *POX* peroxidase, *GR* glutathione reductase, Ca^{2+} calcium, Mg^{2+} magnesium, Na^+ sodium, K^+ potassium, Cl^- chlorine, *B* boron

Rs. 15/kg when using soilrite: cocopeat mixture (https://www.iihr.res.in/large-scale-production-vesicular-arbuscular-mycorrhizal-fungi-finger-millet). Alley cropping of finger millet increased the infective propagules of AM fungi in the soil by 3.8–4.0 folds compared to pigeon pea and peanut (Balakrishna et al. 2017). The fibrous and elaborate root systems of millets make them attractive hosts to bait out AM fungi and large-scale production of AM fungal inoculum.

3.9 Factors Affecting AM Symbiosis

3.9.1 Fungicides

Like other field crops, millets are also affected by a wide range of pathogens causing considerable yield loss. For instance, the finger blast disease caused by *Magnaporthe oryzae* is widespread in finger millet growing areas and is one of the most devastating diseases as it affects the crop during all the growth stages and can reduce the grain yield upto 100% (Mbinda and Masaki 2021). A large number of fungicides are used to control the various pathogens that attack the millets. These fungicides in addition to controlling the desired mycopathogens can also affect nontarget fungi like the AM fungi. Though the role of biocides especially fungicides on soil microflora is well reported, studies on the effect of fungicides on AM symbiosis in millets are very limited (Rejali et al. 2022). Channabasava et al. (2015b) examined the influence of two systemic fungicides (Bavistin and Benomyl) and two nonsystemic fungicides (Captan and Mancozeb) on *R. fasciculatus*-proso millet symbiosis under greenhouse conditions. Application of recommended levels of Captan enhanced the AM fungal colonization of roots, AM spore numbers, growth, and yield parameters in proso millet compared to other fungicides. Contrarily, Benomyl had a significant negative influence on all the AM, plant growth, and yield parameters, and the influence of Bavistin and Mancozeb in proso millet was variable (Channabasava et al. 2015b).

An examination of soil application of systemic fungicide Bavistin and nonsystemic fungicides Copperthom, Cuman, and Sulfex at the prescribed levels indicated an increased AM colonization in finger millet by Bavistin and Copperthom and Cuman in kodo millet and proso millet, respectively, at all stages of plant growth (Udaiyan et al. 1999). Nevertheless, the influence of fungicides on AM fungal spore numbers in the soil varied with fungicides and plant growth stages. For example, Bavistin increased AM fungal spore numbers in finger millet 90 days after seed emergence, such a response in proso millet occurred in Cuman-drenched soils and for kodo millet in Copperthom- and Sulfex-applied soils (Udaiyan et al. 1999). These studies suggest that the influence of fungicides on AM symbiosis in millets could vary with millet species, type of fungicides, and the growth stage of the crop species.

3.9.2 Tillage

Tillage is one of the agronomic practices that are used in the preparation of the field for crop production. Although various types of benefits are ascribed to different types of tillage, it could also affect biological carbon sequestration and greenhouse gas production (Mehra et al. 2018). Studies have shown that efficient tillage along with other agronomic management can increase the growth and yield of millets like pearl millet and finger millet (Sankar et al. 2012; Sidar et al. 2017). However, soil tillage has been shown to negatively affect the AM fungal propagules present in the soil resulting in reduced mycorrhization of roots and early uptake of P by plants (Wilkes et al. 2021). The negative influence of tillage on AM symbiosis was attributed to the disturbance of the extraradical network of AM fungal hyphae in the soil. Another impact of tillage on AM symbiosis is the changes in the composition of AM fungal communities. For example, long-term uses of tillage like mouldboard-plowing have been shown to reduce the percentage of taxa belonging to *Glomus* and *Septoglomus* in the AM fungal community (Lu et al. 2018). In contrast, zero or no tillage conserves the diversity and richness of AM fungal communities and the efficient functioning of the symbiosis (Wilkes et al. 2021). In a study examining the impact of tillage on the diversity of AM fungi associated with certain subtropical crop species, Alguacil et al. (2008) showed that the different types of tillage reduced root colonization by native AM fungi in sorghum by 25%, and the AM fungal taxa colonizing the roots. The number of restriction fragment length polymorphism (RFLP) clones from sorghum roots were 3-folds lower under tillage compared to no-till condition (Alguacil et al. 2008).

3.9.3 Fertilizers

Inorganic fertilizer amendment is inevitable in modern agriculture, which involves plant varieties or cultivars that are bred for quick growth and higher yields. Although millets are often cultivated in marginal land with minimum inputs still inorganic fertilizers are applied for optimum growth and yield of millets (Patil et al. 2015; Juhaeti et al. 2021). Some recent studies have shown inoculation of AM fungi and other growth-promoting microorganisms can reduce the fertilizer need for millets. A field study spanning over two growing in two different sites (Kolli Hills and Bangalore) in India indicated that inoculation of AM fungi *R. fasciculatus* and *A. leptoticha* along with plant growth-promoting bacterium *Pseudomonas* sp. at three recommended levels of mineral fertilization (0%, 50%, or 100% recommended dose of fertilizer) increased grain yields of finger millets when inoculated with both AM fungi and plant growth-promoting rhizobacteria at 50% recommended dose of fertilizer (Mathimaran et al. 2020).

In general, the application of inorganic fertilizers especially phosphatic fertilizers to soils is shown to negatively influence AM symbiosis and mycorrhizal dependency of crop species. Contrarily, the application of phosphatic fertilizers to soils that are deficient in P has shown to positively influence AM symbiosis and plant benefits. Inoculation of pearl millet and sorghum with *F. mosseae* with recommended levels of P in the form of monopotassium phosphate to P-deficient soil did not negatively influence mycorrhization of the millets (Bagayoko et al. 2000). Moreover, the AM-mediated plant growth estimated as the dry mass in pearl millet and sorghum was strongly dependent on P application as *F. mosseae* inoculation failed to increase plant dry weight in the absence of P application. However, in the presence of P application, the shoot and root dry weights of *F. mosseae*-inoculated millets were increased by 7–8 folds (Bagayoko et al. 2000).

3.9.4 Crop Rotation

Cultivating different crops in succession brings about different economic and environmental benefits and also plays an important role in the long-term management of agricultural soils. Nevertheless, crop rotation can have a significant influence on AM fungal symbiosis. This stems from the fact that different crop species differ in their dependence on mycorrhizal symbiosis and their ability to affect the density and diversity of AM fungal propagules in the soil (Castillo et al. 2006; Berruti et al. 2018). Cultivation of non-mycorrhizal hosts like mustard (*Brassica juncea* L.) after finger millet reduced the propagules of AM fungi by 13% affecting the mycorrhization and inoculum buildup by the succeeding crop cowpea [*Vigna unguiculata* (L.) Walp.] (Harinikumar and Bagyaraj 1988).

Cultivation of pearl millet after maize or soybean increased the abundance of indigenous AM fungal spores when compared to other crops like pigeon pea, sunn hemp (*Crotalaria juncea* L.), and Brachiaria (*Brachiaria ruziziensis* Germ. & C.M. Evrard) despite the low AM colonization in roots compared to these plant species (Arruda et al. 2021). Moreover, pearl millet depleted the soil labile P pool minimally when compared to other rotational crop species. This is evidenced by the reduced uptake of nutrients like N, P, and K (Arruda et al. 2021). In another study, cultivation of pearl millet as a winter crop in the soybean-corn summer crop rotation system increased the extraradical soil hyphae of AM fungi by 2–110% compared to other winter crops like oilseed radish (*Raphanus sativus* L.), sorghum, sunn hemp, sunflower (*Helianthus annuus* L.), and pigeon pea (Moitinho et al. 2020). Similarly, sorghum when grown as a winter crop in the same summer crop rotation system increased the number of AM fungal spores by 29–140%. In addition to AM fungal variables, cultivation of pearl millet as a winter crop also increased the total glomalin content of the soil by 3–45% over other winter crops and the extractable glomalin content of the pearl millet soil was similar to those that were under pigeon pea or sunn hemp. In addition, the index for soil aggregate stability and aggregate mean weight diameter was at the maximum for soils under sorghum compared to other winter crops (Moitinho et al. 2020). This suggests that millets in crop rotation affect soil structure compared to positively influencing AM fungal variables.

3.9.5 Intercropping

Intercropping refers to the cultivation of two or more species concurrently in the same field during a cropping season. This cultivation technique results in the more effective use of the resources in addition to improving agricultural productivity, especially in marginal lands (Brooker et al. 2015). In the intercropping system, cereals are normally cropped along with legumes. Experimental studies have shown that the CMN established between the roots of different crop species enables the exchange of resources among them. Pigeon pea plants when grown in the deep compartment with moisture availability at the bottom were shown to uptake N and P and transfer it to the finger millet plants growing in the shallow compartment when connected by a CMN formed by *R. irregularis* (Saharan et al. 2018). A field study conducted over three successive cropping seasons at two different sites in India indicated that bioinoculation of AM fungi *R. fasciculatus* and *A. leptoticha* along with growth-promoting bacteria *Pseudomonas* either individually or in combinations suggested that the effect of bioinoculation was more conspicuous in low-fertility site and dual inoculation of AM fungi and plant growth-promoting bacteria increased the crop yields upto 128% than their inoculations (Mathimaran et al. 2020). Moreover, raising pigeon pea during the preseason in polybags and transplanting them onto the field further increased the grain yield response to bioinoculation across sites upto 267% compared to direct sowing of pigeon pea (Mathimaran et al. 2020). In a recent study, Schütz et al. (2022) showed the rate of spread of hyphae in a simulated finger millet–pigeon pea intercropping system was 4.1 mm/day for the AM fungus *C. etunicatum.*

Intercropping of deep-rooted legumes also benefits the shallow-rooted cereals in the intercropping system during soil water limiting conditions through altered water relations facilitated by AM common mycelial networks. A controlled study performed under greenhouse conditions showed that the deep-rooted pigeon pea can act as bioirrigators for the shallow-rooted finger millet and this phenomenon was more pronounced when the plants were connected by a CMN (Singh et al. 2020). In addition to effective utilization of the resources in an intercropping system, the sharing of resources facilitated by AM fungi strongly depends on the availability of these resources.

3.10 Conclusions and Future Considerations

In conclusion, information available so far indicates that millets are naturally colonized by diverse AM fungi that play an important role in the health and development of millets. Most of the studies examining the role of AM fungi on millet growth are performed under controlled conditions involving selected species of AM fungi. Field studies on AM fungi-mediated growth response in millets are meager when compared to other cereal crops. Similarly, the yield response of millets to AM fungal inoculation both under controlled and field conditions is very limited. More studies are needed to assess and understand the factors that influence AM fungal communities associated with millets. The diversity of endophytic fungi colonizing the roots of cultivated and wild millet cultivars is shown to differ substantially. It is therefore important to assess if such changes also occur for AM fungal communities colonizing millet roots. As millets are frequently cultivated on marginal soils under rainfed conditions of the tropics, more field studies involving AM fungi are needed to evolve strategies to exploit the symbiosis to optimize the yield of millets under resource-limiting conditions. This would substantially improve the economy of resource-poor farmers cultivating these grains. Millets like other cereal crops are infected by several pathogens, but the role of AM fungi in controlling diseases in millets is not adequately examined. The obligate nature of AM fungi is an important bottleneck in the large-scale production of AM inoculum and a major limitation in the widespread use of AM fungi in agriculture. Now there is ample evidence to show that millets could increase the propagules of AM fungi in a short period compared to other plant species that are routinely used in AM inoculum production. Therefore, the development and validation of protocols for large-scale production of AM inoculum are necessary for its common use in agriculture. Future research should focus on the critical assessment of the interaction of AM fungi with other soil microorganisms that are likely to play a pivotal role in nutrient availability and millet fitness. This will contribute greatly to the process of soil microbiome engineering for millets when grown under resource-limiting conditions.

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Chapter 4
Drought-Tolerant Plant Growth-Promoting Rhizobacteria Associated with Millets

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Abstract Millets are drought-resistant, low-maintenance crops, and are a perfect fit for multiple cropping systems under irrigated and dryland farming. Drought resilience in millets is partially attributed to the plant's ability to selectively encourage the growth of drought-adaptive, multifarious plant growth-promoting rhizobacteria (PGPR). Beneficial PGPR plays a key role in aiding millet's growth under water-limited stress conditions as well as protects the plants from various drought-associated biotic and other abiotic stresses. As a component of the plant-soil feedback mechanism, millets actively restructure the rhizosphere microbial assemblages, and their functions through modulations in the composition, and concentration of the root exudates. Therefore, microbiome engineering poses an interesting avenue for formulating a productive abiotic stress management system for crops cultivated under drought-related stress environments. Our current understanding of the complex crosstalk between root-associated microbes and crops are grown under drought is largely drawn from non-millet plants. Limited resources and studies have revealed that effective PGPR employs fundamental mechanisms of drought stress alleviation that include the regulation of phytohormones (auxin and cytokinin), solubilization of phosphate, production of ACC deaminase to lower ethylene level, accumulation of compatible solutes, etc. In this chapter, we discussed how millets exposed to water-limited conditions influence the rhizosphere microbial colonization as a component of the cry-for-help strategy. Additionally, we highlighted the role of

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_4

beneficial PGPR and their core strategies of drought amelioration in various millet crops. We propose that future research efforts should attempt to elucidate the interactions of PGPR strains with the host plant in field conditions and to gain insights into PGPR-induced molecular and metabolic switches in millet crops.

Keywords Millet · Drought · Rhizosphere · Microbiome · Stress alleviation

4.1 Introduction

Millets are a group of diverse small-seeded cereals that include pearl millet [*Pennisetum glaucum* (L.) R. Br.], finger millet [*Eleusine coracana* (L.) Gaertn], foxtail millet [*Setariaitalica* (L.) P. Beauvois], Japanese barnyard millet [*Echinochloa esculenta* (A. Braun) H. Scholz], Indian barnyard millet [*Echinochloa frumentacea* Link], kodo millet [*Paspalum scrobiculatum* L.], little millet [*Panicum sumatrense* Roth ex Roem. & Schult.], and proso millet [*Panicum miliaceum* L.] (Bora et al. 2019). In addition, tef [*Eragrostis tef* (Zucc.) Trotter] and fonio or acha [*Digitaria exilis* (Kippist) Stapf and *D. iburua* Stapf] are grouped under small millets (Maitra et al. 2022; Tadele 2016). Around 97% of the millets are grown in developing countries, especially in the semiarid tropics of Asia and Africa (Thilakarathna and Raizada 2015). Owing to a short growing period (about 110 days) and highly adaptive nature, millets are cultivated in widely diverse and challenging terrains such as dry, high-temperature regions, low-fertile land, rain-fed, and mountainous areas. Millets also perfectly fit into multiple cropping systems under irrigation and dryland farming (Kapoor et al. 2022).

Millets are popularly called “nutri-cereals”‘due to their high nutritional properties including protein (7–12%) with a balanced amino acid profile, dietary fiber (15–20%), essential fatty acids, B vitamins, and various minerals such as calcium, iron, zinc, potassium, and magnesium (Saini et al. 2021). The grains are also rich in multiple health-promoting phytochemicals such as phytoestrogens, polyphenols, phytosterols, lignans, and phycocyanins (Shah et al. 2021). These phytochemicals serve as antioxidants, immune modulators, and detoxifying agents for which a millet-based diet is recommended for patients with age-related degenerative diseases, diabetes, cardiovascular diseases (CVD), cancer, etc. Millet-based products have a relatively low glycemic index and glycemic load, and are safe for people suffering from a gluten allergy and celiac disease (Amadou 2022). The nutritional and gastronomic features empower millets to hold a unique position in the global food security scenario for the people inhabiting densely populated regions, and marginal and underlying residential communities. Covering about 17 million ha ectares of cultivation area, and an annual production of 18 million tons, millets contribute 10% of the total dietary intake in India. The country has witnessed an increase in millet production from 14.52 million tons in 2015–2016 to 17.96 million tons in 2020–2021 (Meena et al. 2021).

The choices for millet cultivation largely depend on the geographical and climatic conditions (especially, precipitation). Sorghum is preferred in areas with an annual rainfall of 400 mm and above, while pearl millet is cultivated in areas with an annual rainfall of 350 mm. Small millets like finger millet, foxtail millet, proso millet, etc. thrive in areas with an annual rainfall below 350 mm. Arid regions experience less than 25 cm of annual precipitation while semiarid regions receive 25–50 cm of precipitation every year (Chaturvedi et al. 2022).

4.2 Drought Adaptations in Millets

Drought is a major abiotic stress affecting crop growth and yield worldwide. It affects morphological characteristics as well as physiological and biochemical processes in plants. Plants under drought stress undergo reduced leaf water potential, stomatal anomaly, cell proliferation, and enlargement. In addition, drought also limits photosynthesis, nutrient and mineral uptake, respiration, and energy budget (Zia et al. 2021). However, despite being sedentary, plants have developed resilience through complex biochemical and physiological strategies to overcome drought stress (Fang and Xiong 2015). The fact that most of the millets in India are grown in water-deficit areas testifies the crop to be the perfect example of drought-resilient crops. Crops employ four core adaptation strategies for tackling drought stress which include (a) escaping drought by reaching maturity before the onset of adverse conditions whereby traits such as rapid growth, early flowering, high leaf nitrogen, and photosynthesis ability help the crop; (b) avoiding drought by maintaining water balance in plant tissues either by reducing transpiration water loss or through high root-shoot ratio; (c) tolerating drought-related low water potential by producing osmoprotectant; and (d) recovering from drought by physiological adjustments for yield and biomass (Shanker et al. 2014; Tyagi and Pudake 2017). In millets, traits related to agronomy (tiller number, panicle size, seed biomass, and yield), morphology (root and shoot length and leaf area), physiology (osmotic adjustment), and biochemistry [antioxidants for scavenging the reactive oxygen species (ROS)] play major roles in drought tolerance. A study conducted in West Africa has revealed that pearl millet primes its panicle development, and flower initiation to coincide with the increasing period of precipitation (Sanon et al. 2014). The drought-tolerant genotypes attain high yield and biomass by increasing their shoot length and leaf tensile strength and decreasing the root length. These genotypes overexpress ROS scavenging enzymes and accumulate other antioxidants to cope with the stress-induced ROS. In addition, a high accumulation of free proline, glycine betaine, and superoxide help to achieve osmotolerance under water-deficit conditions (Tadele 2016).

4.3 Millets Modulate Root Exudate Composition in Response to Drought

Plant root exudate is typically composed of a wide range of metabolites such as mucilages, carbohydrates, tannins, organic acids, vitamins, phenolic acids, terpenoids, steroids, etc. Various factors encompassing the plant genotype, age, soil, and environmental conditions, etc. determine the composition and quantity of exudates (Canarini et al. 2019). Recent studies suggest that the plant can directly influence the composition, and concentration of the compounds in the root exudates. These compounds not only alter the immediate environment but also mediate the multipartite crosstalk between the plant and microbes in the rhizosphere (Ulbrich et al. 2022). Metabolome profiling of drought-sensitive (genotype 843-22B) and drought-tolerant (ICTP8203) pearl millet has revealed that drought stress significantly alters the root exudate composition (especially, phenolics, lignans, and flavonoids) in the contrasting genotypes (Ghatak et al. 2022). Phenolics and flavonoids scavenge the ROS produced under drought stress, thereby playing a direct role in drought tolerance (Brunetti et al. 2013). The antimicrobial activity of flavonoids also inhibits the growth of soilborne pathogens consequently lowering the risk of plant infection under water-limited conditions (Gutiérrez-Venegas et al. 2019). High quantities of citrate and riboflavin expressed by both pearl millet genotypes, that is, 843-22B and ICTP8203 bear signature to such preferential modulation by the plant under a dry environment. In addition, the exudates also contained elevated levels of various organic acids (citric acid, fumaric acid, lactic acid, oxalic acid, malonic acid, and succinic acid) under drought conditions indicating the possibility of plants selectively favoring the colonization of beneficial microorganisms under stress condition (Ghatak et al. 2022). The accumulation of these organic acids also facilitates the drought-stressed plants in osmotic adjustment as well as in repairing the plant root damages caused by the ROS. A study on a PGPR strain, *Bacillus amyloliquefaciens* NJN-6, has reported that the chemotactic response, biofilm production, and successful colonization of the bacterium in banana rhizosphere largely depended on the abundance of organic acid (especially, malic acid) present in the root exudates (Yuan et al. 2015). Later, another study evaluated the biofilm formation ability of a multispecies microbial consortium in response to root exudate of the model plant *Brachypodium distachyon.* This study reported that the plant under polyethylene glycol (PEG)-induced drought influenced the chemotactic behavior of the consortium through modulation in the organic acid (citric acid) present in the root exudate. Increased production of alginate (160% more compared to a stress-free environment) by the consortium could be linked to the compound's role as an adhesive for biofilm formation essential for establishing the "multicellular" system (Saleh et al. 2020). Overall, these studies reinforced the fact that plants under stress conditions can selectively allow the colonization and growth of highly qualified PGPR as a part of their "cry-for-help" strategy.

4.4 Drought-Responsive Root Exudate Composition Influences Millet Microbial Assemblages

Modulations in the root exudate composition favor the growth of a specific group of microorganisms (core microbiota) to the plant's own advantage. For example, organic acids, amino acids, and sugars present in the root exudates function as chemotactic signals for the colonization of beneficial microorganisms (rhizobia and mycorrhizal fungi) (Yuan et al. 2015). Such microbial assemblages influence plant morphology (above- and belowground biomass, root architecture) and plant phenology (flowering initiation), thus indicating their immense potential in determining plant community dynamics and environmental responses (Jeyanthi and Kanimozhi 2018). This response can be well corroborated with the millet selectively recruiting bacteria that modulate soil edaphic factors and enzymes toward accelerated soil maturation and high crop yield (Xu et al. 2019). However, the driving factors for microbial assemblages, composition, and structure in millets grown under droughts still remain elusive to date. Further, omics-based studies on the relationship of rhizosphere microbiome with the plant and drought stress bear significant importance for elucidating their role and responsiveness in abiotic stress alleviation. Toward this, a metagenomics study of the kodo millet rhizosphere has reported the dominance of Actinobacteria (22.76% relative abundance) under drought (Prabha et al. 2018). Exploring the relationship of drought severity and drought localization with that of root architecture, a series of experiments in four millet species revealed that plants can recruit Actinobacteria as a part of the "cry-for-help" strategy. The study also demonstrated that the Actinobacteria enrichment was limited to the root portions experiencing the drought. Overall, this study was successful in showing that Actinobacteria enrichment depends on drought localization rather than on the root developmental stage (Simmons et al. 2020). Another study conducted on nine C3, nine C4 grass, and tomato germplasm under drought too detected a significant enrichment of Actinobacteria in the roots compared to the bulk soils (Naylor et al. 2017). Actinobacteria are known to exhibit multi-trait plant growth-promoting activities as well as antimicrobial properties through the production of an array of antibiotics (Franco-Correa and Chavarro-Anzola 2016). Therefore, their dominance in water-limited conditions is deemed to protect the plant against drought-associated biotic stresses. Functional analysis of the metagenomes revealed several multifunctional traits related to carbon and nitrogen fixation, secondary metabolite secretion, phosphorus solubilization, and defense mechanisms. These traits help the plant to survive and grow in nutrient-deprived, dry, and challenging environments (Prabha et al. 2019).

4.5 Plant Growth-Promoting Rhizobacteria (PGPR): Drought-Stress Alleviation in Plants

The rhizosphere, the immediate zone surrounding the plant roots, acts as a hot spot for microbial diversity and is a dynamic interface of complex plant-microbe interaction. Multiple studies have reported that rhizosphere microorganisms significantly influence seed germination, plant vigor, growth and development, nutrition provisioning, and disease resistance (Mendes et al. 2013). Bacteria in a particular soil sample/rhizosphere may affect plants in one of three ways: beneficial, commensal, and detrimental (Haney et al. 2015). Rhizosphere microorganisms that are well-known for their beneficial effects on plant growth and health are the nitrogen-fixing bacteria, plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, mycoparasitic fungi, biocontrol microorganisms, and protozoa (Mendes et al. 2013). Few agriculturally important rhizobacteria belonging to the genera viz, *Acinetobacter, Agromyces, Entotheonella, Lysobacter, Pontibacter,* etc., have been reported from the millet rhizosphere (Rokhbakhsh-Zamin et al. 2011; Xu et al. 2019). The plant growth-promoting activities of the PGPR mainly involve direct and indirect mechanisms. The production of ACC deaminase, phytohormones (auxin, gibberellin, and cytokinin), N_2-fixation, mineral (P and Zn) solubilization, and siderophore activity comes under the ambit of direct mechanism; while indirect mechanism encompasses multifaceted functions such as evoking the plant defense mechanism (induced systemic resistance), competing for soilborne pathogens for nutrients and space, and biocontrol activities (antibiotics, HCN, and lytic enzyme) (Glick 2012). Promising PGPR isolates may exhibit multi-trait activities under a wide range of environmental and soil (pH, acidity, alkalinity, temperature, etc.) conditions.

4.5.1 Phytohormone Regulation

The production of phytohormones is one of the potential mechanisms by which the PGPR helps the host plant in ameliorating drought stress. Phytohormones like indole-3-acetic acid (auxin), gibberellins, cytokinin, etc. are regularly synthesized as secondary metabolites by the PGPR and released to the rhizosphere (Maheshwari et al. 2015). Rhizobacterial IAA contributes to the plant auxin pool and stimulates root growth, root surface area, and a higher magnitude of exudate production (Coy et al. 2014). IAA also affects root cell division, rate of xylem differentiation, root extension, and overall root architecture leading to improved water and mineral uptake efficiency in plant roots. In this process, tryptophan present in the root exudate functions as a precursor for rhizobacterial IAA production (Kravchenko et al. 2004). Previously, many drought-tolerant rhizobacteria such as *Bacillus altitudinis* (Kumaravel et al. 2018), *Pseudomonas simiae*, and *P. koreensis* (Kumari et al. 2016), *Ochrobactrum pseudogrignonense*, and *Bacillus subtilis* (Saikia et al. 2018) have been reported from various non-millet plants. A study conducted on microbe-mediated drought tolerance in great millet (*Sorghum bicolour* L.) has reported that inoculation of the plants with PGPR strains *Streptomyces laurentii* EU-LWT3-69 and *Penicillium* sp. strain EU-DSF-10 (both were IAA producers) significantly enhanced osmolyte production and chlorophyll (a and b) contents (Kour et al. 2020).

Cytokinins (CKs) are produced in the root tips and translocated to the shoot through the xylem. The phytohormone not only controls cell differentiation in plant meristematic tissues, but also mediates plant tolerance to drought stress (Chang et al. 2019). A study on the expression of cytokinin oxidase/dehydrogenase (CKX) members in germinating embryos of foxtail millet (*Setaria italica*) has revealed that the CKX gene family members were overexpressed under drought stress treatment indicating the role of CKs in drought tolerance (Wang et al. 2014). Multiple studies have reported the expression of CKX genes and the production of CKs in several PGPR (Akhtar et al. 2020). Their treatment of the plant seedlings can largely contribute to the plant's phytohormone pool as evident from a plant-microbe interaction study involving lettuce plants inoculated with *Bacillus subtilis* (Arkhipova et al. 2005). In one particular instance, a genetically engineered strain of *Sinorhizobium meliloti* that overproduces cytokinin was tested to protect alfalfa plants against the senescence resulting from drought stress. The transformed bacterium production of cytokinin was approximately five times more than the production by the wild type (Xu et al. 2012). After extreme drought stress, there was a tremendous increase in the size of the alfalfa plants inoculated with the transformed strain compared to those inoculated with the non-transformed strain. This experiment indicates that rhizobial strains synthesizing higher than normal levels of cytokinin are able to improve the drought tolerance of alfalfa. Genomics and metagenomics analyses of two PGPR strains viz, *Paraburkholderia tropica* strain IAC/BECa 135 and *Herbaspirillum frisingense* strain IAC/BECa 152 revealed zeatin biosynthesis (cytokinin) as one of the putative mechanisms behind growth-promoting activities in sorghum plants grown under phosphate-deficient conditions (Kuramae et al. 2020).

The presence of ethylene hormone in all higher plants testifies to its importance in the modulation of cell development, and growth as well as in helping plants to combat various stress levels (Binder 2020). Insufficiency of water triggers the accumulation of ethylene in plants. Its production above the threshold level in plant tissues causes "stress ethylene," which worsens the effects of the original stress. Higher levels of ethylene inhibit root and shoot growth leading to various physiological and biochemical disorders (Ferrante and Francini 2006). In this regard, bacterial colonization that expresses ACC deaminase and lowers plant ACC (precursor of ethylene) holds promises for decreasing the detrimental effect of "stress ethylene" on plant physiology (del Carmen Orozco-Mosqueda et al. 2020). A study with ACC deaminase producer, *Streptomyces laurentii* has shown such promising scopes of drought stress alleviation in the great millet (Kour et al. 2020). The PGPR utilizes tryptophan present in the root exudate as a substrate for IAA production. The IAA secreted in the rhizosphere adds to the plant's auxin pool contributing to the activation of ACC synthase. This plant enzyme in turn increases the level of ACC in tissues and consequently the amount of ethylene within the plant (Bal et al. 2013). Thus, PGPR that synthesizes IAA from plant tryptophan can both promote plant growth and inhibit plant growth (by inducing ethylene production). Interestingly, PGPR that contains ACC deaminase enzyme inhibits ACC accumulation in the plant, enabling bacterial IAA to take its actual course of plant growth promotion (del Carmen Orozco-Mosqueda et al. 2020).

4.5.2 Lipid Peroxidation

Lipid peroxidation is one of the major underlying factors responsible for impeding a plant's growth under various stressful conditions. It is an important physiological parameter that is indicative of the cell membrane integrity under the threats of several abiotic stresses (Hou et al. 2016). Severe water limitation deactivates the antioxidant systems and induces the generation of free radicals (hydrogen peroxide, hydroxyl radical, singlet oxygen, and superoxide radical) in plants. The first two free radicals, that is, H_2O_2 and the hydroxyl radical have been recognized as the most active and destructive agents for tissue injury (Ishibashi et al. 2011). Millets are comparatively more resistant to oxidative stress and less prone to injuries than other plants as exemplified by sorghum plants exposed to PEG-mediated droughts (Zhang and Kirkham 1994). A study conducted in five finger millet varieties collected from contrasting areas of the Indian subcontinent showed that two drought-resistant varieties, PR202 and VL315, efficiently produced free radical scavenging enzymes viz, superoxide dismutase, ascorbate peroxidase, and glutathione reductase to ameliorate the effects of drought. Nonetheless, all millets are not immune to prolonged exposure to drought as evident from a high accumulation of malondialdehyde and hydrogen peroxide in susceptible varieties (Bhatt et al. 2011). In such a scenario, PGPR inoculation can significantly decrease lipid peroxidation while improving membrane integrity and water status in plants under drought. Previously, a study has reported that the inoculation of drought-tolerant *Pseudomonas* significantly decreased lipid peroxidation and increased proline accumulation in finger millet (Chandra et al. 2018). Earlier, PGPRs producing stress-responsive compatible solutes such as exopolysaccharide, glycine betaine, proline, etc. have been reported to promote plant growth under stress conditions. For example, the application of PGPRs viz*, Pseudomonas fluorescens, Enterobacter hormaechei,* and *Pseudomonas migulae* stimulated seed germination and seedling growth in foxtail millet (Niu et al. 2018).

4.5.3 Mineral Acquisition

Multiple studies in diverse plant species have reported the enrichment of Actinobacteria in the plant root microbiome under drought conditions (Edwards et al. 2015; Xu et al. 2018). This enrichment is proportional to the magnitude and duration of drought and rapidly dissipates when water availability returns to normalcy (Xu et al. 2018). Genome-resolved metagenomics and comparative genomics revealed that Actinobacteria enrichment in the sorghum rhizosphere was directly linked to the loss of iron homeostasis in the plant root and the plant's inability to secrete phytosiderophore for iron transport (Xu et al. 2021). The study also reported that exogenous application of iron disrupted Actinobacteria assemblages while significantly improving the host phenotype. This indicated that microbial enrichment was crucial for facilitating iron availability to the plant under drought. Members of Actinobacteria are well acknowledged for their ability to produce diverse desferrioxamine siderophores such as desferrioxamine G, B, and E (Challis and Hopwood 2003). Earlier, the utility of desferrioxamine B (DFOB) as a shuttle agent for the transportation of Fe to sorghum root cells was reported (Cline et al. 1984). Siderophores are low molecular weight (200–2000 Da) compounds that show high affinity and specificity to iron available in the surrounding environment (Hider and Kong 2010). Although few plants secrete siderophores in the root exudates, the major portion of the iron-chelating compounds is produced by an elite group of microbes popularly known as siderophore-producing microbes (SPM). The ability of SPM to reduce iron deficiency and enhance plant physiology under water-limited conditions projects the group as promising biofertilizer and biocontrol agents toward sustainable agricultural goals (Singh et al. 2022). For example, siderophore-producing mycorrhizal symbionts *Glomus etunicatum* and *G. intraradices* elevated micronutrient levels in sorghum (Aliasgharzad et al. 2009).

Drought stress bears the associated risk of nutrient limitations (mainly, phosphorus), which adversely affect plant growth. Despite the abundance of inorganic and organic phosphates in soils, the availability of the mineral remains limited due to its insoluble form (Sharma et al. 2013). The use of phosphate-solubilizing microbes seems to be an effective approach for availing phosphorus to plants. The phosphate-solubilizing bacteria are an elite group of microbes that convert insoluble organic and inorganic phosphate to a form that can be easily absorbed by the plants (Elhaissoufi et al. 2021). Stress-resilient phosphate-solubilizing rhizobacteria can help the plant in overcoming water scarcity as well as phosphorus unavailability in the soil. The most efficient phosphate-solubilizers belong to the genera *Bacillus, Rhizobium, Pseudomonas, Azotobacter,* and *Azospirillum* (Rodrı́guez and Fraga 1999). The organic acids (gluconic and ketogluconic acids) produced and secreted by such microbes acidify the immediate environment leading to the release of phosphate ions. Several studies have reported the PGPR-mediated amelioration of water deficit-induced adverse effects in tomato (Mayak et al. 2004), pea (Arshad et al. 2008), chickpea and barley (Peix et al. 2001), and chickpea (Kumar et al. 2016). An investigation of the efficiency of stress-adaptive and phosphate-solubilizing rhizobacteria isolated from cereals and pseudocereals prospected 20 isolates to exhibit such capabilities under drought. The drought-adaptive P-solubilizing strains, *Streptomyces laurentii* EU-LWT3-69 and *Penicillium* sp. strain EU-DSF-10, greatly influenced physiological and growth parameters of great millet exposed to drought stress (Kour et al. 2020). Such promising PGPR candidates with drought-adaptive and phosphate-solubilizing abilities can be considered for developing bioinoculum for water-limited ecosystems.

Potassium is a major essential macronutrient vital for optimal plant growth. The mineral is the most profusely absorbed cation in higher plants. Its abundance is necessary for the activation of enzymes, maintenance of cell turgidity, transportation of metabolites, synthesis of starch and proteins, etc. Availability of potassium also determines nitrogen-use efficiency, kernel weight, and straw strength as well as improves disease resistance. Diffusion of the cation directs from high concentration to a lower gradient in the soil, therefore is highly dependent in the developmental stage of the roots. As such, impaired root development in water-limited conditions significantly limits potassium uptake by the plants, restricting their growth and development (Sindhu et al. 2016). Although the efficiency studies of K-solubilizing PGPR in millet crops are scanty, a single study on a silicate-dissolving bacterial strain SBS has reported that inoculation of the bacterium significantly increased K-supply at least by 21–112% in soil. Sorghum plants supplemented with the bacterial culture showed 48%, 65%, and 58% increase in dry matter yield in clay, sandy and calcareous soil, respectively, compared to the uninoculated control (Badr et al. 2006). This indicated that K-solubilizing bacteria (KSB) could be prospected as potential biofertilizers to ensure a continuous supply of available potassium to the plant's developmental stages.

4.6 Conclusion

The rhizosphere microbiome can play an active role in alleviating drought-related abiotic stresses in plants. The harnessing of a drought-adaptive microbiome with multifarious PGP attributes holds promising scopes for bringing out positive physiological changes in plants, improving soil health as well as suppressing the occurrence of associated biotic stresses. As such, microbiome engineering poses an attractive avenue for developing an effective drought stress management practice targeting crops grown in water-limited challenging environments. Translations of such strategies from the laboratory to the field face several drawbacks including limited viability across various environmental matrices, selectivity of host plants, low shelf life, and on-field variations in PGP activities. To resolve such constraints, sustained research efforts are required to gain valuable insights into how PGPR strains interact with the host plant in real-field conditions, which molecular and metabolic pathways are induced in plants upon PGPR treatment, and how their application affects the plant and native microbial population dynamics. In parallel, novel studies on drought-tolerant P-solubilizers, and their interactions with host plants exposed to drought environments will help in formulating wide-spectrum microbial consortia.

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Chapter 5
Identification of Novel Microbial Strains for Reduced Pesticide Use in Millets

Shruti Hazra, Joyeta Dey, Suchismita Mukherjee, Abul Kalam, and Chittabrata Mal

Abstract Millets are worldwide used crops, and more than thousands of varieties of millet crops are cultivated globally because of their beneficial supplement to the human diet. Also, it is rich in micronutrients and protein. Shoot fly, stem borer, and pink borer are the major millets' pests that cause agricultural and economic losses and increase the demand for chemical or synthetic pesticides for millet crop protection. These pesticides affect the environment by manipulating ecosystems. The focus on microbial bioremediation procedures has been increased to maintain sustainable growth and reduce the economic, environmental, and health effects due to the overuse of pesticides. It has been observed that the rhizosphere microbes are the most potent natural bioremediation agent. For example, bacterial genera like Rhizobium (strain RF12 and RZ11), Flavobacterium, Actinobacter, Pseudomonas, Bacillus, Agrobacterium sp., etc., are identified as pesticide-degrading strains. Many microbial and fungal species also degrade major harmful chemicals like acetochlor, carfentrazone, and clothianidin. These chemicals are converted into nontoxic chemical compounds by the enzymatic action of the microorganisms of the Rhizobiaceae group. The researchers are currently focusing on isolating these novel microbial strains. These strains' detailed genetic and molecular studies will help to manipulate their genetic makeup. Thus, the pesticide-degrading effect could be increased and further applied to large-scale use and producing genetically engineered crops as self-herbicide/insecticide/fungicide-resistant plants that eventually lead to reduced pesticide use. A whole microbial community and many novel strains like arbuscular mycorrhizal fungi (AMF) are found in the millet-cultivating soil. This chapter focuses on those novel microbial strains found in millets that can degrade pesticides.

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_5

Several studies can be designed to isolate and characterize for further evaluation that could help to reduce the problems related to pesticide use soon.

Keywords Millets · Pesticides degradation · Rhizosphere · Rhizobiaceae · Arbuscular Mycorrhizal fungal · Genetically modified crop

5.1 Introduction

Millets are generally used as a staple food in the subcontinent area of southern Asia and southern Europe. A high population of the arid and subarid regions of Asia and southern Africa depends on millet grains because of their high carbohydrate, protein, and major nutrient constituents. According to a statistical study, over 28.4 metric tons of eight major millet crops are cultivated worldwide (FAO 2017). Because of its high nutritional value and increasing demand, scientists and farmers focus on higher millet cultivation procedures. A major problem is a destruction or retarded growth of millet crops by over 150 insect pests, of which almost 116 species are reported from India (Nwanze and Harris 1992). To deal with this problem, farmers of millet crops majorly became dependent on chemical pesticides. Chemical constituents of these pesticides, like acetochlor, glyphosate, carfentazone, etc., harm the environment, health, and the economy. Pesticides contaminate the environment by entering the food chain and involving in biomagnification, that is, they increase the accumulation of harmful chemicals in the food chain and cause diseases like cancer, respiratory, nervous problems, and birth deformities, and also can introduce mutation in the genes. To overcome the problem, the necessity of pesticide biodegradation became the focus of researchers. Microbes of different genera have the potential to degrade harmful chemicals and heavy metals. This kind of microorganism performs enzymatic biodegradation. This natural phenomenon of microbes is utilized by scientists to degrade toxic chemicals and is termed biodegradation. For example, biodegrading bacteria, like Bacillus cereus and Bacillus safensis, can degrade carbendazim and imidacloprid; *Pseudomonas sp.* can degrade herbicide Aroclor 1242, *Aspergillus fumigatus* can degrade metribuzin, and *Trichoderma viridae* can degrade endosulfan pesticide (Arya et al. 2017).

The enzyme-catalyzed method is more effective than the chemical method of pesticide degradation. For example, oxidoreductase is a glyphosate pesticide-degrading enzyme found in *Agrobacterium strain T10. Similarly,* monooxygenase enzymes like Ese and P450, found *in Mycobacterium sp. Pseudomonas putida,* can help to degrade endosulfan and hexachlorobenzene.

Increased millet yield is observed in the soil supplied with N, P, and K (nitrogen, phosphorus, and potassium) fertilizer because these nutrients promote the growth of soil microbes, especially microbes of rhizobacteria genera. Studies revealed that these bacteria play a vital role in plant growth by supplying plant growth-promoting factors in the soil or directly in the plant (Xu et al. 2018). Rhizosphere microbes isolated from pearl millet-cultivating soil shows the effecting ring cleavage degradation procedure of parathion (Reddy and Sethunathan 1983). A very diverse species of group arbuscular mycorrhizal fungi (AMF) were found in the minor millet-cultivating species soil. Strains of this AMF group actively participate in plant growth promotion. The soil's varying concentrations of N, P, K, and carbon showed a high species diversity. Almost 24 species of 11 different genera were isolated and showed pesticide-degradation properties with plant growth-promoting factors (Mythili and Ramalakshmi 2022). However, the isolation of these strains is a bit challenging. But these results show the direction to target the millet-cultivating soil. By targeting millet soil with different concentrations of N, P, K, and C, many novel strains, especially from the rhizosphere group, could be found as potent pesticide-degradation organisms. High-throughput molecular and genetic study on these unknown species could give the solution to problems related to pesticide pollution. Recombinant technology can be applied to develop genetically modified plants and organisms to reduce pesticides' use in agriculture.

5.1.1 Various Pesticides Used in Agriculture

Chemical or synthetic pesticides are fast-acting, easy to apply, and cheap making them the primary choice in agriculture (Daly et al. 1998). There are several chemical pesticides vastly used given in Table 5.1 according to their chemical compositions. Due to substantial human manipulation, many pollutants and waste are generated in the environment. More than one billion toxins and inorganic waste are released into the environment indirectly which manipulate our ecosystem (Damalas 2009). As urbanization increases, demand for food increases; as a result, the use of pesticides also increases. Pesticides are majorly used to increase crop production in most areas, and are tiny chemical substances used to eliminate unwanted organisms that hamper the growth and quality of plants (Christos 2009).

Pesticides have a lot of adverse effects on the environment and health. Although pesticides benefit farmers a lot in protecting crops from the damage caused by pests, overuse of pesticides negatively affects crops' health and growth. The chemicals used in pesticides potentially harm our endocrine system, nervous system, and also

Table 5.1 Classification of chemical or synthetic pesticides with their compositions (Adapted from Ortiz-Hernández 2002)

Pesticides	Components
Organochlorine	Carbon(C), chlorine (Cl), hydrogen (H), sometimes oxygen, nonpolar as well as lipid loving (lipophilic)
Organophosphate	Central phosphorus atom, organochlorines stable, merely toxic. Aliphatic, cyclic, and heterocyclic
Carbamates	Structure-based on specific plant alkaloid *Physostigma venenosum*
Copper	Inorganic copper
Dinitrophenols	Reorganize in the presence of NO_2 bound with phenol ring
Urea and their derivatives	Urea bound to aromatic compounds

Table 5.2 Chemicals used in pesticides and their adverse effects

Chemicals used in pesticides	Effects
Acetochlor	Chronic health effects, surface water contaminants, bee poison
Alachlor	Surface water contaminants, wildlife poisons
Carfentrazone	Chronic health effects, groundwater contaminants, wildlife contaminants
Clothianidin	Chronic and acute health effects
Mesotrione	Chronic health effects, long-range transport, groundwater contaminations
Phosphine	Acute and chronic health effects
Glyphosate	Chronic health effects, wildlife poisons

reproductive system. It is also reported that pesticide is a primary reason of life-threatening diseases like Alzheimer's disease, cancer, and congenital disabilities because chemicals used in pesticides cannot be metabolized but instead are stored in the colon (Srivastava and Kesavachandran 2019). The adverse effects of pesticides on the environment and health made scientists curious to study the degradation of pesticides (Table 5.2).

5.1.2 Types of Millets and Their Origin

Millets are coarse grains that are grown globally and a depository of minerals, protein, vitamins, and fiber. Crop *Pennisetum glaucum* is commonly known as pearl millet, spiked millet, bajra, bulrush millet, candle millet, and dark millet. Its probable origin was Tropical West Africa. Origin of *Setara italica* (foxtail millet, Italian millet, German millet, Hungarian millet, and Siberian millet) is eastern Asia (China). Origin of *Panicum sumatrense* (little millets) and *Paspalum scrobiculatum* (kodo millets) are Southeast Asia and India. *Panicum miliaceum* is commonly called proso millet, common millet, hog millet, broom-com millet, Russian millets, and brown corn. Its origin is Central and eastern Asia. *Echinochloa crusgalli is* called barnyard millet, sawa millet, Japanese barnyard millets, and its origin is Japan. The origin of *Eleusine coracana* (finger millet, African millet, Koracan, ragi wimbi, bulo, and telebum) is Uganda or a neighboring region (Sakamoto 1987).

5.1.3 Pesticides Used in Millets

The growth of millets is hampered by different pests. When the damage reaches above 10%, the crop can be sprayed with pesticides for specific pests (Table 5.3). *Atherigona pulla* species is a prevalent pest that harms all the little millets. Also,

Table 5.3 List of pesticides against the grain millets-specific pests

Pests	Pesticides
Headworms: corn earworm, fall armyworm, sorghum webworm	1. Alpha-cypermethrin Fastac CS 2. Chlorantraniliprole 3. Spinosad + gamma cyhalothrin Consero 4. Spinosad Blackhawk (36%)
Stink bugs, leaf-footed bugs	1. Alpha-cypermethrin Fastac CS, other brands 0.83 2. Beta-cyfluthrin Baythroid 3. Spinosad + gamma cyhalothrin Consero 4. Zeta-cypermethrin Mustang
False chinch bugs on seed heads	1. Alpha-cypermethrin 2. Zeta-cypermethrin
Stink bugs, leaf-footed bugs	1. Alpha-cypermethrin 2. Beta-cyfluthrin 3. Spinosad + gamma cyhalothrin 4. Zeta-cypermethrin
Headworms: corn earworm, fall armyworm, sorghum webworm	1. Alpha-cypermethrin 2. Chlorantraniliprole 3. Spinosad + gamma cyhalothrin 4. Spinosad
Stink bugs, zeta-cypermethrin, leaf-footed bugs	1. Alpha-cypermethrin 2. Beta-cyfluthrin 3. Spinosad + gamma cyhalothrin 4. Zeta-cypermethrin 5. Beta-cyfluthrin 6. Spinosad + gamma cyhalothrin

hairy caterpillars, leaf folders, and Noctuidae are common pests that attack almost all kinds of millets (Gahukar and Reddy 2019).

5.1.4 Strategies to Overcome the Problems Related to Pesticides

It is estimated that about 15% of the world's crop yield is lost due to insects or pests. Damage to crops is mainly caused by insect larvae and, to some extent, adult insects (Oerke 2006). Earlier, chemical pesticides were the only means of pest control. For several years, scientists have been looking for alternative pest control methods (Gill and Garg 2014). The alternative methods can be the use of biopesticides and microbial pesticides.

The use of microbial pesticides is a strategy to reduce the use of chemical pesticides. Some genetically modified or naturally occurring microorganisms can

Table 5.4 List of pathogens that can affect the host (insect and pest) larvae (Usta 2013)

Microbes	Pathogen	Host
Bacteria	*Bacillus thuringiensis* var. *kurstaki* (*Bt*)	Caterpillars
	Bacillus thuringiensis var. *tenebrinos*	Colorado potato beetle larvae
	Bacillus thuringiensis var. *israelensis* (*Bt*)	Black flies, *Aedes*, and *Psorophora* mosquitoes' larvae
Fungus	*Lagenidium giganteum*	Larvae of pest mosquito species
	Beauveria bassiana	Mites, white flies, fungus gnats

be directly responsible for the death of insect pests, but these microbes are nontoxic for human health and other nontarget organisms (Table 5.4).

During the cultivation of millet plants, the pesticides used for crop protection is often applied in higher doses. As a result, leftover pesticides remain in the plant body and affects the ecosystem.

Several microorganisms have been characterized to degrade leftover pesticides as nutrients into nontoxic molecules (Iqbal and Bartakke 2014). The two most influential and environment-friendly methods of degrading pesticides are as follows:

1. Microbial degradation method and creating genetically modified microorganisms
2. Producing genetically modified plants with pest-resistant ability.

5.1.4.1 Microbial Degradation of Pesticides

Nowadays, studies have revealed that a certain number of microorganisms have a natural ability to degrade pesticides, and a few can adopt this ability to degrade certain types of pesticides. If these microbes are applied to the soil for a while for a particular duration, they can degrade pesticides enzymatically as the chemicals present in the pesticides provides a suitable carbon source and nutrients for them so that they can grow and become metabolically active to produce enzymes that in turn will degrade those pesticides.

A study found that *Pseudomonas* is the most efficient genus for this process. Three *Pseudomonas* strains were found responsible for degrading Aroclor 1242 (Vásquez and Reyes 2002), a polychlorinated biphenyls (PCB) mixture used in a pesticide responsible for weathering, and short-term exposure to it cause liver damage (Ouw et al. 1976). Another research mentioned common strains like *Aspergillus niger*, *Aspergillus fumigatus*, *Rhizopus microsporus*, *A. terrus,* and *Corymberifera microsporis,* which are responsible for degrading metribuzin. This widely used herbicide control weeds by inhibiting photosynthesis and is also used in the production field of millets like corn (Bordjiba et al. 2001).

The root-associated microorganisms like rhizosphere (Philippot et al. 2013), rhizoplane, and endophytic bacteria play vital roles in plant productivity, nutrition, and disease resistance. This microorganism can replace the use of chemical pesticides, and also plays a vital role in biodegradation.

5.1.5 Rhizosphere and Microbes

The rhizosphere is the soil located adjacent to the plant root system. In the rhizosphere soil, large quantities of metabolic compounds like root exudates and mucilage are released by roots from the fibrous root system. These metabolites help the bacterial movement to the root surface and act as the primary nutrient source to promote plant growth and endurance in the rhizosphere. Bacteria can inhibit this area more than other microbes like fungi, actinomycetes, and protozoa and can colonize the root effectively or the rhizosphere soil (Bakker et al. 2013).

Different groups of microorganisms present in the rhizosphere are bacteria, fungi, actinomycetes, algae, nematodes, arthropods, protozoa, and viruses. Rhizosphere soil is a hot spot for the microbial population than the other soil area. Some examples of rhizosphere microorganisms (Mendes et al. 2013) are given in Table 5.5.

5.1.6 Impact of Rhizosphere Microbes on Millet Plant Growth

Rhizosphere microorganisms can be beneficial, but some microorganisms can be pathogenic for plant and human health (Fig. 5.1). Beneficial microorganisms like

Table 5.5 Examples of different types of rhizosphere microorganisms

Microbes	Examples
Bacteria	*Pseudomonas fluorescens* *Streptomyces filamentosus* *Rhizobium leguminosarum* *Bacillus amyloliquefaciens* *Bradyrhizobium japonicum*
Fungi	*Laccaria bicolor* *Verticillium dahlia* *Sporisorium reilianum* *Metarhizium anisopliae*
Arthropods	*Metaseiulus occidentalis* *Solenopsis invicta*
Algae	*Chlorella variabilis* *Chlamydomonas reinhardtii*
Nematodes	*Caenorhabditis elegans* *Meloidogyne hapla*
Protozoa	*Dictyostelium discoideum*
Viruses	*Pseudomonas phage 73* *Agrobacterium phage 7-7-1* *Rhizoctonia solani virus*

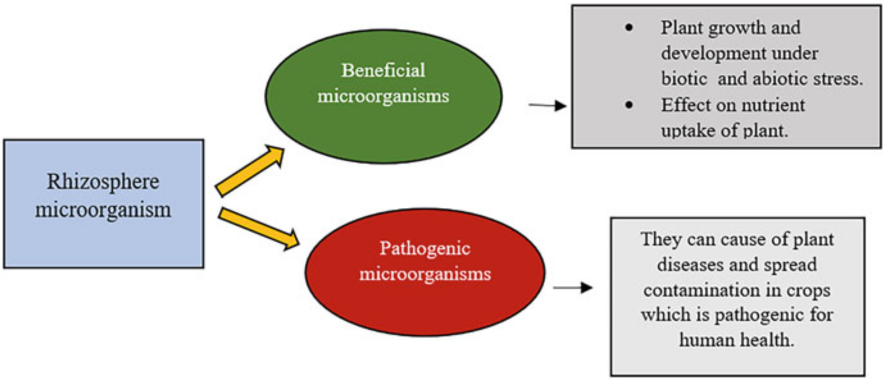


Fig. 5.1 Schematic diagram of the impact of rhizosphere microorganisms

plant growth-promoting rhizobacteria (PGPR) act as a biofertilizer, and they can enhance plant growth by various substances. The PGPR is a very effective microbe for sustainable agriculture.

Some PGPR genera are *Pseudomonas, Azospirillum, Burkholderia, Rhizobium, Flavobacterium, Azotobacter, and Bacillus.*

A wide range of microorganisms of the rhizobacteria family possesses pesticide-degradation potential. Rhizoremediation is an essential tool for sustainable agriculture. This microorganism helps in plant growth and yield by providing plant growth-regulating hormones like auxin and cytokinin. Also, they fix atmospheric nitrogen and enhance soil's nitrogen. Again, they directly control the plant disease by removing the biological agents responsible for plant diseases. However, this plant growth-promoting rhizobacteria (PGPR) family is affected by the chemicals present in the pesticides. However, some research showed that the bacteria of the PGPR family developed pesticide-tolerating or degrading properties by isolating them from the contaminated soil. Molecular studies of pesticide-degrading or tolerating properties of these rhizobacterial strains will soon be an essential tool for the biodegradation of pesticides (Roy et al. 2020). Two strains of bacteria, *Bacillus cereus* and *Bacillus safensis*, belong to the PGPR family and can degrade pesticides, mainly carbendazim and imidacloprid. *Bacillus cereus* could tolerate almost every pesticide, and both possess a single plasmid that could be utilized as a genetic engineering tool (Roy et al. 2022).

Some significant strains have been isolated with plant growth-promoting activity in pearl millets such as *Serratia marcescens* EB 67, *Pseudomonas sp*. CDB 35, and *Bacillus circulans* EB 35 (Hameeda et al. 2006). *Penicillium sp.* has been isolated as plant growth-promoting fungi that can help in the seed germination of pearl millets (Murali et al. 2012). Some pathogenic rhizosphere microorganisms are responsible for the reduction of yield production. Some nematodes and fungi or oomycetes are present in this pathogenic microorganism, but they are agronomically crucial in temperate climates. Downy mildew is a significant millets disease caused by oomycete (*Sclerospora gaminicola*) and *Trichoderma spp.*, isolated from rhizosphere soil with the biocontrol potential against the downy mildew pathogen (Nandini et al. 2021). *Pseudomonas sp. strain MSSRFD41* isolated from rhizosphere soil of finger millet can produce IAA and solubilize phosphate, which can help in the growth promotion of millet. This strain has an antifungal property against *P. grisea*, which is responsible for the blast disease of millet (Sekar et al. 2018). Some bacteria can be responsible for economic damage to crops. Bacterial pathogens (i.e., *Agrobacterium tumefaciens, Ralstonia solanacearum,* and *Pectobacterium atrosepticum*) infect plants via plant roots (Mansfield et al. 2012).

5.1.7 Potential Role of VAM (Vesicular-Arbuscular Mycorrhiza)

Mycorrhiza is a noninfectious producing association, and it is formed between a fungal species and the roots of a plant. The mycorrhizal association is an example of mutualism. Due to this association, plants and fungi both benefit from each other. Mycorrhizal fungi help plants uptaking minerals, whereas these fungi also gain minerals from plants. Two types of mycorrhizae are ectomycorrhiza and endomycorrhiza. Ectomycorrhiza fungi form an external association with roots, and it does not extend the cells of roots. Endomycorrhizal fungi form another association by discerning the cortical cells of the roots (Juwarkar and Jambhulkar 2008).

Mycorrhizal fungi are used as biofertilizers and bioprotectors for sustainable agriculture. A specific structure within arbuscules and vesicles is formed by VAM hyphae, which penetrate the roots of plants. Where nutrient and carbon transfer occur it is known as arbuscles and vesicles are the storage of organs. Generally, VAM is used for maize, millets, barley, and leguminous crops (*Bahunia retusa, Crotolaria albida, Desmodium elegans, D. heterocarpon, and Vicia rigidula*) (Smith 2002). Different roles of VAM are as follows:

1. Enhance uptake minerals and reduce the use of fertilizer.
2. Help uptaking potassium, copper, zinc, nitrate, and ammonium.
3. Soil structure can be improved by external mycelium, which helps to bind the soil particles and microaggregates.
4. Help to reduce the use of pesticides.
5. Promote proper growth of crops.

5.1.8 Rhizobium

Rhizosphere bacteria play a vital role in plant metabolism, pest control, and biodegradation. It promotes plant growth through nitrogen fixation, phosphate solubilizers

Table 5.6 Effect of pesticides on different types of *Rhizobium* sp.

Strains	Effect	Pesticide[a]	References
Mesorhizobium sp.	Pesticide-concentration dependent Progressive-decline in indole-3-acetic acid and siderophore was observed	Metribuzin (H), glyphosate (H), imidacloprid (I), thiamethoxam (I), hexaconazole (F), metalaxyl (F), kitazin	Abd-Alla (1994)
Rhizobium meliloti	The gradual increase in pesticide concentration showed a strong negative effect on the *Rhizobium sp.*	Thiram (F)	Mirza et al. (2007)
Mesorhizobium ciceri	Particular rhizobia population decreases in chickpea	Bavistin (F), thiram (F)	Gaind et al. (2007)
Rhizobium japonicum	Inhibit the growth of particular rhizobium	Captan (F), carbendazim (H) carbendazim being more toxic to the nodule bacterium than Captan	Mirza et al. (2007)
Rhizobium phaseoli	Survival of this rhizobium decreases with increasing concentration of herbicides	Agroxone (H), atranex 50SC (H)	Adeleye et al. (2004)
Bradyrhizobium sp.	Reduce 50% of the growth rate by the production of biochemical reactions in their membrane composition	Mancozeb (F)	Fabra et al. (1998)

aH, F, and I stand for herbicide, fungicide, and insecticide, respectively

(Gopalakrishnan et al. 2015), formation of siderophore (Sinha and Mukherjee 2008), and several phytohormone productions (Mirza et al. 2007).

Rhizobium and *Bradyrhizobium* strains generate extracellular phosphodiesterase and phosphotriesterase enzymes, which can hydrolyze organophosphorus compounds by degrading organophosphorus pesticides (Abd-Alla 1994). Several strains of Rhizobium can implicate pesticide controls. For example, *Rhizobium MRP1. MRL3* (40, 80, and 120 and 400, 80 μg/kg soil) in the presence of herbicides can enhances biomass, nodulations, root and shoot formation in pea and lentil. Various effects of pesticides on different types of Rhizobium sp. are described in Table 5.6.

5.1.8.1 Pesticide-Tolerating *Rhizobium*

Several strains of Rhizobium can tolerate pesticides at specific concentrations like *Rhizobium leguminosarum bv. vicieae* can interact with the leguminous plant *Vicia faba* and degrade pesticides. The mutant of Rhizobium (RZ11 and RH11) is tolerant to malathion (pesticide) at 7000 ppm, whereas Rhizobium (strain RF12 and RZ11) is tolerant to methomyl (pesticide) at 10,000 ppm (Hassan 2010). Chlorpyrifos is a well-known organophosphate pesticide. Vigorous use of this pesticide led to the contamination of soil and water system. Rhizobium biodegrades this pesticide P and its product 3,5,6-trichloro-2-pyridinol (TCP) (Rayu et al. 2017). Different strains of Rhizobium can tolerate pesticides like carbofuran, deltamethrin, and paraquat at soil pH range (3–8) at specific concentrations.

5.1.9 Agrobacterium-*Mediated Degradation*

A soilborne microorganism, *Agrobacterium tumefaciens*, which is gram-negative, has the natural ability to enter the plant genome through a wound site and form a tumor in the plant called the crown-gall disease. Molecular and genetic studies of *Agrobacterium* found the reason behind the formation of gall is the tumor-inducing plasmid (Ti plasmid). T-DNA present in the Ti plasmid gets integrated into the plant chromosome, that carries the genes encoding the proteins involved in the biosynthesis of growth hormones like auxin and cytokines, which causes plant proliferation in turn formation of gall and genes for plant metabolites opines (derivatives of amino acid) and agropines (derivatives of sugar) which act as a carbon and energy sources of *Agrobacterium* (Thomashow et al. 1980). The naturally derived genetic engineering procedure of *Agrobacterium* makes it a widely used gene transfer vector. Several bacteria of Rhizobiaceae proved to have the glyphosate-degrading ability (Pizzul et al. 2009). *Agrobacterium tumefaciens* and *A. rhizogenes* can grow on glyphosate and utilize it as a primary phosphorus source by degrading it (Liu et al. 1991).

5.2 Methodology for Strain Identification

Biodegradation by microorganisms that are present in soils is the most common type of degradation. Many fungi and bacteria use pesticides as their food source. There are several criteria for biodegradation: the bioavailability of target contaminants must be sufficient; soil conditions must be favorable for microbial population/plant growth and enzyme activity.

Many bacterial and fungal species can degrade pesticides. Many pesticides lead to the formation and inflation of metabolites that undergo partial degradation. Fungi can degrade pesticides by minor structural changes to the chemical form of pesticides and make them nontoxic. Thiram is a fungicide and is degraded by the *Pseudomonas aeruginosa* (Elhussein et al. 2011). Like this, any novel strain can be present in pesticide-contaminated soil. For example, *Bacillus thuringiensis* strain *ZS-19* is a novel strain, and this strain was isolated from pyrethroid contaminated areas with the help of the enrichment culture technique (Melo et al. 2016).

5.2.1 Isolation, Purification, and Screening of the Pesticide-Degradable Microorganisms

5.2.1.1 Bacteria

Isolation of pesticide-degrading bacterial strain has been done by the enrichment culture method, In this method, samples need to be collected from pesticide-contaminated millet rhizosphere soil and these soil samples can further be treated with same pesticides in a laboratory scale to improve the probability of detecting pesticide-degrading microbiota. In the 100 mL of mineral salt medium (MSM) containing selected pesticides as the source of carbon and phosphorus, 10 g of each soil sample is inoculated. The flasks must be incubated in a proper environment for 7 days. Inoculation-free media act as a control. At the final concentration of different pesticides, pesticide-tolerant cultures are used to study further. The whole experiment should be performed in triplicate (Sowunmi et al. 2021).

One milliliter of each enriched culture is inoculated on nutrient agar media plates and incubated at 37 °C. Single colonies can be picked and streaked on solid media and then incubated at 37 °C for 48 h to get pure culture. The minimum inhibitory concentration (MIC) determines bacteria resistance to the pesticide. This method selects bacteria showing 100% growth in a high concentration of pesticides for further study (Hussaini et al. 2013).

5.2.1.2 Fungi

The soil samples are redressed with the addition of an adequate concentration of selected pesticides, and this mixture is incubated at room temperature for 2 weeks. The digested soil samples are washed with sterile distilled water and kept for 30 min until the soil debris has settled down. Then the supernatant can be transferred into a sterile test tube and serially diluted. The serially diluted soils are plated in pesticide-containing potato dextrose agar medium. The number of fungal strains can be observed after 6–7 days. The most noticeable fungal colonies are isolated and maintained as a pure culture. The isolated colonies can be transferred to PDA slants and then further tested their growth in the presence of selected pesticides by culturing with the pesticides containing PDA medium, which cultures can show the highest tolerance ability, those are selected for further study (Iqbal and Bartakke 2014).

5.2.2 Morphological Study

Bacterial and fungal morphology is studied macroscopically and microscopically. Macroscopically, isolated colony features are observed by their color, shape, elevation, texture, etc. using Bergey’s manual (Table 5.7).

Table 5.7 Bergey's manual characterization

Margin	Color	Elevation	Texture	Shape
Curled	Orange	Raised	Slimy, moist	Round
Entire (smooth)	Red or pink	Umbonate	Matte, brittle	Punctiform
Filamentous	Black	Flat	Shiny, viscous	Rhizoid (rootlike)
Undulate (wavy)	Brown	Convex	Dry, mucoid	Filamentous
Lobate	Opaque or white	Pulvinate (cushion-shaped)	Translucent	Irregular
Erose (serrated)	Milky	Growth into a culture medium	Iridescent (changes color in reflected light)	Spindle

Table 5.8 Biochemical characterization

Activity test	Process
Starch hydrolysis test	Helped on starch agar media
Cellulase production test	Helped on Congo red agar media
Catalase test	Few drops of 3% H_2O_2 are added on microscopic slides of the culture strain
Citrate test	Isolated strains are inoculated on Simmon's citrate agar media

Microscopically bacterial colonies are investigated using gram staining under a compound microscope. Crystal violet and safranin are used in gram-staining methods to determine the gram-positive and gram-negative bacteria, and iodine is used as a mordant.

The fungal colonies are microscopically investigated using lactophenol cotton blue-stained slides (Alsohaili and Bani-Hasan 2018).

5.2.3 *Biochemical Characterization*

Bacterial isolates can be characterized by biochemical tests, for example, amylase, catalase, and cellulose. It is a beneficial technique for classification and identification. This biochemical characterization analyzes the metabolic and nutritional capabilities of the isolates, and it helps to determine the genus and species of the isolates (Table 5.8). This biochemical activity test is performed with the help of different media (Surekha Rani et al. 2008).

5.2.4 Molecular Characterization

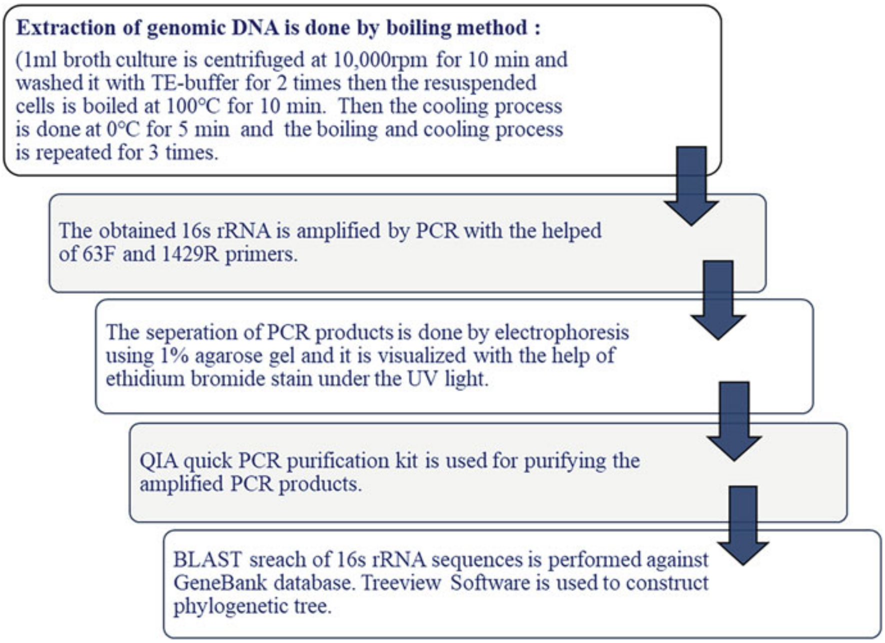
5.2.4.1 16s rRNA Sequencing

Molecular characterization is done by the 16srRNA sequencing method to identify the isolates. 16s rRNA sequencing is a recently developed and accurate method to identify any strain. This process is done by PCR amplification of 16srRNA of isolated species. Based on this sequencing method, a phylogenetic tree can be formed for identification. The overall process of 16s rRNA sequencing is described in Fig. 5.2 (Sangwan et al. 2021).

5.3 Bioremediation Assay of Pesticides Through Bacterial and Fungal Isolates

5.3.1 Pesticide Degradation Using Fungi

Bioremediation of pesticides using fungi needs two different culture media (triplicate). One media is used, which contains only PDA, and another contains PDA and



Extraction of genomic DNA is done by boiling method :
(1ml broth culture is centrifuged at 10,000rpm for 10 min and washed it with TE-buffer for 2 times then the resuspended cells is boiled at 100°C for 10 min. Then the cooling process is done at 0°C for 5 min and the boiling and cooling process is repeated for 3 times.

The obtained 16s rRNA is amplified by PCR with the helped of 63F and 1429R primers.

The seperation of PCR products is done by electrophoresis using 1% agarose gel and it is visualized with the help of ethidium bromide stain under the UV light.

QIA quick PCR purification kit is used for purifying the amplified PCR products.

BLAST sreach of 16s rRNA sequences is performed against GeneBank database. Treeview Software is used to construct phylogenetic tree.

Fig. 5.2 A schematic flowchart of the process of 16s rRNA sequencing pesticide at a definite volume (w/w) inoculated with spores of specific fungi. Then both plates are incubated at 28 °C for 8 days.

Generally, fungi are better degraded than bacteria for their specific characteristics, like bioactivity specific-growth morphology, and resistance power are very high in the presence of a high concentration of pollutant (Hussaini et al. 2013).

5.3.2 *Pesticides Degradation Using Bacteria*

Previously, isolated bacterial isolates were picked from pure culture and then inoculated into 100 MSM broth that contained a high concentration of selected pesticides to determine the degradation ability for 10 days at 30 °C, and every 24 h, samples are analyzed using GC-ECD. For this analysis, samples are prepared by the extraction of 2 mL culture with 4 mL ethyl acetate and kept for evaporation until dryness. Then the extract is again dissolved with 2 mL hexane (Asim et al. 2021).

The GC-ECD method analyzes the degradation of pyrethroid and organochlorine pesticides that are frequently used in millets.

$$\text{Percentage of degradation rate} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

5.4 Mechanism of Pesticide Biodegradation

5.4.1 *Mechanism of Microbial Degradation of Pesticides Generally Used in Millets*

As pesticides are chemically very complex, in some cases, more than two strains of microorganism are required to degrade chemical components of pesticides. Naturally, microbial populations degrade the substrate in a correctly ruled pathway. They convert the toxic chemical into a simple nontoxic compound or degrade the chemical through a systematic metabolic pathway. The process in which the organisms degrade toxic to nontoxic compounds but do not use as their nutritional source is termed as co-metabolism. Microorganisms can also change a chemical's structure either physically or chemically, and this procedure is called biotransformation. Biotransformed chemicals are further degraded by another group of microorganisms (Ortiz-Hernández et al. 2013).

Besides bacteria, a few groups of fungi and actinomycetes also produce some extracellular enzymes to degrade pesticides. White rot fungi play a vital role in degrading pesticides when bacteria fail to degrade atrazine, diuron, aldrin, dieldrin, mirex, chlordane, etc. (Sharma et al. 2019).

Table 5.9 List of microorganisms involved in the degradation of organochlorine pesticides

Degrading pesticides	Microorganisms
DDT in soil	*Escherichia coli, Enterobacter aerogenes, Enterobactercloacae, Klebsiella pneumonia, Pseudomonas putida, Bacillus species and Hydrogenomonas*
DDT (activated sludge)	*Pseudomonas sp., Pseudomonas aeruginosa, Micrococcus, and Flavobacterium sp.*
Endosulfan	*Pseudomonas sp., Flavobacterium sp., and Bacillus sp.*
Lindane	*B. thiooxidans* and *Base thiooxidans, Sphingomonas*

5.4.1.1 Organochlorine

Organochlorine pesticides are composed of hydrogen, carbon, and chlorine. Different organochlorine pesticides are lindane, endosulfan, heptachlor, and DDT. These organochlorine pesticides are used as insecticides in agriculture, which cause several health problems for their toxicity. Several microorganisms have been isolated, which can degrade the toxicity of organochlorine pesticides (Table 5.9), but the breakdown rate of these pesticides is prolonged. It is observed that organochlorine pesticide β-endosulfan was detected with a higher concentration in millets from northern Cameroon (Sonchieu et al. 2010). This β-endosulfan pesticide can be degraded by bacterial genera like *Klebsiella, Flavobacterium, Actinobacteria, Pseudomonas,* and *Bacillus* (Kafilzadeh et al. 2015).

5.4.1.2 Pyrethroids

It is a synthetic pesticide, an organic compound used for commercial and household insecticides (Robert et al. 2000). It is an artificial insecticide found naturally from chrysanthemum flowers, derived from pyrethrins (Sogorb and Vilanova 2002). Pyrethroids are nonpersistent, and sodium channel modulators are more toxic than organophosphates and carbamates.

Pyrethroids target CNS in target and nontarget organisms; these compounds are generally applied to household pests (Gilbert and Gill 2010). Some examples are cypermethrin, cyfluthrin, deltamethrin, etofenprox, fenvalarate, permethrin, phenothrin, prallethrin, resmethrin, and tetrafenprox (Ortiz-Hernández et al. 2013). Cypermethrin and cyhalothrin are used in millets, mainly sorghum and pearl millets. *Bacillus sp.* can degrade cypermethrin within 15 days with a 90% degradation rate (Jambagi et al. 2022). Chemical degradation mechanism of cyhalothrin is described in Fig. 5.3.

5.4.1.3 Neonicotinoids

It is a neuroactive insecticide chemically close to nicotine (Adegun et al. 2020). It belongs to the family of acetamiprid, clothianidin, imidacloprid, nitenpyram,

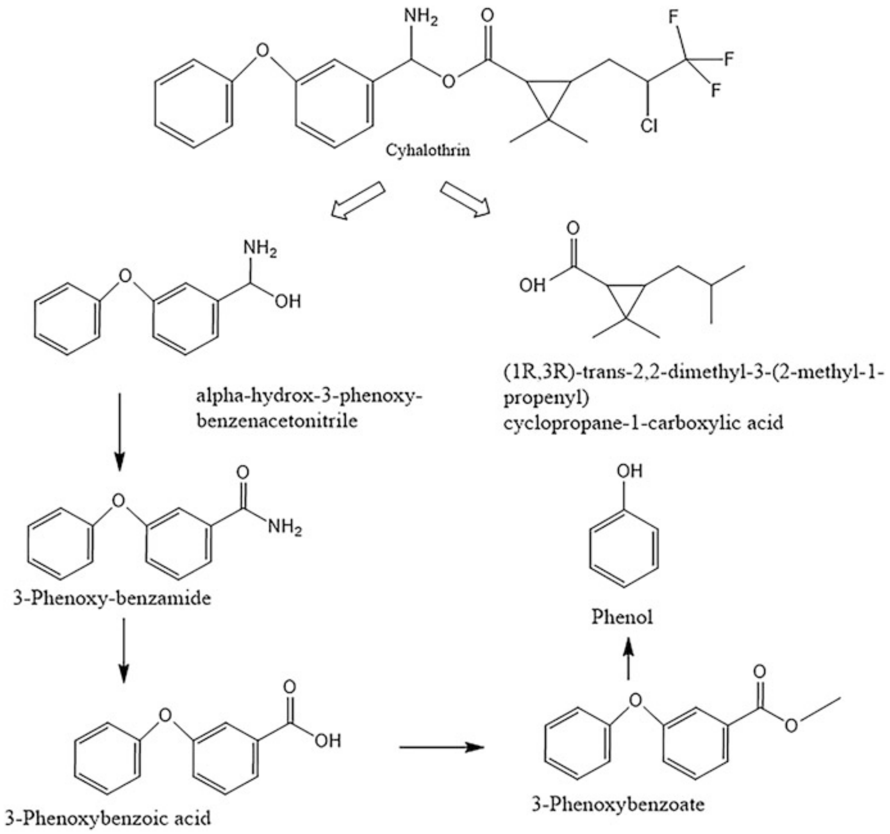


Fig. 5.3 Degradation pathway of *Cyhalothrin* (adopted from Jambagi et al. 2022)

nithiazine, thiacloprid, and thiamethoxam. Imidacloprid has been used widely throughout the world since 1999 (Yamamoto and Casida 1999). Imidacloprid is the new generation of neonicotinoid pesticides used in millets. Degradation mechanism of imidacloprid is shown in Fig. 5.4. Neonicotinoids are degraded by *Achromobacter, Agromyces, Ensifer, Mesorhizobium, Microbacterium*, and *Pseudoxanthomonas* (Gupta et al. 2016).

5.5 Enzymatic Degradation of Pesticides

As mentioned earlier, pesticides are degraded by organisms through metabolic pathways. Enzymes are the critical factor in these metabolic pathways. Esterase, hydrolases, cytochrome P450, GST (glutathione S-transferases), and mixed function oxidase (MFO) are the main enzyme groups that play a vital role in pesticide degradation (Bass and Field 2011). Enzymology study is the most recent and

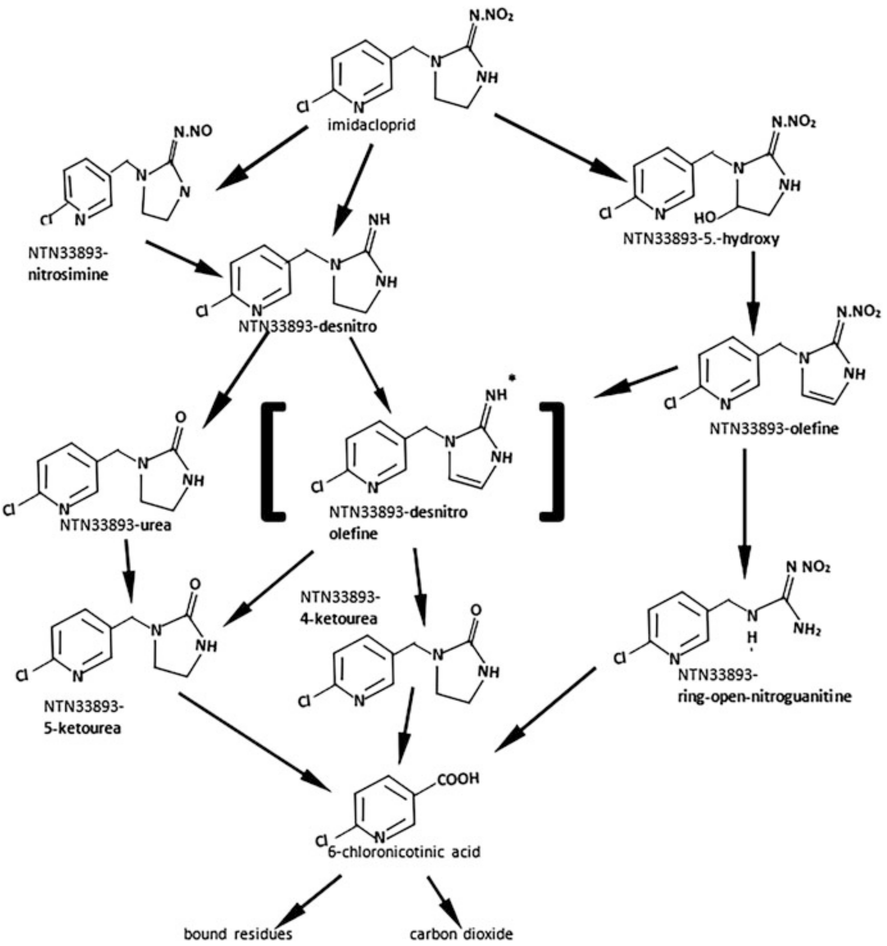


Fig. 5.4 Degradation pathway of imidacloprid (Adapted from Gupta et al. 2016)

innovative technology that is helping scientists to solve many major problems like pesticide degradation, which is more valuable than any chemical-mediated degradation method. Enzymes act on the pesticides in two ways: enzymes act on the pesticide and activate them in situ or enzymes target pesticides with particular physiological roles. These findings suggest the following:

1. Enzyme groups of transferase, isomerase, hydrolases, and ligases play a role in the central metabolism of biodegradation pathways.
2. Metabolism pathways synthesize some intermediate compounds, maintaining a topology between all the metabolism pathways.
3. To understand the exact pathway involved in the degradation, all the intermediates and all required enzymes should be considered (Ramakrishnan et al. 2011).

Table 5.10 Examples of pesticide-degrading enzymes produced by microorganisms

Enzyme	Organism	Pesticide	References
Oxidoreductase	*Agrobacterium sp.*	Glyphosate	Masotti et al. (2021)
Phosphotristerase	*Agrobacterium radiobacter* *Flavobacterium sp.*	Insecticide phosphotriester	Masotti et al. (2021)
Atzr A, B, C, D, E	*Pseudomonas strain AKN5*	Atrazine	Sharma et al. (2019)
DMO	*Pseudomonas maltophilla*	Dicamba	Ortiz-Hernández et al. (2013)
LinB	*Sphingomonas sp.*	Hexachlorocyclohexane	Manickam et al. (2008)
Ese	*Pseudomonas fluorescens* *Arthrobacter sp.*	Aldrin	Pang et al. (2022)
P450	*Pseudomonas putida*	Hexachlorobenzene and pentachlorobenzene	Kunze et al. (2009)
E_3	*Lucilia cuprina*	Synthetic pyrethroids and insecticides phosphotriester	Bhatt et al. (2019)

High-throughput techniques like MALDI-TOF could analyze responsible enzymes involved in the metabolic pathway of organisms for the degradation of pesticides.

Catalytic mechanisms of the total metabolism of pesticides can be divided into three phases. In the first phase, the main toxic compounds of the pesticide undergo hydrolysis or oxidation-reduction reaction on enzyme action and become water-soluble and transformed into a less toxic product. Generally, MFO, hydrolases, and esterase participate in this phase. In the second phase, organic products like amino acids and carbohydrates are attached with pesticides or the products formed in the first phase to make them more water-soluble so that they become less toxic. Mechanisms like dehalogenation, oxidation of amino, sulfur, and nitro groups, side chain metabolism, and ring degradation by adding water molecules in the ring take place by the action of the GST group of enzymes.

Furthermore, in the final phase, microbes produce and secrete some extracellular enzymes like peroxidases, hydrolytic, etc. to convert phase 2 metabolites further into nontoxic products (Ortiz-Hernández et al. 2011). Some pesticide-degrading microorganisms and enzymes involved in the degradation procedure are given in Table 5.10.

5.6 Genetic Studies of Pesticide-Degradation Mechanisms

To utilize the enzymatic-degradation pathway and to enhance pesticide-reduction efficiency, it is crucial to focus on the genetic basis or find the genes responsible for the use of pesticides. Identification of the genes that are present in the plasmid is more accessible than the genes that are present in the chromosomes. The microbes of the rhizobacteria family possess their pesticide-degrading or tolerating genes in their plasmids. With the help of recent advanced "omics" studies like metagenomics, transcriptomics identification of pesticide-degrading genes has become possible. One such important, highly conserved gene, the "opd" gene encodes the protein for the enzyme organophosphorus hydrolase found in many soilborne microorganisms like, *Pseudomonas diminuta, Agrobacterium radiobacter, Lactobacillus brevis, Enterobacter sp., Aspergillus niger, and penicillium lilacinum,* are responsible for degrading organophosphorus by utilizing it as a carbon source (Singh and Walker 2006). Methyl parathion hydrolase-encoding gene (mpd gene) is another such example, where methyl parathion is degraded by the microorganisms of the family *Pseudaminobacter, Ochrobactrum sp., Rhizobium radiobactor, Pleisomonas, Brucella,* etc. (Zhongli et al. 2001). Novel genes from the pesticide-degrading microorganisms could be identified and applied in the genetic engineering studies of pesticide degradation. One such study on cowpea was done, where the cowpea plant of Africa is naturally resistant to herbicide due to the presence of trypsin inhibitor-encoding genes. The genetic sequence of this gene could be put into a plasmid containing pesticide-tolerating microbes to enhance its pesticide-degradation ability.

5.7 Conclusion

Pesticide biodegradation has the potential to remove the problems related to environmental health. Adapting high-throughput technologies like "omics" technology helps to understand the enzymatic-degradation mechanisms of pesticides and the proteins or genetic materials involved in the process. This genetic information could be applied to generate either transgenic plants or genetically modified organisms for large-scale application and enhanced degradation efficiency. Recent research showed the positive sites of these genetic studies. Transgenic crops like the Bt crop were synthesized by incorporating the gene encoding the cry protein in the plant genome (which showed insecticidal properties) from the novel strain *Bacillus thuringiensis* (Bt). It is herbicide resistant and proven that Bt acts as a plant growth-promoting factor (Kumar et al. 2008). MCm5 strain of *Acinetobacter calcoaceticus*, FCm9 strain of *Brevibacillus parabrevis*, and *Sphingomonas sp*. RCm6 are identified as novel strains as they fall under the PGPR group and are efficient depredators of cypermethrin (Akbar et al. 2015). Another research group developed transgenic herbicide-resistant pearl millet *Pennisetum glaucum L* using the *Bar* gene (Girgi et al. 2002). However, the application is limited to the laboratory scale.

Isolation of novel microbial strains for biodegradation and enhancement of plant growth-promoting factors are important which might be found from millet soil as recent research isolated almost 24 species of 11 different genera of arbuscular mycorrhizal fungi (AMF) from the minor millet soil from Tamil Nadu, India. That indicating to focus on the millet-cultivating soils for isolation of a large number of novel microbial strains as pesticide-reducing agent (Mythili and Ramalakshmi 2022). These unique biodegrading and plant growth-promoting features of these novel strains could further be implicated in the bioremediation process, and also the alternative of pesticide could be achieved by incorporating these genes into plants and developing herbicide-resistant plants. This problem addresses focusing the research adaptation on the soil microbial ecosystem simultaneously with the enhanced degradation efficiency for field application.

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Chapter 6
Current Insights into the Role of Rhizosphere Bacteria in Disease Suppression in Millets

Vellaichamy Mageshwaran, Santhappan Paulraj, and Yalavarthi Nagaraju

Abstract Millets are considered as smart food or nutri-cereals and they are rich in micronutrients and vitamins and a better substitution of major cereals. There is an increasing awareness among the farmers, scientists, policymakers, and other stakeholders more particularly the consumers about the beneficial role of millets in day-to-day life. The millets are moderately to highly resistant to most of the pathogens due to their hardy nature and mostly grown under rainfed conditions. However, certain diseases of millets are considered as economically important due to the severity of yield losses it causes. The long-term use of fungicides to control the diseases causes irreparable damage to the environment. Though the development of resistant cultivars against the emerging pathogens is a superior strategy as part of integrated disease management, the occurrence of a wide pathogenic variability and the development of resistance in different populations of pathogens present a serious threat to the development of resistant cultivars. Biological control of millet diseases with rhizobacteria or endophytes is a novel tool for eco-friendly management of diseases. The foremost diseases of millets, which cause great economic losses are charcoal rot in sorghum, grain mold in sorghum, anthracnose in sorghum, downy mildew of pearl millet, blast of finger millet, and foot rot in finger millet. *Bacillus* sp., *Pseudomonas* sp., and *Trichoderma* sp. are the successful rhizobacteria used as biocontrol agents for the management of these diseases. The mechanism of biocontrol of these destructive diseases by the rhizobacteria involves production of hydrolytic enzymes, induction of systemic resistance through synthesis of pathogenesis-related proteins and defensive enzymes, production of antimicrobial compounds, hydrogen cyanide, and siderophore production. In addition, these rhizobacterial agents promote the plant growth by secretion of growth hormones (IAA, cytokinin,

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_6

gibberellins, etc.), solubilization of mineral nutrients, nitrogen fixation, etc. The present chapter deals with the biocontrol of major diseases of the millets by rhizobacteria and their mechanism.

Keywords Biological control · Disease · Lipopeptides · Mechanism · Millets · Phytopathogens · Rhizobacteria

6.1 Introduction

By 2050, world's population is expected to grow to almost 10 billion warranting the agricultural demand to 50% in the scenario of modest economic growth (FAO 2017). Agriculture remains as the primary occupation and more than 50% of population directly or indirectly depends on agriculture. In India, about 17–18% gross domestic product (GDP) depends on agriculture. In the present scenario of changing climate, there is an increasing threat to the major food grains production. Millets are considered as smart food or nutri-cereals and they are rich in micronutrients and vitamins and a better substitution of major cereals. There is an increasing awareness among the farmers, scientists, policymakers, and other stakeholders more particularly the consumers about the beneficial role of millets in day-to-day life. Every year, more than 40% of crop losses occur due to insect and plant diseases. Farmers use chemical pesticides to overcome this problem, but these chemicals rigorously pollute the environment and deteriorates soil fertility. Though, the development of resistant cultivars against the emerging pathogens is a superior strategy as part of integrated disease management, the occurrence of wide pathogenic variability and the development of resistance in different populations of pathogens present a serious threat to the development of resistant cultivars. The millets are moderately to highly resistant to most of the pathogens due to their hardy nature, and mostly grown under rainfed conditions. However, certain diseases of millets are considered as economically important due to the severity of yield losses it causes. The foremost diseases of millets, which cause great economic losses are charcoal rot in sorghum (*Macrophomina phaseolina*), grain mold in sorghum (*Fusarium* sp., *Curvularia lunata*, *Alternaria alternata*, *Phoma sorghina*, *Bipolaris* spp., *Aspergillus* spp., etc.), ergot in sorghum and pearl millet (*Claviceps pupurea*), anthracnose in sorghum (*Colletotrichum graminicola*), downy mildew of pearl millet (*Sclerospora graminicola*), blast of finger millet (*Pyricularia grisea*), and foot rot in finger millet (*Sclerotium rolfsii*). These pathogens often form resting structures such as microsclerotia, sclerotia, chlamydospore, or oospores and survive in plant debris, soil organic matter, etc. for longer periods.

Biocontrol is an eco-friendly way to reduce the use of chemical pesticides in agriculture. Plant growth-promoting rhizobacteria (PGPR) are considered as potential biocontrol agents, which is the primary indirect mechanism for promoting plant growth (Ramakrishna et al. 2019). The mechanism of biocontrol of plant pathogens and induction of plant growth by PGPR include synthesis of antimicrobial compounds (both extracellular and volatiles), hydrogen cyanide, siderophore, production of phytohormones, nitrogen fixation, mineral solubilization, etc. In addition, PGPR induce systemic resistance in host plant through synthesis of antioxidants, phenols, pathogensis-related (PR) proteins, hydrolytic enzymes, and the expression of genes related to jasmonic acid and ethylene pathways. These multiple mechanisms offer resistance to the host plant against pathogen invasion and supportive role in plant growth and performance (Jiao et al. 2021).

Bacterial endophytes are a new tool to control pathogenic microbes and enhance plants growth. Bacterial endophytes are present in almost all plant species. They present inside the root, stem, leaves, and fruits. They produce plant growth-promoting substances and antimicrobial compounds. Some of them are also responsible for phytoremediation and xenobiotic degradation (Morales-Cedeño et al. 2021). Bacterial endophytes show plant growth-promoting traits, including phosphate solubilization, N_2 fixation, phytoremediation and produce antimicrobial compounds, that have use in agriculture and medicine.

Santos et al. (2018) reviewed the metabolic effect of endophytic bacteria to the plant during its colonization in plant tissue. Bacterial endophytes showed antagonistic property against plant pathogens, increased plant growth traits such as biomass, grain production, and root length. Similarly, Rosenblueth and Matrinez-Romero (2006) reviewed the effect of bacterial endophyte on growth of the plant. Bacterial endophytes eliminate plant pathogens and solubilize minerals like phosphate, zinc, and potassium. Most of the endophytes have metabolic mechanism to colonize in plant tissue, but some of them are seedborne. Firdous et al. (2019) and Malea and Serepa-Diamini (2019) highlighted the importance of bacterial endophytes in plant growth promotion through phosphate solubilization, siderophore production and phytohormone production etc. Endophytes induce systemic resistance in plants, increase plant stress tolerance and able to degrade xenobiotic pollutant in its proximal environment. In one of the studies, a total of 360 fungal endophytes were obtained from tissues of leaf, stem, and root of sorghum plants. The endophytic fungal isolate *Trichoderma asperellum* showed broad spectrum of activity against grain mold pathogens. The study showed eco-friendly approach of sorghum disease management (Rajini et al. 2020).

Bacillus and *Pseudomonas* are most abundant bacteria in rhizospheric soils and frequently occur in isolation and screening of plant growth-promoting rhizobacteria. The major functions of these bacteria in agricultural soil are nitrogen fixation, nutrient supplementation (major and micronutrients), synthesis of antimicrobials, phytohormones production, plant growth support. They also play important role in plant defense against pathogens through induction of systemic resistance by synthesizing related metabolites and pathogenesis-related proteins. They have the ability to colonize the plant tissues as endophytes and do all the functions within the plants as described here (Govindasamy et al. 2010; Hyung et al. 2016). The bioagents employ multiple mechanisms for the biocontrol plant pathogens. The antagonistic property of *Bacillus* isolates included β-1,4 glucanase, chitinase, siderophore, hydrogen cyanide, ammonia, and other biocidal and thermostable, nonvolatile antifungal metabolites (Kumari and Khanna 2016). The fluorescent *pseudomonas* isolates suppress the most destructive diseases of the grain smut disease and brown spot disease of barnyard millet at the mid-hill regions of Uttarakhand, India through seed treatment, soil application of value-added vermicompost impregnated by fluorescent *pseudomonas*, and foliar application (Rawat et al. 2018). Among the bioagents, *Trichoderma* spp. is considered as popular bioagent for the control of soilborne pathogens. Rudresh et al. (2005) reported potential *Trichoderma viridie* and *T. harzianum* strains for the biocontrol of wilt complex disease in chickpea. Though *Trichoderma* sp. are reported as potential biocontrol agent, *Bacillus* and *Pseudomonas* are frequently encountered as rhizobacteria and endophytes and have multiple traits of plant growth and biocontrol of plant diseases.

6.2 Major Diseases in Millets

6.2.1 Charcoal Rot of Sorghum

Charcoal rot is the major disease impacting the root and stalk with great destructive potential on sorghum caused by *Macrophomina phaseolina*. Its host range includes legumes (groundnut, soybean, pigeon pea, and chickpea), cereals (sorghum, finger millet, and maize), fruits (banana, orange, pear, and apple), and vegetables (pumpkin, tomato, and cabbage). It is a serious disease in the Americas, Australia, Asia, and other dry continents. *Sorghum bicolor*, which covers more than five million acres in the Indian states of Maharashtra, Karnataka, and Andhra Pradesh, suffers a significant yield loss due to the disease. Perhaps, root exudates from seedlings of sorghum will cause the sclerotia of *M. phaseolina* to germinate. Seedling blight may result from the germinated sclerotia infecting the main root (Das et al. 2008a). The plants perish if the infection takes place before secondary roots form.

However, seedlings with less-severe infections live, form additional roots, and eventually develop into plants. Few isolates are host-specific, and the rest have a broad host range; however, the general biology is similar among the isolates. The major impediment in the control of this disease is the nonavailability of highly resistant cultivars. The pathogen also produces phaseoline, which can cause anemia in mice. Therefore, the disease has an indirect impact on animal health. Particularly in cultivars with large yields, where the infection becomes severe and destructive, there is a lack of genetic resistance to the disease at a high level (Das et al. 2008b). The term “charcoal rot” comes from the overgrowth of sclerotia in the vascular bundles, which gives the afflicted region a charcoal look. The symptoms are sometimes inconspicuous, making them difficult to diagnose. The affected plants look pale and thin, and the seedlings’ infection results in the symptoms’ damping. The yield losses may be partial to full when mechanical harvesting is adopted.

The goal of eco-safe disease control is to protect the root against fungal infection using microbial inoculants. *M. phaseolina* is a soilborne root pathogen that naturally forms heat-tolerant sclerotia. The pathogen survives predominantly as small, black sclerotia in diseased root and stem debris or soil after the decay of the plant material in which they were formed, and serves as the primary source of charcoal rot infection. The isolates have little or no saprophytic activity, thus the sclerotia acts as a primary source of inoculum for the disease initiation. In the infection site, isolations revealed the association of other fungi like *Fusarium moniliforme, Rhizoctonia solani*, *Helmenthosporium sativum*, and *Nigrospora sphaerica*. *F. moniliforme* is dominant under low soil temperatures; contrastingly, the charcoal rot pathogen prefers to grow in the presence of high temperatures. The pathogen targets plants that have lost strength due to unfavorable situations such as drought. The pooled effect of charcoal rot and lack of moisture result in yield reduction. The signs of the disease include root rot-caused plant lodging, pith and cortex disintegration, early stalk drying, and poorly developed stalks with low grain quality.

6.2.2 Grain Mold

One of the most significant diseases affecting sorghum is grain mold, which is brought on by a variety of different fungi. Infections in India are mostly caused by the genera *Fusarium*, *Curvularia*, and *Alternaria*, accounting for 80–90% of the infections (Das et al. 2020). Additionally, irregular, low-frequency detections of *Aspergillus*, *Cladosporium*, *Penicillium*, *Olpitrichum*, *Phoma*, *Drechslera*, *Botrytis*, and *Bipolaris* species have been made (Das et al. 2020). Depending on the cultivars and season, the disease can result in yield reductions of 30–100% (Kalaria et al. 2020). Grain mold causes losses in seed weight, grain density, germination, and seed viability, which significantly reduces seed-quality metrics during the wet season (Nida et al. 2019). The infected grain of sorghum is toxic and inappropriate for use as animal and bird feed (Das et al. 2020). The most economical, effective, and environmentally beneficial control technique is host-plant resistance (Mofokeng et al. 2017). Grain mold disease resistance in sorghum is facilitated by traits such panicle compactness, glume cover, glume color, grain hardness, polyphenols (tannins), flavonoids (flavan-4-ols), and antifungal proteins (chitinases, glucanases, sormatin, PR-10, and RIPs).

To enhance sorghum, it is important to avoid relying solely on the few sources of grain mold-resistant genes and alleles that are now accessible. In this regard, crop wild relatives and landraces provide a significant amount of potential as gene reservoirs (Brar and Khush 2018; Kyratzis et al. 2019). The need to gather, screen, and identify new sorghum germplasm carrying the grain mold-resistant trait that may be used for adaptation to rainfed agroecologies of India has arisen because of the intensifying effects of climate change. The pathogens can survive in crop residues and soil and spread through the air, and mainly cause discoloration of grains. It affects seed mass, grain density, seed germination, storage quality, food- and feed-processing quality, and market value.

6.2.3 Ergot or Sugary Disease

The fungus of the *Claviceps* genus is responsible for the fungal disease known as ergot, affecting grasses and cereals. Ergot or sugary disease is a major problem in millet production. It commonly occurs in sorghum and pearl millet. The sclerotia produced in the infection spread through seed and soil and have collateral host. Honeydew secretion attracts molds and insects and causes black discoloration in leaves and grains. The disease is caused by several distinct fungus species, however *Claviceps purpurea* (Fr.) Tul is the most prevalent according to ergot surveillance in Canada (Liu et al. 2020). The pathogen *C. purpurea* is a homothallic ascomycete fungus infecting susceptible hosts' florets by windborne ascospores. The species have been separated into numerous distinct lineages, each with a unique range of hosts, some of which can spread disease to a wide variety of cultivated and wild grass hosts (Shoukouhi et al. 2019). Different grass species have been shown to vary in their ability to withstand illness, with rye being more vulnerable and having more special reports of ergot toxins.

There is heterogeneity in the susceptibility levels between cultivars of grain species, showing the possibility of reducing the infection via breeding (Menzies and Turkington 2015). However, as our grain production systems expand to satisfy the demands of an expanding global population and changes in environmental and climatic circumstances, there is a forecast rise in the pressure from fungal diseases. Ergot is present on plant spikes as black masses (ergot sclerotia). The disease gets its name from these sclerotia, which grow instead of healthy kernels and resemble cock's spurs (or "argot" in French). When soil moisture and temperature conditions enable the release of wind-dispersed ascospores that infect florets during blooming, ergot sclerotia germinate during the disease cycle. The substance known as "honeydew" is secreted from infected florets because the ergot pathogen produces masses of conidia in a sugary matrix throughout the infection phase. Insects and rain splashes propagate the conidia in the honeydew, which causes secondary infections such as spreading sick grass to nearby cereal crops. During the growth season, ergot sclerotia form from the infected florets, replacing the kernels. They eventually fall to the soil's surface and overwinter in the field, producing a new source of ascospores the following year (Menzies and Turkington 2015). During the harvesting process, sclerotia can also be gathered with healthy grain.

Ergot sclerotia can form instead of healthy kernels, reducing the yield of cereal grains. The effect of ergot on yield losses for rye grown in Alberta in the middle of the 1970s was investigated by Harper and Seaman (1980). Due to fewer healthy kernels on infected spikes paired with a lower weight of healthy kernels on infected spikes as compared to uninfected spikes, they recorded a yield loss of 32%. These statistics were extrapolated to the field size, and it was determined that rye crops with 0.5–3.3% infected spikes would have a 0.16–1.1% lower grain production. Ergot's toxin generation by the fungus is one of the main causes of worry. Ergot alkaloids, which *C. purpurea* generates as secondary metabolites, have a tetracyclic indolequinoline ring structure as a structural defining characteristic. Honeydew contains ergot alkaloids in quantities of up to 1 part per billion, whereas sclerotia have amounts of up to 1 part per million (Orlando et al. 2017). In their excellent summary, Florea et al. (2017) covered the production of ergot alkaloids and the evolution of the genes involved.

Ergotism, a condition marked by swelling of the extremities, scorching agony, and the possible loss of limbs, hallucinations, delirium, muscular spasms, and convulsions, has been brought on by eating meals containing ergot alkaloid-contaminated grain (Pitt and Miller 2017). Animals ingesting feed tainted with ergot alkaloids experienced stunted development, interruptions in milk production in calves, and circulatory problems, resulting in gangrene and increase in body temperature. Grain functioning and quality are both impacted by ergot; the primary effects on quality are related to color and the presence of black specks in grain products.

6.2.4 Blast of Finger Millet

The most detrimental disease influencing the output and productivity of finger millet is finger millet blast, which is brought on by the filamentous fungus *Magnaporthe oryzae* (sexual amorph *Pyricularia oryzae*) and is widespread throughout all finger millet-growing regions. A finger millet plant's growth stages are infected by *M. oryzae*, which can eliminate the crop's grain output (Takan et al. 2012). Effective disease management strategies are required to assure global food security due to the relevance of finger millet blast disease, especially in arid and semiarid parts of Africa and Asia where the crop is mostly grown. A finger millet blast occurs at all growth stages, from seedling to grain formation, and causes significant yield loss to the tune of 28% (Netam et al. 2016). Blast disease brought on by *M. oryzae* impacts finger millet development and yield. The fungus infects numerous commercially significant crops, including wheat (Cruz and Valent 2017), foxtail millet, barley, rice, and various grass species in the Poaceae family (Han et al. 2018).

The disease may infect the leaves, stem, collar, node, neck, fingers, and roots under the right circumstances, leading to significant crop losses in all finger millet-growing regions. The early signs of the disease, known as little gray or brownish spots on the leaves, are caused by blast infections, which primarily affect the leaves. The pathogen spreads through crop residues and airborne conidia. In leaves, it causes spindle-shaped spots with brown margins and gray centers. It causes chaffiness or partial filling of grains. *Pyricularia grisea* is the most devastating (Cruz and Valent 2017), leading to yield loss of up to 100%, resulting in economic loss to farmers and, ultimately, a food crisis (Prajapati et al. 2013). The disease is common on finger millet in India and Africa. Blast pathogen is susceptible to minute temperature changes and other environmental factors. Control of blast disease is a serious and challenging issue relying heavily on chemical pesticides like organophosphorus fungicides, which have been reported to be highly effective (Kumar et al. 2019).

6.2.5 Foot Rot of Finger Millet

One of the significant new ragi diseases, foot rot caused by *Sclerotium rolfsii*, has become more prevalent recently, especially in irrigated and heavy rainfall areas (Nagaraja and Anjaneya Reddy 2009). The disease has been observed to result in more than 50% yield losses. Sclerotium-producing fungi, the fungus characterized by small tan to dark brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus *Sclerotium*. This pathogen affects a range of hosts, causing symptoms such as leaf spot in *Lotus meliloti*, finger millet foot rot, collar rot in chickpeas, and southern blight in sugar beets.

The use of fungicides may not be cost-effective since *Sclerotium rolfsii* is a soilborne disease and requires a field-wide drench, and fungicide resistance development was a prevalent issue. Considering *Sclerotium rolfsii* broad host range and ability to infect more than 500 crops, cultural measures, including crop rotation, were also ineffective against it. Although soil solarization is one of the most efficient methods for reducing soilborne diseases, it is only recommended for use in nurseries and is not practical at the field level. Studies were conducted to determine the optimum integrated management practices for preventing *Sclerotium rolfsii*-caused foot rot disease of finger millet while keeping these factors in mind. The important diseases in pearl millet are downy mildew or green ear caused by *Sclerospora graminicola*, rust caused by *Puccinia pennisetii*, smut caused by *Tolyposporium penicillariae*, and ergot/sugary disease caused by *Claviceps fusiformis*.

6.3 Biocontrol of Major Diseases in Millets by Rhizobacteria

The commonly used fungicides for the control of millet diseases are Thiram/Captan/Carbendazim (2–4 g/kg) in seed treatment, while Metalaxyl/Edifenphos/Mancozeb (500 g/ha) in case of field application. Systemic fungicides such as propicanozole, hexaconazole, and difeconazole , and contact fungicide, Mancozeb, were found to control soilborne pathogens. The long-term use of these fungicides affects the ecosystem and causes irreparable environmental damage. The development of resistant cultivars is considered a superior integrated disease management strategy. Due to high variability in pathogenesis, adequate host resistance is lacking to control soilborne diseases of millets like charcoal rot in sorghum and sheath rot in minor millets. These pathogens form resting structures such as sclerotia, resist extreme environmental conditions, and survive as viable propagules for years. When a conducive environment is created, these resting structures start germinating and invade the crop plants. Therefore, biocontrol agents are considered as the most sustainable strategy for managing these diseases.

6.3.1 Sorghum Charcoal Rot

The lack of suitable management practices instigates alternate strategies for investigation. After numerous biocontrol agents were field- and greenhouse-tested, biological suppression has emerged as a dependable element of integrated disease management of phytopathogenic fungi. The most important and potentially fruitful category of rhizobacteria implicated in the biocontrol of plant diseases among the bacterial biocontrol agents is fluorescent *Pseudomonas* spp. (O'Sullivan and O'Gara 1992). Considering how quickly and aggressively these bacteria colonize the root, they are perfectly suited as soil inoculants. Due to its ability to stop harmful soil microbes from colonizing the root surface, this property alone is proposed as a disease control strategy. Additionally, it has been suggested that the antagonistic effects of fluorescent pseudomonas are mediated via secondary metabolites such as antibiotics, cyanide, and siderophores.

The synthesis of siderophores, volatile compounds, extracellular antibiotics, efficient colonization of roots, and survival in the sorghum rhizosphere were only a few of the mechanisms that worked to suppress the diseases. *P. chlororaphis* strains are proven effective biocontrol agents for fungal root rot in tomato plants. It is recognized that none of the biological control mechanisms (such as antibiosis, competition, parasitism, and induced resistance) is mutually exclusive. Furthermore, a single biocontrol agent typically exhibits many modes of action (Whipps 2001). A rare actinomycete *Amycolaptopsis* BCA-696 had significantly reduced the incidence of charcoal rot disease in sorghum, in an experiment conduced for two seasons. The suppression of the disease by the actinomycete in sorghum was anatomically illustrated using scanning electron microscope (SEM) in which the conducting tissues, xylem and phloem vessels, were intact in actinomycete-treated plants while most of the stem tissues damaged in positive control (only *M. phaseolina*) inoculated plants (Gopalakrishnan et al. 2019).

Streptomyces albus CAI-21 exhibited significant biocontrol activity against charcoal rot pathogen *Macrophomina phaseolina* in sorghum. The number of internodes infected and length of infection was significantly reduced (>50%) in sorghum seeds treated with a cell suspension of *S. albus* CAI-121 compared to control (pathogen alone). The strain also showed similar biocontrol activity in other crops like sunflower, chickpea, pigeon pea, and rice (Gopalakrishnan et al. 2020, 2021; Ijaz et al. 2021). Similarly, Das et al. (2008a) isolated 126 fluorescent pseudomonades from sorghum fields in India. The fluorescent pseudomonas strains, viz., SRB 129, SRB 288, and SRB 127, had significantly inhibited the mycelial growth and germination of microsclerotia of *M. phaseolina*. The strain *Pseudomonas chlororaphis* was able to colonize the root tissue of sorghum plants which was confirmed through SEM. The antagonistic potential of *Trichoderma* spp., against the charcoal rot pathogen *M. phaseolina* in sorghum, was demonstrated by Gavali et al. (2021) and Yassin et al. (2021).

6.3.2 Sorghum Grain Mold

The development of fungus reduces grain production and quality, changing the chemical and nutritional composition. It causes grain discoloration, a reduction in dry matter owing to carbohydrate consumption, protein breakdown, and lipid degradation, all of which affect the product's digestibility and produce volatile metabolites that emit odors (Orina et al. 2017). The generation of heat and moisture in cereals during a mold infestation also adds to product spoiling, which has an impact on grain grade, marketing price, and client satisfaction. The primary cause for worry is the creation of dangerous secondary fungal metabolites (mycotoxins) that have a severe impact on both human and animal health (Mwakinyali et al. 2019). Mycotoxins, which harm cereal grains during growth or after harvest, are mostly generated by fungus of the genera *Aspergillus, Penicillium, Alternaria*, and *Fusarium* (Mikusova et al. 2013). Four biological control agents, viz., *Pseudomonas fluorescens*, *Trichoderma viride*, *Trichoderma harzianum,* and *Bacillus subtilis*, were evaluated for biocontrol of grain mold in sorghum, and the results showed *P. fluorescens* was found to be best among the four bioagents (Desai and Rakholiya 2021). The tripartite interaction of volatile compounds produced by antagonistic actinomycete *Streptomyces rochei* with the grain mold pathogens of sorghum, viz., *Fusarium moniliforme* and *Curvularia lunata*, was studied. The results showed the production of volatiles, furan 2-methyl (6.60%), benzene (4.43%), butanol, 2-methyl (18.67%), and myrcene (1.14%) which are involved in sesquiterpenoid and alkane biosynthetic pathway and the oxalic acid degradation pathway (Sudha et al. 2022).

6.3.3 Sorghum Anthracnose

Sorghum anthracnose disease caused by *Colletotrichum graminicola* is a fungal disease that causes significant yield loss and depends on the heavy use of chemical fungicides for its control. Although there is a large supply of germplasm resistant to the sorghum disease, little is known about the molecular genetics of anthracnose resistance. Typically, pathogen-derived chemicals that are sensed and the kinetics of immune responses are used to divide up plant immune pathways into two types. Pathogen-associated molecular patterns (PAMPs), which are pathogen-derived signature molecules, are recognized at the cell surface by pattern recognition receptors, which then activate pattern-triggered immunity (PTI). PTI is connected to different levels of quantitative resistance. Quantitative resistance to *C. graminicola* has been connected to phytoalexin 3-deoxanthocyanidin accumulation at the site of infection (Cui et al. 2015).

Effector trigger immunity (ETI), a powerful and frequently extremely specific disease resistance, is initiated by the second route, which is mediated by nucleotide-binding leucine-rich repeat (NLRs) proteins that recognize pathogen effectors inside of cells. The hypersensitive reaction (HR) and enhanced tolerance of infection (ETI) are related. An arms race between plants and diseases is fueled by the NLRs' high polymorphism and the infections' strong selection pressure. Both qualitative and quantitative resistance mechanisms are involved in the defense against anthracnose (Nelson et al. 2018). Twenty *Trichoderma* spp. were isolated from rhizosphere soil of sorghum. *Trichoderma* sp. T3 exhibited maximum inhibition of the anthracnose pathogen, and the lowest incidence of 54.9% was observed in *T. asperellum* T3 bioprimed plants 75 days after sowing (Manzar et al. 2021). Similarly, Teja et al. (2020) reported that among the various species of *Trichoderma* tested, *T. harzianum* was found to be a promising antagonistic against *Colletotrichum graminocola*, which causes anthracnose in sorghum. The major mechanism of inhibitory action of *Trichoderma* against the pathogen is extracellular production of hydrolytic enzymes. The culture filtrate of *T. asperellum* and *T. harzianum* at 10 and 25% concentration in the medium had 60–80% inhibition against *C. graminicola* (Manzar and Singh 2020). The integrated use of neem and fungicides were found to be effective for the management of foliar diseases (anthracnose, gray leaf spot, and zonate leaf spot) of sorghum (Atri et al. 2022). Thus, the reports clearly showed the use of biocontrol agent integrated with chemical fungicide was found to be effective in the management of foliar diseases in sorghum.

6.3.4 Downy Mildew of Pearl Millet

Downy mildew of pearl millet *Pennisetum glaucum* (L.) caused by *Sclerospora gruminicola* (Sacc.) Schroter is the most devastating disease in major pearl millet-growing areas of the world. The bacterial endophytes *Bacillus amyloliquefaciens, B. subtilis*, and *B. cereus* isolated from pearl millet showed multiple plant growth-promoting traits including HCN, siderophore and IAA production, 1-amino cyclopropane-1-carboxylate (ACC) deaminase activity, and mineral solubilization capability and protect the host plant from abiotic and biotic stresses (Kushwaha et al. 2020a, b; Sangwan et al. 2021). *P. fluorescens*, a biocontrol agent isolated from the rhizosphere of pearl millet, was effective against downy mildew disease of pearl millet. Among the treatments, viz., seed treatment alone, foliar spray alone, and seed treatment with a foliar spray, the application of seed treatment with foliar spray was found effective in suppressing the disease. The application of talc-based formulation of *P. fluorescens* in seed treatment (10 g/kg of seed) followed by foliar spray (1 × 10^8 CFU/mL) resulted in a decrease in percent incidence of downy mildew disease from 90% (control-untreated) to 85% (Umesha et al. 1998). The effect of seed biopriming with biocontrol agents on suppression of downy mildew disease in pearl millet was demonstrated (Atri et al. 2019). Among fungi, *Trichoderma* spp. has been widely studied for biological control of downy mildew in pearl millet. The oligosaccharides and/or total crude protein (TCP) extracted from *Trichoderma* spp. and *Trichoderma atroviridie* elicited the systemic resistance in pearl millet against the downy mildew pathogen *Sclerospora graminicola*. The peroxidase and lipoxygenase levels were elevated in

Trichoderma-inoculated plants (Nandini et al. 2017a, b). The effect of *Trichoderma*-mediated selenium nanoparticles (SeNPs) and *Trichoderma asperellum* as biocontrol agent studied individually and in combination to evaluate the suppression of downy mildew pathogen under greenhouse conditions. The results showed that the combination of SeNPs and *T. asperellum* resulted in significant suppression of downy mildew disease compared to individual application (Nandini et al. 2017c). In a similar attempt, trichogenic-lipid nanoemulsion elicited resistance against pearl millet downy mildew disease (Nandini et al. 2019). Similarly, anti-oomycetes secondary metabolites were reported from *Trichoderma* spp. and their role in biological control of downy mildew disease in pearl millet (Nandini et al. 2021a). The distribution and diversity of *Trichoderma* from rhizosphere samples of pearl millet grown under different agroclimatic conditions was studied and the results showed that *Trichoderma* spp., *T. asperellum* and *T. harzianum*, were found in higher frequency (Nandini et al. 2021b).

Attempts were also made to evaluate fungal endophytes on suppression of downy mildew disease in pearl millet. The fungal endophytes *Fusarium oxysporum*, *T. asperellum*, and *Acremonium* sp. were found to suppress downy mildew disease by 36% under greenhouse conditions (Nandhini et al. 2018). The seed biopriming with endophytic *T. hamatum* UOM 13 in pearl millet showed enhanced seed germination, seedling vigor, and suppression of downy mildew disease. The unveiling of mechanism of disease suppression showed the elicitation of defense enzymes, overexpression of pathogenesis-related proteins, and upregulation of salicylic acid biosynthetic pathway gene, isochorismate synthase (Siddaiah et al. 2017). The endophytic actinomycete isolates obtained from pearl millet roots showed antagonism against downy mildew disease in pearl millet. The seed coating of either spores or cell-free extract of actinomycete, *Streptomyces griseus* SJ_UOM 18-09 induced the systemic resistance which aids in biocontrol of downy mildew disease in pearl millet (Jogaiah et al. 2016). The systemic protection against downy mildew disease in pearl millet induced by conidial suspension of *Penicillium oxalicum* (Murali and Amruthesh 2015), cell wall glucan elicitors from *T. hamatum* UOM 13 (Lavanya et al. 2017, 2022) were reported.

6.3.5 Finger Millet Blast

Control of blast disease by employing biological agents can enhance agricultural productivity in crops such as wheat, rice, millet, and barley and is reported to protect against yield loss (Kumar and Kumar 2011; Prajapati et al. 2013; Cruz and Valent 2017). Microbes capable of colonizing the rhizosphere and plant roots can protect the plants from pathogens through antagonistic interaction (Buchenauer 1998; Whipps 2001) and induce systemic resistance to the plants, which can reduce fungal infection (Compant et al. 2005) by helping in reducing the pathogenic attack on the plant (Weller 1983).

Many studies have reported pseudomonads as bioinoculants with the potential to manage phytopathogens and promote crop growth under different agroclimatic conditions (Yasmin et al. 2017; Wang et al. 2015). Pseudomonads produce a wide range of metabolites, including antibiotics (2,4-DAPG, HCN, PLT, and PCA) and enzymes that exhibit antagonistic activity against phytopathogens (Müller et al. 2016; Vacheron et al. 2017; Yan et al. 2017). The disease incidence increases with increasing N levels (Kumar and Rashmi 2012). Management practices such as plant spacing and regulating the amount of nitrogenous fertilizer are essential measures to minimize the occurrence of blast disease. Nagaraja et al. (2012) observed that seed treatment to resistant varieties with either carbendazim at 2 g/kg or *Pseudomonas fluorescens* 6 g/kg was found to reduce blast disease incidence by two and a half times over control besides recording a high mean yield of 25.67 and 24.98 quintals/ha as against 21.06 quintals/ha in susceptible variety. Studies to evaluate the impact of bioinoculants on the control of blast disease in the finger millet have shown disease suppression in the range of 16–54% (Radjacommare et al. 2004; Kumar and Kumar 2011; Waghunde et al. 2013). *Pseudomonas* sp. MSSRFD41 seed priming and root dipping for control of blast disease and growth promotion in finger millet, which protected the finger millet against blast disease in the initial growth stage and foliar spray at the later growth stage through induced systematic resistance (Sekar et al. 2018). *Pseudomonas fluorescens*, *Trichoderma viridie*, and *Trichoderma harzianum* are reported to effectively control blast disease. Two sprays of *P. fluorescens* at 3 g/L were at par with carbendazim treatment (Netam et al. 2016). The combination of seed treatment at 10 g/kg seed followed by two sprays of *Pseudomonas fluorescens* was found to be at par with chemical fungicides treatment for the management of blast in finger millet (Prajapati et al. 2020). Rawat et al. (2022) reported that seed biopriming with *Trichoderma* was found useful in overcoming the salt stress conditions and providing protection against finger millet blast. The rhizospheric *Pseudomonads* exhibited excellent antifungal activity against fungal pathogen, *Magnaporthe grisea*. The *in-planta* assay showed more than 80% suppression of blast finger millet due to seed treatment and foliar spray with *Pseudomonas fluorescens* (Negi et al. 2017).

6.3.6 Foot Rot of Finger Millet

The contact fungicide Mancozeb was found effective for controlling soilborne pathogen *Sclerotium rolfsii*, causing foot root rot in finger millet. As an alternative to fungicides, the biocontrol agent, *Trichoderma harzianum* (GKVK), was found effective in managing this disease (Manu et al. 2012). Senthil et al. (2012) reported the application of rhizobacterial agent (*Pseudomonas fluorescens* Pf 1) talk formulations (0.6%) performed at par with a combination of fungicides (Carbendazim + Mancozeb) at 0.2% on effective control of blast disease in finger millet. The application of chitosan and bioagents, viz., *Trichoderma harzianum* and

P. fluorescens, had synergistic effect on inhibition of fungal root disease causative agent, *Sclerotium rolfsii* in finger millet (Malagitti et al. 2021).

6.4 Mechanism of Biological Control of Soilborne Phytopathogens by Rhizobacteria

The rhizobacteria possess different antagonistic traits for suppression of pathogens, including competition for colonization site or nutrients, production of volatile/diffusible antibiotics, synthesis of PR proteins in host plants, and production of enzymes and biocidal compounds. According to Zaim et al. (2013, 2016), the synthesis of enzymes that break down fungal cell walls, hydrocyanic acid (HCN), antibiotics, induction of systemic resistance (ISR), and antagonism against siderophores are some of the mechanisms used by plant growth-promoting rhizobacteria to suppress soilborne fungal pathogens. Figure 6.1 shows the mechanism of rhizobacteria's biocontrol of plant diseases. Additionally, the rhizobacteria and PGPR emit phytohormones, including IAA, gibberellic acid, and other similar substances, fixing atmospheric nitrogen and directly promote the development of host plants. In this chapter, we discuss antimicrobial metabolites, induction of systemic resistance, and HCN and siderophore production by antagonistic rhizobacteria as the primary mechanism of suppression of potential soilborne fungal pathogens. The frequency of soilborne diseases can be decreased by lowering the pathogen's inoculum density in the soil.

6.4.1 Antimicrobial Metabolites

Endophytes and other biocontrol agents are reported for synthesizing antimicrobial compounds of small molecular weights, usually less than 3.5 kDa. They are generally classified as lipopeptides (LP)-containing aminoacid chains with lipid moiety. It comprises a hydrophobic tail, usually a fatty acid, linked to a hydrophilic head between 4 and 12 amino acids. The significant microorganisms producing LPs are *Bacillus*, *Pseudomonas*, yeasts, etc. The cyclic lipopeptides contain a lactone ring in the aminoacid chain. The cyclic lipopeptides reported in rhizobacteria are bacilysin, subtilin, fengycin, surfactin, iturins, lichenysins, viscosins, amphisins, etc. (Biniarz et al. 2017). The structural details of selected cyclic lipopeptides are presented in Fig. 6.2.

An endophytic bacterium *Bacillus amyloliquefaciens* ES-2 isolated *from Scutellaria baicalensis* produced two families of secondary metabolites with broad-spectrum antibacterial and antifungal activities. This bacterium showed an antagonistic effect on plant pathogens, food spoilage bacteria and fungi, and foodborne pathogens. The electrospray ionization/collision-induced dissociation

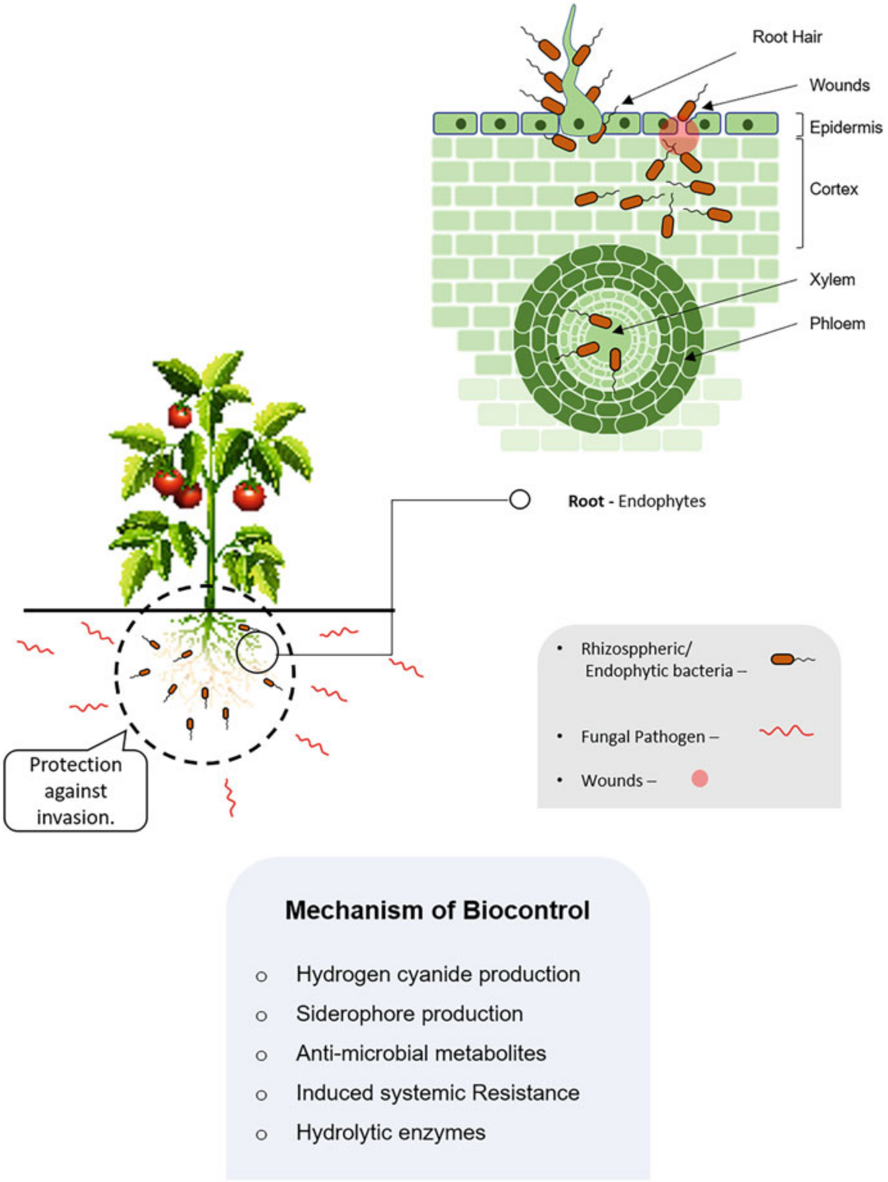

Fig. 6.1 Mechanism of biocontrol of plant pathogens by rhizobacteria

spectrum analysis revealed the antimicrobial metabolites belong to fengcyin and surfactin homologs, respectively. These lipopeptide antibiotics could be used against fungal plant diseases and in food preservation (Sun et al. 2006). Mageshwaran et al. (2012) isolated an antimicrobial compound from the endophytic bacteria *Paenibacillus polymyxa* HKA-15. The antimicrobial compound produced by

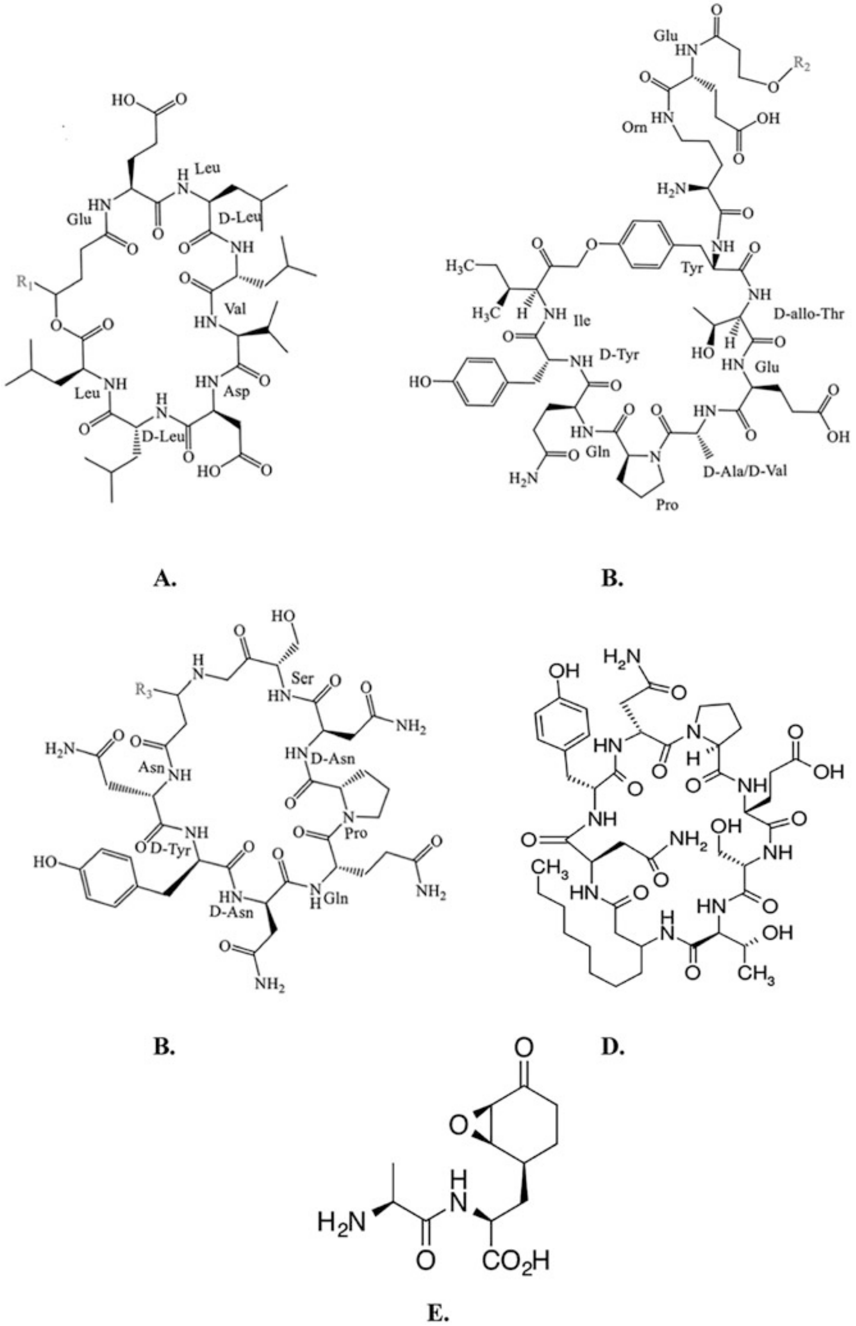

Fig. 6.2 Structural details of cyclic lipopeptides: Surfactin (**a**), Fengycin (**b**), Iturin (**c**), Bacilomycin (**d**), and Bacilysin (**e**)

P. polymyxa HKA-15 was partially characterized using mass spectrophotometry and SDS-PAGE. The lipopeptide compound produced by *P. polymyxa* HKA-15 showed antagonism against plant pathogenic fungi and bacteria, viz., *Xanthomonas campestris* pv. *phaseoli* M-5*, Xanthomonas oryzae, Ralstonia solanacearum*, *Xanthomonas campestris pv. phaseoli CP-1-1*, *Fusarium udum, Rhizoctonia bataticola,* and *Macrophomina phaseolina*.

Hyun et al. (1999) isolated an antibiotic compound from *Bacillus polymyxa* strain KB-8. The isolated compound was active against *Fusarium oxysporum* f. sp. *sesame,* causing Fusarium wilt of sesame. The media and pH were optimized for large-scale production of antibiotics produced by *B. polymyxa* KB-8. The optimized parameters were yeast-malt extract medium, pH 5, and 13-day incubation. The isolated antibiotic inhibited the growth of *F. oxysporum, Rhizoctonia solani, Alternaria mali, Colletotrichum gloeosporioides,* and *Phytopthora sp.* Soil drenching of lipopeptide antibiotic at 13.0 μg/mL and 26.0 μg/mL effectively inhibited the Fusarium wilt of sesame under greenhouse conditions. Kim et al. (2003) isolated and characterized antibiotics produced by *Bacillus* strain GB-0356 and GB-017. The partial characterization of antibiotic revealed that the antibiotics belongs to lactone and polyene groups. Both strains showed antifungal properties against *Rhizoctonia solani, Fusarium sp., Pythium sp., and Botrytis cineria.*

Screening of antimicrobial peptide genes elucidates the potential of a biocontrol agent for the synthesis of antimicrobial peptides which are active against pathogens. The biocontrol potential of *B. subtilis* RH5 against sheath blight of rice caused by *R. solani* was evaluated through the presence of antimicrobial peptides (AMP) biosynthetic genes (bacylisin, surfactin, and fengycin) (Jamali et al. 2020). *Pseudomonas brassicacearum* strain YC5480 inhibited the growth of plant pathogenic fungi like *Phytophthora capsici, Colletotrichum gloeosporioides,* and *Fusarium oxysporum,* but they also produce some compounds which inhibit the growth of seed germination. These compounds identified KS-1and KS-2 and chemically found to be 2, 4-diacetylphloroglucinol (DAPG) and 2, 4, 6-trihydroxyacetophenone (THA), respectively. Tendulkar et al. (2007) reported that *Bacillus licheniformis* BC98 could produce surfactin with molecular mass of 1035 Da and suppress the phytopathogens *Magnaporthe grisea*, *Curvularia lunata,* and *Rhizoctonia bataticola*. The endophytic *B. amyloliquefaciens* strain VB7 was ably harboring 10 diverse antibiotic biosynthesis genes, namely, *ituD*, *ipa14*, *bacA*, *bacD*, *bamC*, *sfP*, *spaC*, *spaS*, *albA*, and *albF*, which correspondingly produces the antibiotics iturin, bacilysin, bacilomycin, surfactin, subtilin, and subtilisin (Vinodkumar et al. 2017). Superior disease suppression activity was shown in the *Pythium*-cucumber and *Fusarium*-tomato pathosystems when biocontrol pseudomonads were able to synthesize the antifungal substance 2,4-diacetylphloroglucinol (Rezzonico et al. 2007).

6.4.2 Induced Systemic Resistance

The expression of certain defense-related genes in the host plant indicates the resistivity of the plants against the invading pathogens. Gurjar et al. (2011) studied the expression of defense-related genes during wilting in chickpea caused by *Fusarium oxysporum* f. sp. *ciceri*. The upregulation of defense-related genes such as chalcone synthase (*CHS*) gene, isoflavone reductase (IFR) gene, and 60s ribosomal protein (60srp) in chickpea root tissues is resistant cultivar (Digvijay) than the susceptible cultivar (JG62). The presence of plant growth-promoting bacteria as endophytes inside the host plants stimulates the production as well as activity of pathogenesis-related (PR) proteins such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), chitinases, lipoxygenases, and glucanases to suppress the invading pathogen and host plant self-defense mechanism. The inoculation of antagonistic bacteria (*Bacillus* and *Pseudomonas*) along with *Mesorhizobium* in chickpea plants pre-challenged with *Fusarium oxysporum* f. sp. *cicero*-induced PAL, malonic aldehyde concentrations in stem tissues revealed the role of inoculated bacteria on plant defense response to reduce the disease incidence and to improve plant growth and yield (Kumari and Khanna 2019). Nagendran et al. (2014) reported that in rice plants pre-challenged with *R. solani* causing sheath blight disease, higher induction of defense-related enzymes, PO, PPO, and PAL and higher accumulation of total phenols were observed in *B. subtilis* var. *amyloliquefaciens* (FZB24)-inoculated rice plants than in untreated (pathogen alone). Similarly, Jamali et al. (2020) reported that in addition to enhancing plant growth-promoting traits, the inoculation of *B. subtilis* RH5 triggered defense-related enzymes, PO, PPO, and PAL in rice plants pre-challenged with *R. solani*. The endophytic strain *Bacillus* sp. 2P2 showed strong inhibition against collar rot pathogen in tomatoes (Sahu et al. 2019). The strain-induced systemic resistance in the host plant elicited PAL, PO, and PPO and upregulated pathogenesis-related proteins PR1a, PR2a, and PR3, which are responsible for the synthesis of glucanases and chitinases. Sahu et al. (2020) reported that the PAL and PO activity was higher in *R. solani* pre-challenged, and endophytes-inoculated rice plants, and the value recorded was 30 nM cinnamic acid h^{-1} g^{-1} fresh weight and 3.2 units min^{-1} mg^{-1} fresh weight, respectively.

6.4.3 HCN and Siderophore Production

Hydrogen cyanide (HCN) is the secondary metabolite produced at the end of the exponential phase and the start of the stationary phase. It is synthesized by *hcn* ABC, which oxidizes glycine to produce HCN and CO_2 (Laville et al. 1998), and it is volatile and controls the growth of surrounding microorganisms (Akhtar and Siddiqui 2006). HCN inhibits electron transport, which disrupts the cell's energy supply and causes cell death. Especially, cytochrome oxidase and other metalloenzymes are inhibited by HCN and hence are toxic for all aerobic organisms. HCN production has been demonstrated in many bacterial genera and endophytes, including *Alcaligenes, Aeromonas, Bacillus, Pseudomonas*, and *Rhizobium* species (Bhuiyan et al. 2008). According to several studies, volatile compounds may also help to prevent several plant diseases (Hammerbacher et al. 2019). According to

Ramette et al. (2003), fluorescent *Pseudomonads* associated with several plants used HCN, a broad-spectrum antibacterial chemical, to control root diseases biologically. HCN inhibits electron transfer, and through reversible mechanisms of inhibition, it interferes with the efficient functioning of the enzymes and natural receptors (Corbett 1974). For example, *Macrophomina phaseolina* can be efficiently controlled by *Pseudomonas fluorescence* production of HCN (Reetha et al. 2014). Many rhizobacteria produce hydrogen cyanide (HCN), which is known to be involved in the biological control of pathogens (Defago et al. 1990). *Phytophtora infestans* were inhibited by pseudomonas strains associated with potatoes at various developmental stages. Multiple mechanisms, including the production of hydrogen cyanide, were identified as potentially contributing to this anti-oomycete activity using a comparative genomics approach. In this study, HCN-negative mutants (*Dhcn*) were generated and compared their activities to those of their corresponding wild types to quantify the contribution of HCN in biocontrol (Anand et al. 2020). Numerous species have evolved defense mechanisms against cyanide poisonings such as cyanide-insensitive oxidases or chemical conversion of HCN to thiocyanate by the rhodanese enzyme (Cipollone et al. 2007; Frangipani et al. 2014; Cunningham et al. 1997).

Iron is a vital element. All living organisms require iron for many cellular processes such as the electron transport chain and as a cofactor for many enzymes (Litwin and Calderwood 1993). Microorganisms growing under aerobic conditions need iron for various functions, including the reduction of oxygen for the synthesis of ATP, the formation of heme, and other essential purposes. Siderophores are the low molecular weight iron-binding substances secreted by endophytes for the acquisition of iron present in the environment, thereby limiting iron availability to the plant pathogens in the same niches. Thus, endophytes suppress the pathogens by limiting iron availability in the environment. Under iron-limiting conditions, siderophores, a low molecular weight ferric iron-chelating compound, are secreted extracellularly, with their main objective being to provide iron to iron-deficient cells (Sessitsch et al. 2004). Three primary forms of siderophores—catecholate, hydroxymates, and carboxylates—are formed depending on the functional group. Using radio-labelled ferric siderophores as a sole source of iron demonstrated that plants could take up labelled iron by PGPB. Some PGPR strains form siderophores that bind Fe^{3+}, reducing their availability to specific local microflora species (Kloepper et al. 1980). When grown on chrome azurol S with an iron deficiency, *P. fluorescens* produced extracellular siderophores (Suryakala et al. 2004).

The other genera reported are *Aeromonas*, *Azadirachta*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Serratia,* and *Streptomyces* sp., etc. Siderophores, which provide iron to plants, also may assist in reducing the stresses that high soil levels of heavy metals place on plants. Soil bacteria enhance plant iron intake (Diels et al. 2002). According to O'Sullivan and O'Gara (1992), chemicals like siderophores are mostly produced during the exponential growth phase, when the population needs more nutrients for cell division. Like most secreted pseudobactin molecules bind to Fe in the media, the pseudobactin Fe complex has a high stability factor (Chen et al. 1994; Loper and Henkels 1999). The antagonistic bacteria may scavenge most of the available iron and prevent the growth of fungal pathogens since the siderophores produced by biocontrol bacteria have a higher affinity to iron than those produced by fungal pathogens. Recently, Ji et al. (2014) reported that the endophytic diazotrophs *Klebsiella pneumoniae, B. subtilis*, and *Microbacterium* sp. displayed antagonistic behavior toward *R. solani* as a siderophore producer.

6.5 Conclusion and Future Scope

PGPR are a group of bacteria that colonize the plant roots, promote plant growth, and protect the plants against invasion of pathogens. Certain PGPR are considered as endophytes that have the unique ability to enter the host plant and resides inside different plant parts of host. PGPR provide direct and indirect benefits on growth and development of crop plants. The direct benefits include fixation of atmospheric nitrogen, solubilization of nutrients, secretion of phytohormones and antimicrobial metabolites, and synthesis of ammonia, siderophore, and HCN,. which are helpful in plant growth and yield and provide protection against invading pathogens. The indirect benefits include induction of systemic resistance through synthesis of PR proteins and phenols, PAL, PPO, and PO, thus offering resistance against biotic stress. The *Bacilli* are reported for the synthesis of novel cyclic and acyclic lipopeptides, which are receiving much attention in biotechnological applications in agriculture and beyond especially in medical fields due to increasing resistance of pathogens against conventional pesticides and chemicals. The genera *Bacillus, Pseudomonas*, and *Trichoderma* are frequently encountered while screening for microbial inoculants in agricultural applications. *Bacillus, Pseudomonas*, and *Trichoderma* offer benefits to the host plant directly and indirectly through multiple plant growth-promoting traits as described here. Therefore, the microbial inoculants *Bacillus*, *Pseudomonas*, and *Trichoderma* are deemed to be considered as best for plant development and biocontrol of key diseases in millets and other crop plants, the authors conclude.

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Chapter 7
Comparison of Rhizospheric Functional Diversity Between Chemically Fertilized and Bioinoculated Millet

Rohini Mattoo and Suman B M

Abstract Millets are ancient nutritious grains widely used as food and fodder. Millets grow well in most climates and easily yield sufficiently even on nutrient-deficient soils and rain-fed conditions with no chemical inputs. They have been used in intercropping and mixed cropping practices. However, little is known about the rhizosphere-microbe interactions and the type of microbial abundance in the soils of millets. The rhizosphere is a unique niche, where diverse microbes and macroorganisms thrive under the influences of plant roots and in turn are affected by climatic and anthropogenic influences. While chemical fertilizers helped achieve faster growth and yields during the mass famine, continuous and excessive fertilizers create a hazardous environment and thus diminishing soil health, in turn affecting microbial diversity in the plant rhizosphere. Diminishing microbial diversity in the soil in turn affects soil properties, biogeochemical cycles, and reduction in soil flora, leading to nutrient imbalances. Biological solutions such as bioinoculants serve as alternate and sustainable methods in order to engineer the agroecosystems to enhance crop yields and benefit the environment. This chapter highlights and compares the functional microbial diversity between chemically treated and bioinoculated millets. We also discuss future prospects for sustainable methods in order to improve microbial diversity and hence, food security despite climate change.

Keywords Millets · Rhizosphere microbes · Bioinoculants · Microbial diversity

7.1 Introduction

Millets are ancient grains predominantly cultivated and consumed in India. They are so ancient in India and China that they are still not known by their English names. Millets were counted as the major grains in India as about 50 years ago and

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_7

Table 7.1 The nomenclature of Indian millets

Sl. No.	Common names in India	Scientific name	English common names
1.	Rajika, ragi, nachni	*Eleusine coracana*	Finger millet
2.	Varjari, bajra	*Pannisetum glaucum*	Pearl millet
3.	Kodara, haraka, kodra, kodua	*Paspalum scrobiculatum*	Kodo millet
4.	Chinaka, baragu	*Panicum miliaceum*	Broomcorn/proso millet
5.	Kanguni, kakum, kangni, navane	*Setaria italica*	Foxtail millet
6.	Yaavanala, jowar, jola	*Sorghum bicolor*	Sorghum
7.	Syamaka, sanva, madira	*Echinocloa esculenta*	Banyard millet
8.	Korale	*Brachiaria ramose*	Brown top millet
9.	Sama, moraiyo, kutki, shavan	*Panicum sumatrense*	Little millet
10.	Kuttu	*Fagopyrum esculentum*	Buckwheat
11.	Rajgira, ramdana, chola	*Amaranthus hypochondrius*	Amaranth millet

accounted for 40% of the grains; but post the green revolution, production of rice increased doubly and wheat tripled, causing many local food grains to be suppressed. The ancient farming of millets promoted multi-crop farming. There are many varieties of millets, and this has provided many economic, environmental, and health benefits.

With recent advances and awareness in our understanding of crops for climate adaptation among the many crops, millets are gaining recognition for their ability to grow in conditions where there is insufficient water, high temperatures, etc. India alone accounts for about 40.62% of total millet production in the world (Chandra et al. 2021). Millets could resist pests and diseases with a short growing season compared to other cereals, resulting in low production costs (Hassan et al. 2020). Further to this, pearl millet, also a drought-tolerant cereal, can yield more grain and thrive under rainfall as low as 200–250 mm (Bidinger and Hash 2004). Depending on the conditions and soil type, millets exhibit variations in their nutritional content. The factors contributing to the nutrition of millets are not completely known; however, the soil type is important for the mineral composition of millets. Given the current challenges of climatic changes and water scarcity, coupled with overpopulation, interests have been developed in millets. Hence, farmers, scientists, policymakers, and processed food industries must unanimously come together to ensure research and development of the nutritional value of millets for feed and fodder.

These have been grown locally in all states of India although their cultivation was abandoned and suppressed in many states due to changes in agricultural practices, cultural changes, etc. The nomenclature of some important millets grown in India is given in Table 7.1.

Due to their tolerance to biotic stress like pathogen attack and abiotic stress such as drought, millets have been successfully grown since ancient times even in arid and semiarid regions with significant yield under low-quality land and minimum input

(Saxena et al. 2018). Unlike most modern varieties of rice, wheat, maize, etc., millets do not require large chemical applications and yield sustainably under good soil conditions. Compared to nitrogen requirements for rice, maize, and wheat, which are 90–120 kg N/ha, 125–160 kg N/ha, and 70–200 kg N/ha, respectively, for millets it has been observed to be 40–60 kg N/ha (Prasad et al. 2014; Wang et al. 2018). Similarly, phosphate requirements for rice, maize, and wheat are 30–40 kg P/ha, 55–80 kg P/ha, and 20–40 kg P/ha, respectively, while for millets, it is 12–25 kg P/ha (Prasad et al. 2014; Wafula et al. 2016). Along with this, millets have reduced dependence on pesticides due to their disease-resistant traits Thus they are less vulnerable to pest attack when compared to other cereals and they also have several years of shelf life due to their resistance to storage pests (Antony Ceasar and Maharajan 2022). Millets have deep roots, about 90 cm, compared to other crops such as groundnut, which is 70 cm deep (Gregory and Reddy 1982). The deep and branched rooting system in certain millets is beneficial in the uptake of nutrients like nitrogen, phosphorus, and potassium from the soil, again highlighting why millets do not have a requirement for high quantities of fertilizers, unlike other cereals (Wang et al. 2018).

Addition of chemical fertilizers has been considered a threat to the traditional cultivation of millets which has so far been free of chemicals. But with the recent goals of higher production, farmers may include quick measures such as synthetic fertilizers and chemical pesticides without considering natural methods of yield enhancement.

7.2 Why Chemical Fertilizers Are added to Millets?

Chemical fertilizers consist of synthetic macronutrients (e.g., nitrogen, phosphorus, and potassium) and micronutrients applied to crops to promote rapid plant growth and productivity. The Green Revolution led to excessive use of chemical fertilizers, which was successful in overcoming global issues like food scarcity and hunger (Sharma and Singhvi 2017). The application of synthetic fertilizers directly for cultivation of crops encourages higher yields per hectare. Although crops like millets are capable of growing under low-nutrient conditions, the addition of chemical fertilizers by farmers boosts their yield and productivity.

7.2.1 Effects of Chemical Fertilization on Microbial Diversity in Millets

Insights from the rhizosphere of finger millets treated with synthetic chemicals (NPK (100:50:50) yielded data on the effects of these on plant growth and soil microbial communities. The treatment using chemical fertilizer increased the plant height and yield compared to the control plants where no chemicals were added and compared to the treatment carried out in the presence of bioinoculants. However, the chemical treatment led to disturbance in soil microbial populations where it was observed that a reduction in beneficial microorganisms associated with soil health, fertility, and crop productivity occurred. Also, the treatment simultaneously increased opportunistic pathogenic species like *Flavobacterium* in the rhizosphere. In addition, the chemical fertilizer led to an increase in the growth of *Firmicutes* and *OD1*, which seemed to have been favored due to anoxic conditions that resulted in the soil following the addition of chemical fertilizers to the finger millet. Further, it was observed that the *Bacteroidetes* and *ZB2* involved in degradation of compounds and fermentation enriched in the millet rhizosphere treated with chemical fertilizer, indicating an environment containing complex compounds (Mattoo et al. 2021) since these get accumulated in the soil for a long time. The NPK-balanced fertilizer treatment combined with the organic manure can significantly increase the enzyme activities, bacterial community compositions, and yields in foxtail millet. Higher abundance of the *Actinobacteria, Bacteroidetes, Verrucomicrobia* phyla, and genera *Devosia*, *Mycobacterium, Opitutus*, *Chitinophaga*, *Rubrobacter*, and *Nonomuraea* was observed when the foxtail millet was treated with chemical fertilizer (NPK treatment) (Xu et al. 2019).

Chemical fertilizers (N and P) alone lower the population of nitrogen-fixing bacteria like *Azotobacter* and phosphate-solubilizing bacteria (PSB) in pearl millet-wheat intercropping system. Integrated use of chemical fertilizers and organic manure sources like FYM, and wheat straw had a stimulating effect on the population of *Azotobacter* and PSB and improved the viable counts thereby improving the soil fertility and crop productivity due to increased availability of utilizable nutrients for the plant growth (Kumar et al. 2021).

Compound fertilizer treatment (750 kg ha^{-1}, N-P_2O_5-K_2O, ratio: 22:9:9) in broomcorn millet showed higher abundance of *Actinobacteria*. *Phormidium sp*., which survives in desiccated environments, and *Cellulosimicrobium sp*., which can degrade complex compounds, were the bacterial genera found in high abundance in rhizospheric soil (Cao et al. 2022).

Acidobacteria, *Chloroflexi*, *Nitrospirae*, and *Planctomycetes* were more abundant in rhizosphere of proso millet treated with nitrogen fertilizer (150 kg ha^{-1} N) and phosphorus fertilizer (100 kg ha^{-1} P_2O_5) compared to organic fertilization (15,000 kg ha^{-1} of organic fertilizer consisting of 40.0% organic matter and 50 million effective microorganisms) (Liu et al. 2020).

Chemical treatment (130 kg of urea-N ha^{-1}) lowered the abundance of *Actinobacteria* and *Firmicutes* in rhizosphere of sorghum while *Proteobacteria* was enriched (Lavecchia et al. 2015). To this date, most studies reveal that there are disturbances when chemical treatments to enhance crop growth and yield are undertaken. The balance between the beneficial bacteria and those found in anoxic conditions get not only disturbed but also pose challenges to eradicating the excess chemicals from soil which otherwise lead to eutrophication. Also, methods to combine treatments may be useful to millets in order to rapidly enhance their yields; however, since India has achieved grain sufficiency, the need for sustainable natural methods is the need of the hour. Rejuvenation of soil health should be prioritized in order to avoid excess use of chemical fertilizer.

7.3 Why Bioinoculation of Millets?

Although the Green Revolution made India self-independent in the production of food grains, the indiscriminate application of synthetic fertilizers to increase crop productivity over the years is associated with high environmental risks and modification of soil physiochemical and biological properties. Continuous use of chemical fertilizers alters soil pH and increases acidification, resulting in decline of soil organic matter, thereby reducing the quality of agricultural soils (Pahalvi et al. 2021; Mattoo and Gowda 2022). Chemical inputs suppress beneficial microorganisms, causing the predomination of pathogenic microorganisms that negatively impact plant and soil health (Mattoo et al. 2021). This is also illustrated in Fig 7.1.

The increasing risks associated with chemical fertilization have shifted the focus toward the use of alternative treatments like bioinoculation (Mattoo and Gowda 2022). Beneficial microorganisms support plants to adapt to biotic and abiotic stress in addition to improving growth and yield characteristics (Sagar et al. 2020). Bioinoculation enhances the beneficial microbial counts and keeps a check on pathogenic microorganisms, thereby preventing the emergence of diseases resulting from climate change and anthropogenic factors. On the other hand, chemical fertilization contributes to increased pathogen load leading to spread of diseases in terrestrial and aquatic environments (Fig. 7.2).

Nitrogen-fixing and phosphate-solubilizing bacteria *like Azospirillum lipoferum, Azotobacter chroococcum, Acetobacter*, and *Bacillus megaterium* have shown to significantly increase the yield of pearl millet (Latake et al. 2009). Co-inoculation of *Azospirillum* and AM fungi enhanced finger millet yield by 36.8% compared to uninoculated finger millets (Bama and Ramakrishnan 2010). Application of recommended dose of fertilizer (50:40:25 NPK) along with 7.5 tonnes/ha of farm yard manure produced significantly higher grain yield and farm yield (Govindappa et al. 2009). When FYM and biofertilizers (Azotobacter/Azospirillum and phosphate-solubilizing bacteria) were used, it had resulted in a nonsignificant increase in nutrient uptake over control; however, when they were used with 50% NP + 100% K dose, better nutrition of the crop was observed (Dwivedi et al. 2016).

Bioinoculation studies of some important millets and their effect on plant growth and yields are summarized in Table 7.2.

Few studies based on the effects of chemical treatment and co-inoculation on millet growth and yield parameters are summarized in Table 7.3. Chemical treatment ensures faster yields but can be harmful in the long run. Co-inoculation with microorganisms is a better choice as it exerts beneficial effects on soil and plant health, in addition to high yields.

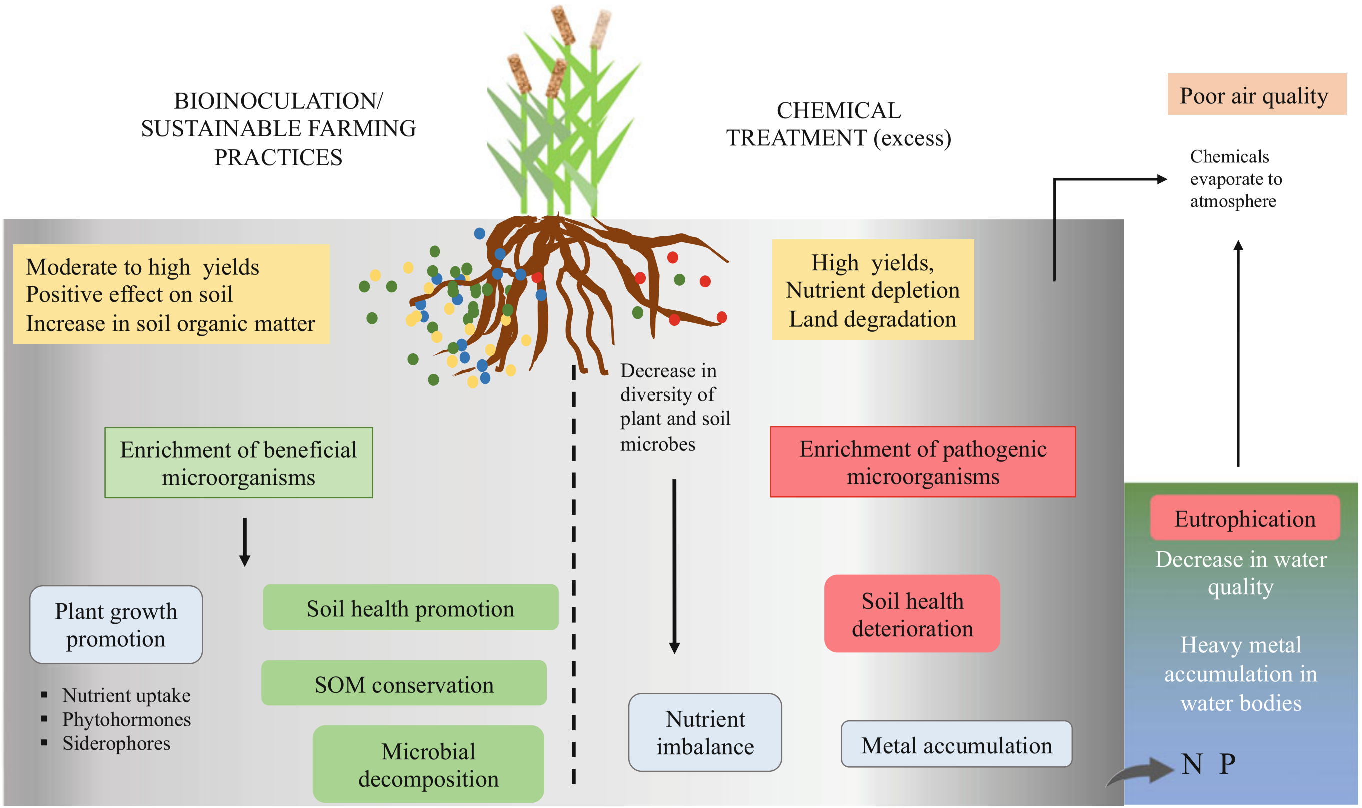

Fig 7.1 Effect of sustainable methods and chemical treatment on soil health and plant productivity. Bioinoculation and sustainable farming practices positively influence soil and plant health. Beneficial microorganisms enriched in the soil contribute to plant growth promotion, decomposition, and organic matter conservation. Chemical treatment decreases the diversity of plant and soil microbes, resulting in pathogenic load and nutrient imbalance. Toxin accumulation and subsequent release into water bodies and atmosphere decrease water and air quality

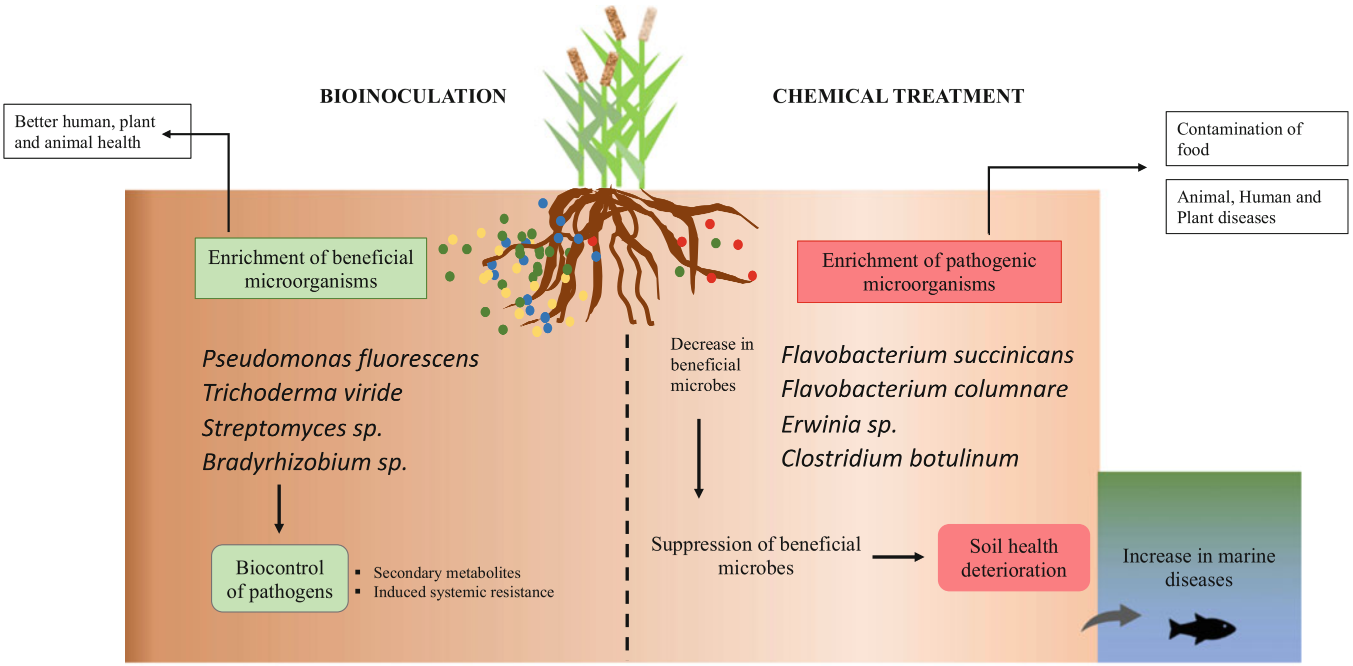

Fig 7.2 Effect of bioinoculants and chemical treatment in disease prevention. Microorganisms used for bioinoculation produce secondary metabolites, antimicrobial compounds, and induce systemic resistance in plants against pathogens resulting in better plant health. The use of chemical treatment for cultivation of millets results in suppression of beneficial microbes leading to decreased plant health. Chemical contamination of food increases animal and human health risks. Leaching of chemicals into water bodies affect marine animals

Table 7.2 Effect of bioinoculation of microorganisms on millets

Sl. No.	Millet type	Bioinoculant	Effect	Role	Reference
1.	Pearl millet	*Pseudomonas fluorescens Azotobacter chroococcum Azospirillum lipoferum Acetobacter diazotrophicus Trichoderma viride*	Increased plant height, dry weight, ear length, grain and stover yields	Nitrogen fixation Phosphate solubilization Biocontrol	Singh and Raghuvanshi (2016)
		Azotobacter sp. *Azospirillum sp.*	Increases plant height, dry weight, test weight, grain yield, straw yield	Nitrogen fixation Phytohormone production	Mourya and Singh (2022)
2.	Sorghum	*Azospirillum sp.*	Increased crude protein content and yield, crude fiber content, ash content	Nitrogen fixation	Paul et al. (2019)
		Azospirillum sp. Phosphate-solubilizing bacteria	Enhanced plant height, biomass, grain and stover yields	Nitrogen fixation Phosphate solubilization Drought tolerance	Rani et al. (2021)
3.	Finger millet	*Azotobacter chroococcum* *Bacillus megaterium* *Frateuria aurantia* *Pseudomonas fluorescens* *Trichoderma viride*	Increased plant height, shoot weight, root weight, root length, number of leaves	Nitrogen fixation Phosphorus solubilization Potassium solubilization Biocontrol	Mattoo et al. (2021)
		Rhizophagus irregularis, Pseudomonas fluorescens	Increased root length, root and shoot biomass	Nitrogen fixation Phosphorus solubilization Drought tolerance	Saharan et al. (2018)
		Rhizophagus intraradices	Increased seedling height, shoot and root weight, root length, phosphorus content	Phosphorus solubilization Drought tolerance	Tyagi et al. (2021)
		Rhizophagus intraradices, Piriformospora indica	Increased shoot and root length, biomass chlorophyll content	Nutrient acquisition Drought tolerance	Tyagi et al. (2017)

4.	Foxtail millet	*Rhizobium sp.* *Bacillus sp.*	Increased grain yield, dry weight, chlorophyll content and protein content in seeds Reduced maturation time	Nitrogen fixation Nutrient uptake	Khatri et al. (2016)
		Azospirillum lipoferum A1, Azospirillum lipoferum A2, Phosphate-solubilizing bacteria	Increased plant height, shoot and root dry weight, panicle, and seed weight	Nitrogen fixation Phosphate solubilization Phytohormone production	Rafi et al. (2012)
5.	Barnyard millet	*Methylobacterium sp.*	Increased seed germination, root length, panicle length, no. of tillers	Nitrogen fixation Phytohormone production Siderophore production	Poorniammal et al. (2020)
6.	Kodo millet	*Azotobacter sp.* *Azospirillum sp.* Phosphate Solubilizing Bacteria	Increased grain yield, straw yield, nutrient uptake, and mineral content	Nitrogen Fixation Phosphorus solubilization Potassium solubilization	Dwivedi et al. (2016)
7.	Amaranth millet	*Pseudomonas gessardi* *Bacillus* sp. *Erwinia rhapontici*	Increases shoot and root length, fresh and dry weight, chlorophyll, sugar, phenolics and flavonoid content	Phosphorus solubilization Nitrogen fixation Potassium solubilization	Devi et al. (2022)
8.	Proso millet	*Pseudomonas fluorescens*	Increased plant height, number of tillers, seed yield, chlorophyll content	Nutrient solubilization Production of plant growth regulators	Sumalata et al. (2020)

Table 7.3 The effect of chemical treatment and co-inoculation on millet characteristics and microbes

Sl. No.	Millet	Treatment		Effect on		Other comments	Reference
		Chemical	Bioinoculant	Plant	Microbes		
1.	Pearl millet	NPK (30:45:30 kg ha^{-1})	—	Increased plant height, biomass, panicle density, number of grains per panicle	—	Fertilization enhanced early growth, but plants were N-deficient at later stages	Hirooka et al. (2021)
		NPK (0.117 kg N, 0.117 kg P_2O_5, 0.117 kg K_2O/18 m^2 combined with 46 kg/18 m^2 of urea)	Arbuscular mycorrhizal fungi (AMF)	Comparatively increased shoot length, number of leaves, root length, biomass in NPK treatment	—	Biofertilization increased root colonization Biofertilization showed better results than organic manure	Ndirmbula et al. (2022)
2.	Sorghum	100 kg N ha^{-1}	—	—	*Pseudomonas* and *Herbaspirillum* increased while *Bacillus* and *Erwinia* decreased	No significant effect on shoot diversity	Mareque et al. (2018)
		NPK (25%, 50%, 75%, 100%)	AMF *Azospirillum sp.* *Klebsiella sp.*	Increased shoot height, number of leaves, plant fresh weight, and plant dry weight	Increased AMF with decreased NPK 25% NPK increased *Azospirillum sp.* *Klebsiella sp.*	25% NPK increased all plant parameters Combination of bioinoculants increased root P content	Ramadhani et al. (2019)
3.	Finger millet	NPK (50:25:25 kg ha^{-1} N/P_2O_5/K_2O)	—	Increased straw yield, root and shoot biomass, macronutrient contents	—	Integrated use of FYM and NPK enhances yield attributes along with nutrient content	Thilakarathna and Raizada 2015
		Urea (30 kg ha^{-1} 60 kg ha^{-1} 90 kg ha^{-1})	*Trichoderma harzianum*	Increased plant height, root length, shoot-to-root biomass ratio	—	FYM + *Trichoderma*, normal nitrogen and high nitrogen treatments improve yields	Gupta et al. (2011)

		NPK 100:50:50 (76 g of 10:26:26 and 70 g urea/plot and 38 g of 10:26:26 and 35 g of urea/pot)	*Pseudomonas fluorescens* *Azotobacter chroococcum* *Azospirillum lipoferum* *Acetobacter diazotrophicus* *Trichoderma viride*	Increased plant height and shoot weight in chemical treatment Increased root length and root weight in bioinoculant treatment	Chemical treatment enriched *Flavobacterium, Bacteroidetes, ZB2, OD1, Firmicutes* Bioinoculation enriched *Planctomycetes, Acidobacteria, Actinobacteria*	Chemical treatment increased pathogenic microorganisms, while bioinoculation enriched beneficial microorganisms	Mattoo et al. (2021)
4.	Foxtail millet	NPK (40:20:0 and 20:10:00 kg N:P_2O_5: K_2O/ha)	*Azotobacter* PSB ZnSB	Increased root growth and enzyme activities	Bioinoculation enhanced bacterial population in rhizosphere	Application of bioinoculants and bioenhancers like cow dung along with chemical fertilizer boosts yield and soil activity	Veerendra et al. (2022)
5.	Browntop millet	NPK (75%, 100%, 125% of 40: 20:00 kg ha^{-1})	—	Increased plant height, number of tillers, finger length and number, grain yield	—	—	Siddiqui et al. (2020)
6.	Little millet	NPK (20:20:20 kg ha^{-1})	*Azospirillum* PSB (Combined with organic manures)	Comparatively higher grain yield, straw yield, plant height and number of productive tillers in NPK treatment	—	Organic carbon and soil nutrients increased in organic treatment after 5 years	Rani et al. (2020)
7.	Barnyard millet	NPK (40:20:00 kg ha^{-1})	*Azotobacter* (Combined with organic manures)	Comparatively higher growth parameters, grain yield, in NPK treatment	—	Organic fertilization with bioinoculation is better alternative to inorganic fertilization with no significant losses in yield and soil health	Yadav and Malik (2010)

(continued)

Table 7.3 (continued)

Sl. No.	Millet	Treatment		Effect on		Other comments	Reference
		Chemical	Bioinoculant	Plant	Microbes		
8.	Kodo millet	NPK (40:30:10 kg ha^{-1} N/P$_2$O$_5$/K$_2$O)	*Aspergillus awamori* *Azospirillium brasilence* (Combined with inorganic sources)	Increased grain number, panicle length, grain weight and test weight	—	—	Choudhari et al. (2018)
9.	Amaranth millet	Calcium ammonium nitrate (80 kg ha^{-1} 120 kg ha^{-1})	—	Enhanced grain yield and N uptake	—	Low-harvest index inhibited higher grain yields and nitrogen utilization efficiency	Kaul et al. (2005)
10.	Buckwheat millet	Calcium ammonium nitrate (30 kg ha^{-1} 60 kg ha^{-1})	—	Slight increase in dry matter, no influence on grain yield or harvest index	—	N application caused problems like grain scattering and lodging	Kaul et al. (2005)

7.4 Sustainable Agricultural Practices for Millets

Conventional agricultural practices and extensive use of chemical fertilizers have negatively impacted the ecosystems as evidenced by deterioration of soil and plant health, nutrient leaching, human health risks, emission of greenhouse gases, and climate change (Mattoo and Gowda 2022).

Crop diversification methods like intercropping is considered the most suitable method for sustainable millet productivity. It maximizes resource use efficiency, enhances functional diversity, and minimizes the risk due to biotic and abiotic factors (Maitra 2020). In arid regions, intercropping pearl millet with legumes (mung bean) in different ratios produced higher yields than mono cropping of pearl millet (Ram and Meena 2015). Similarly, intercropping of little millet with green gram and black gram improved growth parameters, yield attributes compared to the sole cropping of little millet (Sharmili and Manoharan 2018).

The use of organic and natural treatments is highly beneficial in low-fertilizer-requiring crops such as millets. Green manure, farmyard manure, compost, etc. enhance the biological properties of soil, thereby encouraging good yields. Application of farmyard manure along with neem cakes are beneficial in maintaining productivity of millets and soil fertility compared to inorganic treatments (Shivakumar et al. 2011). Bioenhancers like *jeevamrutha* (mixture of water, cow dung, cow urine, ant hill soil, and lime) and *beejamrutha* (mixture of water, cow dung, cow urine, ant hill soil, jaggery and flour of any pulse) are effective in enhancing bacterial counts and maintaining nutrient balance and soil health thereby boosting millet cultivation in semiarid regions (Veerendra et al. 2022). Integrated nutrient management, involving the combined use of organic and inorganic treatments, is successful in maintaining productivity of millets and decreasing the chemical load into the environment (Veerendra et al. 2021)

Conservation/zero tillage practices in millet cropping systems improve millet productivity, nutrient concentration, nutrient uptake, and micronutrient biofortification when compared to conventional tillage practices (Faiz et al. 2022). In addition, these practices require less fuel, resulting in lower CO_2 emissions (Hobbs et al. 2008). Use of crop residue as much is another sustainable approach toward millet cultivation to retain soil moisture and nutrients as it effectively moderates soil temperature, maintains soil profile moisture, and increases soil fertility (Singh et al. 2018). These practices along with reduced mechanisation reduce greenhouse gas emissions.

Other interventions include regenerating degraded lands, enriching poor and nutrient-deficient soils by natural methods, and effectively increasing the soil organic matter to increase crop productivity. These practices not only mitigate the effects of climate change but reduce greenhouse gas emissions, reduce volatilization of chemicals to atmosphere, and in turn are beneficial to the environment and climate.

Traditional agricultural methods and use of agrochemicals for enhanced yields play a critical role in climate change due to increased emissions of greenhouse gases and toxin accumulation. Thus, cultivation of millets through sustainable agricultural practices offers opportunities in remedying problems related to climate change and anthropogenic influence (Fig. 7.3)

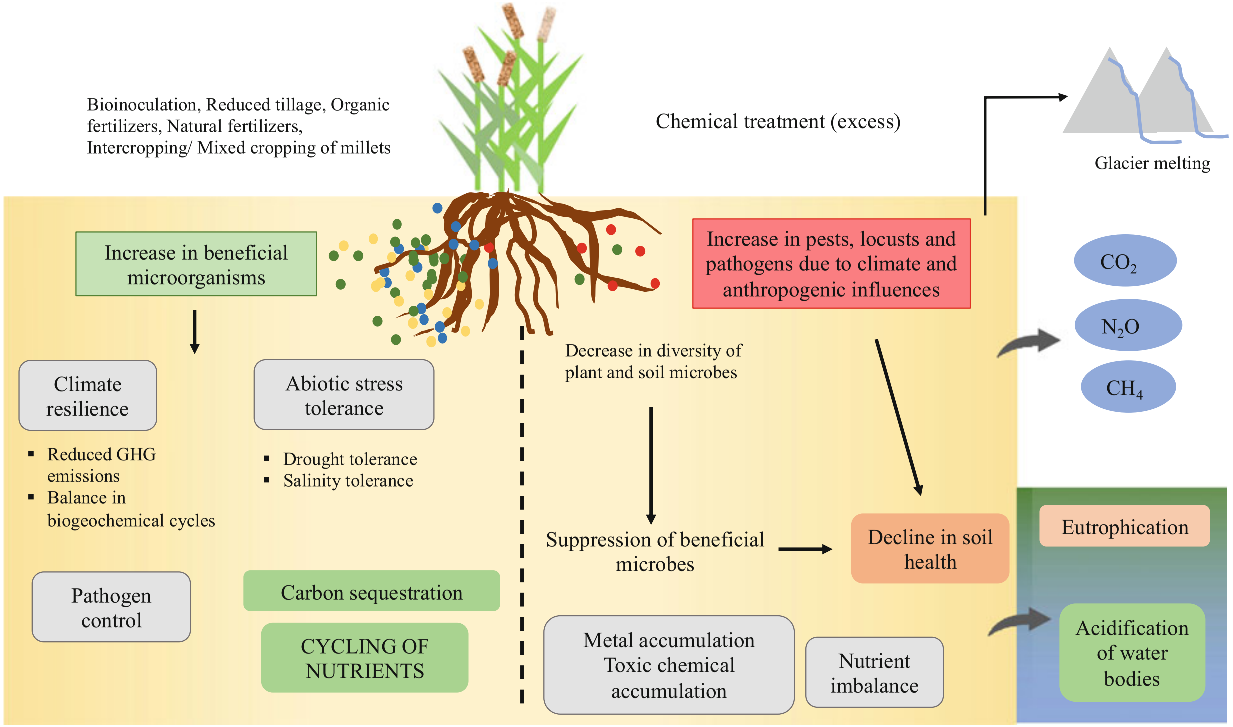

Fig 7.3 The effect of sustainable millet agroecosystems and chemical inputs on climate change. Sustainable practices for cultivation of millets are a climate resilient approach as it regulates biogeochemical cycles, reduces greenhouse gas emissions, and enhances carbon sequestration. Microorganisms confer climate-related stress tolerance in plants. On the other hand, excess use of chemicals creates nutrient imbalances, acidification of soil, and increases greenhouse gas emissions and these effects are observable through physical processes such as increasing melting of glaciers, warming of climate and soil, air, and water pollution.

7.5 Comparison of Microbial Diversity in the Ragi/Finger Millet Rhizosphere Under Chemical Treatment and Bioinoculant Treatment

Ragi or finger millet is a C4 crop displaying a high tolerance to diverse biotic and abiotic conditions. The grains demonstrate better suitability for cultivation on varied soils under adverse climatic conditions compared to other cereals. Finger millet has been conferred with the title of "nutri-millet" as the grains are laden with proteins, minerals, calcium, iron, and vitamins. The higher fiber present in these aids in preventing constipation, high cholesterol formation, and intestinal cancer (Malleshi and Hadimani 1993).

Metagenome studies by Mattoo et al. (2021) were carried out in order to improve the understanding of microbial diversity and compare the differences in the soil rhizosphere when the millets were grown in the presence of chemical fertilizer and/or bioinoculants. Further, microbial analysis was performed using high-throughput whole genome sequencing along with computational methods to elucidate the microorganisms in the rhizosphere of ragi. Metagenomic studies on rice have provided important hints on the properties of its soil microbes. Recent metagenomic studies have shown that monocrops of rice along with nonscientific application of fertilizers and improper land management methods lead to a reduction in the population of beneficial microbes. The application of bioinoculants in the rhizosphere of agroecosystems is considered to have the potential for further improving plant growth and health in extreme environments. Deciphering bacterial abundance and community structure in rhizosphere of any millet is important not only for knowing which bacteria are present but also their contribution to crop plants in stress conditions such as the pre-monsoon when water levels are low and land is dry. Till date, most of the studies (in vitro) on finger millet have centered around its genetic transformation (Ceasar and Ignacimuthu 2008; Hema et al. 2014; Satish et al. 2015, 2016)

In the finger millet rhizosphere using metagenome sequencing, it was found that the soil samples comprised of phyla; *Planctomycetes*, *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, *OD1*, *Verrucomicrobia*, *Actinobacteria*, *Acidobacteria*, *Elusimicrobia*, *WS6*, *Armatimonadetes*, *Cyanobacteria*, *OP11*, *Crenarchaeota*, *Gemmatimonadetes*, *Nitrospirae*, *Chlamydiae*, *TM7*, and *Firmicutes*, in samples, are treated with chemical fertilizer and bioinoculants. The bacterial population were characterized using illumina sequencing of 16S ribosomal RNA gene. Comparing the treatments, *Chitinophagaceae* and *Betaproteobacteria* were found more in abundance in the bioinoculant treatment as compared to chemical treatment. In contrast, members of *ZB2* and *Flavobacterium* are the highest in chemical fertilizer treatment. Another important comparison is that at the phylum level, the sample with bioinoculants shows close values to that of untreated soil suggesting that the population of chloroflexi probably reverts back to its initial original state even after the treatment with beneficial microbes. The differences were prominent at species level.

Flavobacterium succinicans, *Flavobacterium columnare*, *Candidatus_Nitrososphaera SCA1145*, *Verrucomicrobium spinosum*, *Trachelomonas volvocinopsis*, *Solitalea canadensis*, *Panacagrimonas perspica*, *Candidatus_Nitrososphaera SCA1170*, *Reyranella massiliensis*, *Magnetospirillum magnetotacticum*, *Candidatus_Nitrososphaera gargensis*, *Ilumatobacter fluminis*, *Peredibacter starrii*, *Phaselicystis flava*, *Gaiella occulta*, *Lacibacter cauensis*, *Bacillus fumarioli*, *Streptomyces reticuliscabiei*, *Cystobacter minus*, and *Nitrospira calida* were most dominant of the species in the finger millet rhizosphere. The chemical fertilizer graphs show a distinct profile with an increased relative abundance of *Flavobacterium succinicans* and *Flavobacterium columnar*e as compared to the bioinoculant treatment where the major species were *Candidatus_Nitrososphaera SCA1145* (32.44%), *Candidatus_Nitrososphaera SCA1170* (35.09%), and *Candidatus_Nitrososphaera gargensis* (34.94%).

Another comparison with respect to the treatments is that Acidobacteria, Planctomycetes, and Rhizobiales were enriched in rhizosphere treated with bioinoculants. Acidobacteria play key functions in carbon, hydrogen, and nitrogen cycles (Greening et al. 2016). They can be easily cultured under low-nutrient conditions and contribute toward resistance to desiccation and aiding in soil structure (Ward et al. 2009). *Planctomycetes* are involved in the nitrogen cycle and include important denitrifying organisms in soil (Tsoy et al. 2016), while *Rhizobiales* comprise the methylotrophic bacteria involved in fixing atmospheric nitrogen. Rhizobiales are often thrive as symbionts in nodules of leguminous plants where they contribute to providing essential nutrients, nitrogen, etc. (Venkataraman and Tilak 1990; Black et al. 2012); again, validating those beneficial microorganisms were more abundant in the bioinoculant treatment. Other dominant microorganisms in bioinoculant treatment of finger millet include, *Proteobacteria*, *Reyranella massiliensis*, and *Magnetospirillum magnetotacticum*, *Gaiella occulta*, *Solitalea canadensis* including *Candidatus_Nitrososphaera SCA1145*, *Candidatus_Nitrososphaera SCA1170*, and *Candidatus_Nitrososphaera gargensis*, which may possess vital roles in carbon cycling (Chen et al. 2019). The studies also hinted that the presence of *Trachelomonas volvocinopsis*-free-living euglenoids could be diminished due to the addition of chemical fertilizers (Poniewozik and Paska 2013). *Actinobacteria* was also abundant in bioinoculant treatment of finger millet, and these are important for secondary metabolites and antibiotics, along with other compounds suggesting that microbial abundance in bioinoculant treatment helps sustain soil rhizosphere.

In contrast, the presence of *Bacteroidetes*, *ZB2*, *OD1*, *and Firmicutes* in chemical fertilizer treatment hints at that the environment created due to chemicals leads to the formation of anoxia in soil. Also, compared to the positive environment by the bioinoculants, the chemical fertilizer treatment led to the growth and abundance of opportunist pathogens and suppression of microbes of carbon, nitrogen cycles pressing on the need to avoid excess chemical fertilizers for millets. Additionally, continuous application of synthetic fertilizers could lead to enrichment and subsequent presence of those bacteria which are opportunists. This could lead to their accumulation in soil making it tedious task to decimate them from the rhizosphere.

7.6 Studies Highlighting Microbial Roles When Bioinoculants Are Added

Combination of beneficial microorganisms as bioinoculants could be useful in the future in order to engineer soils to overcome deficiency of nutrients or enhancement in soil organic matter. For example, combining nitrogen-fixing bacteria and phosphorus solubilizers together could support growth of millets by increasing the availability of nitrogen and phosphorus, which are essential for plant development. Studies employing co-inoculation of *Azospirillum,* a nitrogen fixer, and phosphorus mobilizer AM (*Arbuscular mycorrhizae*) fungi in finger millet not only increased its biomass, root, and shoot, but also its grain productivity by 36.8% compared to nitrogen and phosphorus fertilization (Bama and Ramakrishnan 2010). *Azospirillum* aided in nitrogen uptake and making it available to the plant and promoting growth inducing IAA and GA, while the AM fungi increased the mobilization of phosphate so as to enhance plant growth and productivity (Bama and Ramakrishnan 2010). Sorghum seeds when treated with *Azospirillum* and barnyard millet with Methylobacterium, manifested higher germination, vigour index and increase in yield parameters (tillers, plant height, and grain yield). Surprisingly, a comparative reduction in flowering and maturity time was also observed upon biofertilization. In addition, enrichment of beneficial microbial population in the rhizosphere of barnyard millet was observed perhaps due to increase and type of exudation at the root interface (Poorniammal et al. 2020). Rani et al. (2019) led to an enhanced plant height (195.3 cm), biomass (8.1 t/ha), and grain yield (2.33 t/ha) compared to control plants (160.4 cm height; 6.01 t/ha biomass; 1.47 t/ha grain yield). Bioinoculation was thus responsible in increasing the microbial activity in the sorghum rhizosphere which resulted in enhanced solubilization and uptake of nutrients. Further, improved seed germination, vigor, and productivity (Rani et al. 2017) were also observed demonstrating that biofertilization is beneficial for millets. In pearl millet, inoculation of *Azotobacter chroococcum, Azospirillum lipoferum, Acetobacter sp.,* and *Bacillus megaterium (*phosphate solubilizer) at 25 g/kg of seed showed that there were synergistic effects due to combined inoculation and improved pearl millet yields compared to inoculation with single species. The positive effects of combining microorganisms and joint administration of bioinoculants enhance nitrogen fixation and phosphate solubilization, thus reducing the heavy reliance on chemical treatments (Latake et al. 2009). Another interesting study on millets of foxtail exhibited that when the seeds were treated with *Rhizobium sp.* and *Bacillus sp.* isolated from the rhizosphere of horse gram, there was improved yield, increased nutrient uptake, better chlorophyll content, and more concentration of protein in the seeds when compared to the seeds which were not bioinoculated. Also, the total dry weight of plants which were treated with *Rhizobium* increased by 122.78% and 86.07% with those treated with *Bacillus*, followed by the grain yield which increased by 76.29% and 36.55%, respectively. It was observed that inoculation with Rhizobium reduced the maturation time of the foxtail millet by 15 days indicating its valuable potential to be employed as an alternative practice to chemical fertilizers

(Khatri et al. 2016). As a biofertilizer for proso millet in semiarid Mediterranean lands under water-stressed conditions, AMF increased the plant biomass and yields highlighting directly that AMF could be utilized as an efficient growth promoter (Caruso et al. 2018). As observed for kodo millets, *Azotobacter* in combination with a phosphate solubilizer, kodo gave better grain yields (695 kg/ha) in comparison to the control (620 kg/ha). Other combinations along with the bioinoculants was also observed to increase the kodo (1585 kg/ha) yields significantly (Dwivedi et al. 2016).

7.7 Future Prospects

Reliance on a limited number of major cereals like rice and wheat impacts global food and nutritional security. The cultivation of alternative crops like millets will address the inadequacies, provided their cultivation is increased and improvement strategies are employed. The potential health benefits and high nutritional profile of millets when compared to other food grains is gaining popularity in recent times. Considering the severe effects of traditional farming systems on resource depletion and climate change in the years to come, the only solution is to shift to more improved farming practices that are more resilient and diversified to meet the needs of nation and ensure sustainable use of resources while benefiting the lives of farmers. Millet farming is indeed a rising and necessary approach toward achieving this objective, in addition to enhancing farmes's income. Intervention of governmental and nongovernmental bodies in dedicated programmes with proper training and initiatives will ensure the urge for farmers to move from traditional crops to more diversified, environmentally friendly, and nutritionally rich millets. Efforts should be made on improving traits in millets and alternative cereals and shifting the scenario toward cultivation of these underutilized crops to meet future needs.

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Chapter 8
Deciphering the Role and Diversity of Microbes Present in Millet Rhizosphere

Vashista Kotra, Monika Singh, Chitranjan Kumar, and Sangeeta Pandey

Abstract Millets are cereal crops that are grown in tropical and subtropical regions of the world most popular in China, India, and Africa. The millet rhizosphere refers to the area in the soil surrounding millet roots, and it can be quite helpful in managing some pest problems and encouraging healthy growth. One reason it grows so well in these areas is that it can thrive in high temperatures and low humidity. This chapter outlines what we need to know about the millet rhizosphere, from its structure to its microorganisms and more. Soil biodiversity can be restored by using millet crops as their rhizosphere has a variety of microbial populations which can only be seen in the areas/soil where millet crops are grown. The rhizosphere is an area of soil that surrounds plant roots and is shaped by their biological responses. In millet crops, it has been shown that the rhizosphere influences root growth, influencing nutrient uptake and stress resistance. Several microbes have been found in millet rhizospheres, some of which are only found in this environment. This is important as it can help us understand how these microbes affect plants in terms of plant nutrition and plant health. The rhizosphere of millets is a habitat for bacteria, fungi, and other microorganisms. These organisms play an important role in soil quality through their interaction with plants. Some organisms help plants from getting resistance to various soil-borne diseases. There is not much information/research available on millet rhizosphere due to its less consumption. This chapter concentrates on the benefits of microbial populations in the millet rhizosphere and their importance with all the available information.

Keywords Millet crops · Plant biomass · Plant nutrition · Rhizosphere · Rhizospheric microbes · Rhizospheric pH · Soil-borne diseases · Stress-resistance

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_8

8.1 Introduction

Microorganisms make up more than 90% of the living mass on earth (Bar-On et al. 2018). Despite their tremendous numbers, most microorganisms cannot be observed by the human eye because of their small size and tendency to thrive in areas where there is no light, such as the depths of ocean water or beneath layers of soil on land. The rhizosphere, the area around plant roots, turns out to be teeming with microscopic organisms that assist plants in acquiring nutrients from the soil (Lidbury et al. 2022), fighting off disease-causing microbes (Gao et al. 2022), and even helping plants communicate with one another (Estabrook and Yoder 1998; Mageshwaran et al. 2022). Microorganisms make up a diverse and unseen world (Tavares 2022). They are responsible for several tasks in the natural system. We know less about them than other life forms, but without their contributions, our lives would be very different. This means it is important to continue studying microbes. Here, we will explore soil microorganisms and how they affect millet growth.

Soil health indicators based on microbiological parameters, such as active biomass and respiration, are very important for assessing plant growth concerning soil quality (Serri et al. 2022). Researchers have conducted studies to examine how plants grow on different soils, studying their composition, nutrient content, and microbial activity (Tian et al. 2022; Schloter et al. 2003). They found that the millet rhizosphere has a certain amount of microbial activity and a large number of microorganisms like bacteria and fungi are associated with its roots (Tian et al. 2022). However, there is a lot of further research that must be done. Research has shown that microbial populations can positively influence plant growth and yield, but little is known about these microbial communities (Tian et al. 2022). It is clear that there are both beneficial and harmful microbes in a rhizosphere, but what determines how they interact? What traits do beneficial microbes have that increase their chances of survival? And how can we best cultivate these microbes to help our crops thrive? More research is needed to answer these questions (Mythili and Ramalakshmi 2022).

One reason for this might be that many millets are considered to be weeds and are not studied in detail (Pal et al. 2022). There is also little research into how these microbes affect the growth of the plant. Earlier report had provided insight into what types of microorganisms live in the rhizosphere and how they affect plant growth and soil nutrient availability (Kelly et al. 2022). The authors concluded that more studies need to be done on millet soils before we can make any conclusive claims. However, they do suggest that if certain populations are found to increase crop yields, then it would be worthwhile trying to cultivate those microbial populations in other areas or using them as inoculants when planting new fields (Kelly et al. 2022). Knowing more about these microbial populations and their roles within crops could help improve yields, maximize fertilizer use, and reduce pesticide application. This chapter serves as a first introduction to understanding which microbes live around millets and their roles. We have also included some recommendations for future research or technologies we can use to better understand our rhizosphere microbes.

We can start making strong connections between soil microbiology, food production, and environmental health at scales relevant to policy.

8.2 Chemical and Biological Responses Influenced by Millet Rhizosphere

8.2.1 Chemical Responses Influenced by Rhizosphere

8.2.1.1 Soil pH and Electronic Conductivity

In agricultural production, acidic soils are considered a key limiting factor for plant growth. Crops do not grow well in acidic soils that have a pH of 5.5 or lower. Few researchers also consider that 50–65% of agricultural soils are acidic in nature (Zhao et al. 2022). Highly acidic soil becomes increasingly difficult for farmers with each passing year. To properly diagnose, manage, and maintain optimal soil health on any given farm, it is important to understand what causes soil acidification to occur in agricultural fields as well as how we can prevent it from occurring (Geng et al. 2022). Every living organism, including plants, has to maintain a delicate pH balance to survive (Börjesson and Kirchmann 2022). To understand how important pH is for plant growth, it is important to know that pH stands for the negative logarithm of hydrogen ions (Dong et al. 2022). Soil pH plays an important role in the growth and development of all plants (Mondal et al. 2022). However, inefficient agricultural practices accumulate more organic and inorganic acids drastically eventually, leading in increase of free H^+ ions in the soils (Hussain et al. 2022). The world's soils are becoming increasingly acidic (Dong et al. 2022). Although this trend has been occurring for thousands of years, scientists are predicting that the rate of change will only increase in the coming decades, potentially affecting global agriculture and other industries that rely on healthy soil (Wang et al. 2022). To understand why agricultural soils are becoming more acidic, we need to know how and why soils become acidic in the first place (Rathnathilaka et al. 2022). Each year, farmers apply millions of tons of fertilizer to their fields. Fertilizers contain chemicals that stimulate crop growth—but they also carry oxygen-containing compounds called anions that flow away from a field when it rains (Maroušek and Trakal 2022). These anions have negative charges, so they bond with positively charged particles in the soil, causing them to become more acidic. Apart from this various other processes, like the leeching of nutrients, decomposition of organic material, and weathering of rocks/soil also contribute to an increase in the level of pH (Wang et al. 2022).

The soil PH scale, which ranges from 0 to 14 (Ye et al. 2022), indicates the acidity or alkalinity of the soil. Each whole number represents 10 times the concentration of hydrogen ions in the soil. A pH less than 7 indicates an acidic environment and a pH greater than 7 indicates an alkaline environment (Liu et al. 2022). The ideal pH for millet production is 6–6.5, but some varieties may be more resistant to environmental challenges and thus have a higher tolerance to pH level changes compared to other varieties of millet (Ali et al. 2022). The nutrient availability in the soil (the growth potential) will be affected by PH. Plants are not able to absorb nutrients from too much or too little of any one thing in solution, and as a result, growth is severely stunted when nutrient availabilities are out of balance (Rengasamy et al. 2022). The majority of soils have a Ph value between 6.5 and 7.0, which means they are more acidic than neutral (Saleem and Khan 2022). Since millets are grain crops, their requirement for high productivity demands adequate availability of several nutrients in favorable concentrations (Alegbeleye et al. 2022). One important nutrient for crop growth and development is phosphate (P). The quantity of P absorbed by crops depends on availability, solubility, and mobility in the soil as well as uptake by plants (Saleem et al. 2023). Soil pH plays a key role in determining these factors which influence P uptake by plants (van der Bom et al. 2022). If available P levels fall below critical thresholds at the root zone, yield losses could occur due to reduced plant uptake of P.

Millets have been proven to be hardy, but they may not be performing as well in acidic soils as they could. An underperforming millet crop can result in reduced yields (Muleya et al. 2023). Their nutrient content will be much lower in acidic conditions due to a lack of essential microorganisms that aid proper growth. Without these nutrients present, there is little chance for optimal production. Even though plants produce reduced nutrient value in produce, every plant can adjust pH by precipitating cations, thereby raising or lowering pH. In liming soils, a decrease in hydrogen ions lowers pH (Maithani et al. 2022). Similarly, a deficiency of hydrogen ions will lower (acidify) plants can also acidify their surroundings in other ways: they can take up hydrogen ions from their environment and convert them to organic acids through metabolism (Yadav et al. 2020) they can excrete organic acids or chelating agents that help lower soil pH by forming insoluble precipitates with free hydroxyls millets also have same (Emami-Karvani and Chitsaz-Esfahani 2021; Etesami and Adl 2020).

Millets grow, and they build carbohydrates and protein in their roots. This absorbs many elements from the soil and builds up organic matter in root systems (Yaashikaa et al. 2022). However, during its growth process, millet consumes HCO_3^- ions from underlying calcium carbonate ($CaCO_3$) layers. In a natural climate, $CaCO_3$ will be dissolved slowly to generate CO_2 for plant respiration and release Ca ions into root zones for uptake by plants (Naorem et al. 2022). That is why vegetation normally causes a drop in pH value in upper levels of soils (Madejón et al. 2021). As millet crops absorb more Ca than other crops, millet grows better on acidic soil (low pH) with low basicity and low buffering capacity (Hussain et al. 2022). As acid soils are deep layers over limestone bedrock with a low buffering capacity of 100~300 uS/cm and basic soils (alkaline) have a large amount of Mg and Fe that interferes with the absorption of calcium by plants (Hussain et al. 2022). Both conditions lead to poor absorption ability by millets, which results in worse yield performance. However, it does not mean all acidic soils can raise yields of millet. The microbe-induced drought tolerance mechanism by millets is shown in Fig. 8.1.

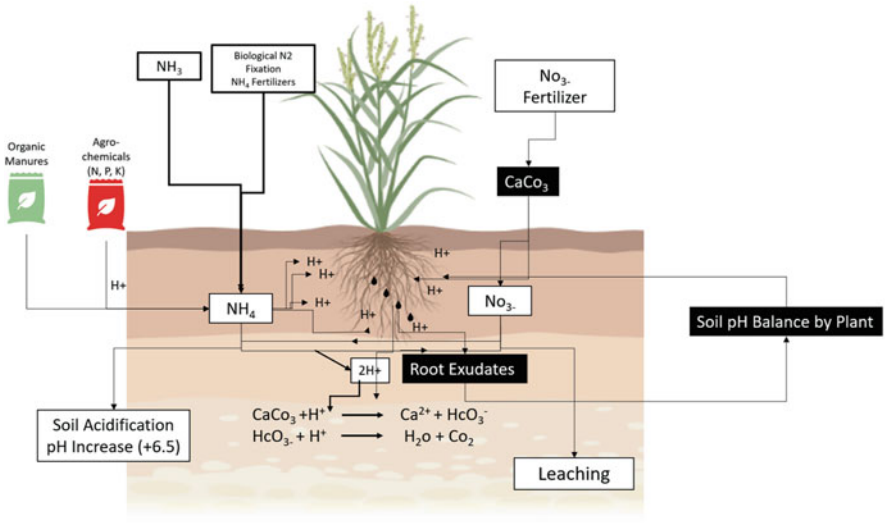

Fig. 8.1 Microbe-induced drought tolerance in millets

8.2.1.2 The Carbon-to-Nitrogen Ratio is Influenced by the Rhizosphere

Soil organic matter, including living organisms and decaying remains of dead plants and animals, helps create a suitable growing environment for crop plants (Tarafdar 2022). It provides a habitat for beneficial soil organisms and retains water so that it is available to roots when needed (Banerjee and van der Heijden 2022). A variety of factors influence whether or not organic matter will break down in soils, including plant material (or mulch), climate, pests, and diseases, as well as weather. C:N refers to how much carbon there is compared to nitrogen in organic matter found in agricultural fields.

Millets have a low nutrient requirement and return nitrogen to the soil when they are grown. This can be an advantage for other crops that may follow in their rotation because it means less nitrogen needs to be added to maintain high yields (Anas et al. 2020). The best way to manage millet's role in making nutrients available to later crops is by rotating them with other legumes or small grains that can fix atmospheric nitrogen, such as wheat or barley. However, since they are not considered a true legume, millets do not fix as much N as other common cover crops (Bettiol et al. 2022).

Compared to other crops, millets have a low C:N ratio and are therefore more vulnerable to N deficiency (Huang et al. 2022)s. Low nitrogen availability in their environment leads to reduced photosynthesis rates, subsequently lowering yields, as a result, the EC value of soil is reduced by continuous cropping without intercropping and crop shifting (Liu et al. 2022). The fact is that millets have a low C:N ratio when compared to other cereals, legumes, and oil seeds. This is attributed to poor N-fixation by microbes. Millets have similar growth habits to maize; millets and other cereals are grown together in a rotation system resulting in stable or increasing available soil nitrogen levels at depths from 10 to 30 cm increasing yields with crop rotation (Silva et al. 2022). In comparison, continuous sorghum monoculture led to increasing C: N ratios and decreasing yields (Liang 2022). The changes were most likely due to the mineralization of soil organic matter with time, due to N mineralization by different species, and the depth of incorporation of residues (Butterly et al. 2022).

8.2.2 Biological Responses Influenced by Rhizosphere in Millet Crops

The rhizosphere is the narrow region of soil or substrate that is directly influenced by root secretions and associated soil microorganisms known as the root microbiome. Various biological responses have been induced by the rhizosphere as follows:

1. Biocontrol of fungal pathogens (Ashajyothi et al. 2022; Mageshwaran et al. 2022)
2. The rhizosphere helps millet crops in nutrient uptake (Mageshwaran et al. 2022).
3. Bio-stimulation of plant growth, nitrogen fixation, and plant root and shoot development (Vaccaro et al. 2022; Ammar et al. 2022).
4. Retrieves soil degradation due to nitrates (Panchal et al. 2022).
5. Plant growth and yield improvement via the biodegradation of pollutants including heavy metals, toxic dyes, herbicides, insecticides, etc. (Takio et al. 2021).
6. Induced systemic resistance of plants against pests and diseases through the generation of phytohormones like jasmonic acid (Yu et al. 2022).
7. Induced systemic resistance against pests by modulating signal transduction mechanism (Parmagnani and Maffei 2022; Thankappan et al. 2022; Thankappan et al. 2022).
8. Synthesis of disease resistance genes into crops by lateral gene transfer (Agarwal et al. 2021).
9. Increase biomass, nutrient accumulation, and growth rate of crops through carbon and mineral nutrition (Khan et al. 2022; Wafula et al. 2016).
10. Acetylenic amino acids produced by actinomycetes activate the expression of defense-related genes, leading to the induction of specific immunity which reduces infections when grown on healthy rhizosphere soils (Gupta et al. 2022).
11. Root colonization by beneficial microbes may result in the modification of root physiology and morphology thereby enhancing plants' ability to adapt or survive under extreme environmental condition.
12. Microbiome in the rhizosphere of millets synthesizes different phytochemicals which protect plants from the detrimental effects of abiotic stress, insects, and other pathogens (Vandana et al. 2021; Nandhini et al. 2020).

13. Enhancement of ethylene production resulting from the symbiotic association of rhizosphere microbes with roots of millet crops (Noman et al. 2021).
14. Induction and maintenance of growth regulators such as cytokinins, auxins, and gibberellins (Jamra et al. 2021).
15. Plant pathogen recognition systems lead to altered susceptibility against certain pathogens (Yu et al. 2022).
16. Regulation of secondary metabolites (Prabha et al. 2019).
17. Promotion of cell division and cell elongation during flowering is an essential requirement for increased flowering and seed production (Wahab et al. 2022; Prasad et al. 2020).
18. Increased agronomic performance by promotion of interaction between flowers and pollen grains by increasing pollen deposition on stigmatic surfaces (Nawaz et al. 2020).
19. Greater intrinsic tolerance of plants to exogenous stresses (Alharby et al. 2021).
20. Increase biomass, nutrient accumulation, and growth rate of crops through carbon and mineral nutrition (Nawaz et al. 2020).
21. Improved crop yields, nutrient and water use efficiencies, and weed suppression by promoting fungal diversity (Zhu et al. 2022).
22. Rhizobacteria produce plant growth-promoting substances such as plant hormones which in turn reduce plant diseases (Arora et al. 2020).
23. Phosphorus solubilization, P uptake, and transfer from senescent leaves to young leaves in plants growing on phosphorus-deficient soils are enhanced by rhizobacteria.
24. The ability of rhizobacteria to modify soil characteristics for better physical, chemical, microbial, and biological functioning of soil (Ayangbenro and Babalola 2021).
25. Improvement in plant growth and quality through plant activators released by rhizobacteria (Borah et al. 2023).
26. Production of phytohormones [e.g., auxin, ethylene, cytokinins] (Maheshwari et al. 2015).
27. Improving chemical and physical properties of soil directly or indirectly by the production of extracellular polymeric substances (EPS), extracellular enzymes, and bio-stimulants (Ayangbenro and Babalola 2021).
28. Increases the availability of nitrogen and phosphorous by fixation, mineralization, degradation, and immobilization in soil (Wang et al. 2022).
29. Alteration of rhizosphere pH toward optimum growth conditions for some crop plants.
30. Protecting plants against abiotic stresses such as drought, salinity, cold, UV radiation, and oxidative stress (Khan et al. 2020; Hasanuzzaman et al. 2021).
31. Enhance soil structure by the formation of EPS (Extracellular Polymeric Substances) and fimbriae which help in keeping soil aggregated resulting in reduced soil erosion. The EPS produced by plants such as millets which are commonly grown in Rajasthan is significantly greater than wheat. The EPS shows a high specific surface area and large interfacial area which may help in retaining water and stabilizing soil structure (Ayangbenro and Babalola 2021). Microbial

signaling, interaction, and nutrient cycling in the millet rhizosphere are schematically presented in Fig 8.2.

8.2.2.1 Role of Microbes in Millet Rhizosphere

Root-to-microbe Signaling and Root-to-microbial Interaction

During time and space changes in millet rhizosphere, root exudates to microbe signaling participated in a variety of processes, such as symbiosis and competition for nutrients between plant roots and microbes, food absorption by plants via mediating soil organic matter decomposition, nitrogen fixation or nutrient mineralization from organic and inorganic forms (Pantigoso et al. 2022). Root exudation can induce changes in microbial population growth and activity by different signal molecules. The pathways responsible for signal interactions were direct cell-to-cell contact or signal exchange through volatile compounds via a quorum sensing system within a relatively short distance (Vandana et al. 2021). Signal hormones from bacteria can influence rhizosphere microbiomes by changing metabolisms in the millet soil ecosystem while maintaining homeostasis. A series of chemical signals called phytohormones are released by plant roots into their surroundings which serve as interspecies communication between plants and microbes. These phytohormones control many aspects of plant development including seed germination, flowering, senescence, pest resistance, and pathogen defense. Phytohormones also regulate growth and virulence gene expression in some types of beneficial microorganisms that live near host plants. Bacterial populations in the millet rhizosphere are influenced significantly by Cd stress or N nutrition status. Therefore, understanding how these factors affect bacterial communities will help us to improve millet production (Wu et al. 2022). This study provides insights into how environmental factors modulate root exudation and its effects on microbial community structure under different conditions. Understanding the function of those species may lead to novel approaches for enhancing millet production efficiency. Molecular mechanism/ Signaling pathway: There are several plant hormones, including abscisic acid (ABA), gibberellin (GA), and salicylic acid (SA), which play an important role in regulating diverse biological processes in millet plants (Salvi et al. 2021).

Role of Microbes in Fixation/Cycling of Nutrients in Millet Rhizosphere

The rhizosphere is defined as the soil surrounding a root and is comprised of macronutrients and trace elements that are involved in both: cycling of nutrients and also in immobilizing them in complexes that cannot be utilized by plants (Halim et al. 2020). In millet, microbes play an important role to help plant growth as well as fixing nitrogen for plant growth. Activated microorganisms that are present in the millet rhizosphere can help in the fixation/ cycling of nutrients for millet plants. As

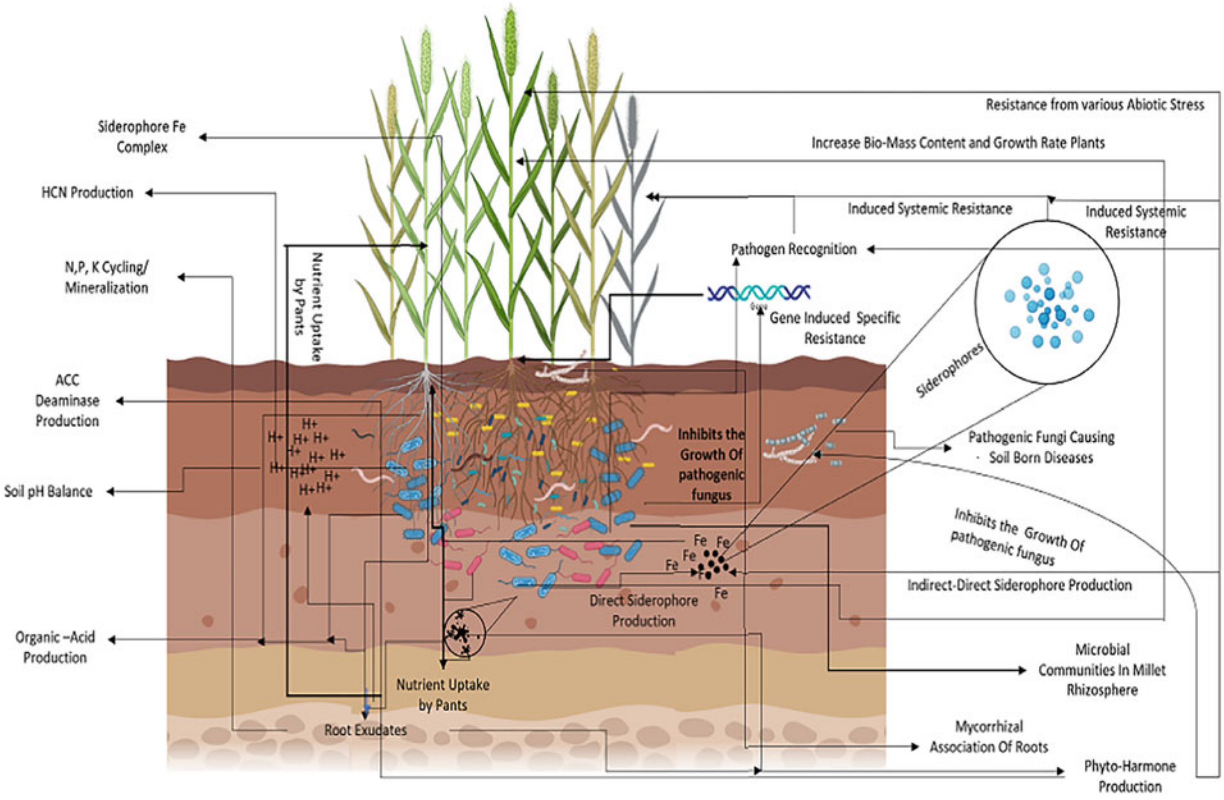

Fig. 8.2 Microbial signaling, interaction, and nutrient cycling in the millet rhizosphere

discussed earlier, minerals from soil get released into the water due to the degradation of organic matter by microbes. These dissolved minerals then become available to plants through absorption through roots (Prabha et al. 2019). On another hand, insoluble mineral such as phosphate gets converted into a soluble form (phosphate ions) by microbes and hence become available for plant uptake. Thus, it can be concluded that microorganisms have a vital role in the fixation/cycling of nutrients in the millet rhizosphere and hence helps the overall growth of millet plants (Salvi et al. 2021).

Role of Microbes in Macronutrient Cycling

Although microbes in the millet rhizosphere play an important role in nitrogen mineralization, evidence regarding their function in phosphorus cycling and carbon cycling is lacking. The exact mechanism by which microorganisms are involved in macronutrient cycling needs further investigation. It has been found that there are a lot of different soil microbial communities present at most or all of these sites (Gurung et al. 2023). This indicates that factors other than management practices were responsible for those changes. However, it was not possible to identify any specific factor(s) because of a lack of data. In addition, these authors reported that no relationship existed between community structure and site fertility status. A better understanding of microbial diversity in the millet rhizosphere may help improve our knowledge about nutrient cycling and be useful for improving crop production under low-input conditions.

Role of Microbes in Micronutrient Cycling

Most micronutrients in the millet rhizosphere are mobile and circulated between macro-organisms (plant, animal), soil environment, and nutrients. As mentioned before, soil microbes play a significant role in element cycling. The mobility of nutrients for plants can be increased by microbial transformations or release through the decomposition of macromolecules (White et al. 2018). They act as a potential source of minerals for plants. For example showed that microbe-mediated mineralization is an important factor for organic matter production during the cultivation of winter wheat on acid soils with low pH values in millet rhizosphere (Hayat et al. 2010). Apart from this, mineralization plays an important role in nutrient availability during the millet crop growth cycle from the seedling till the maturity stage (Patel and Thakker 2021). It was reported that there is a high concentration of metals such as Cu, Fe, Mn, and Zn in water bodies which may become toxic to crops if they are not properly chelated. Therefore, it is essential to understand how microbes function to cope with these challenges. Therefore, it will be necessary for agriculturalists to use and strengthen the theoretical and practical knowledge of alternative strategies such as using biofertilizers instead of chemical fertilizers.

8.2.3 Plant Growth-promoting Activities of Microbes in Millet Rhizosphere

8.2.3.1 Resistance to Soil-borne Diseases

Several studies have shown that there is an interaction between soil-borne fungi and plant roots which helps in creating resistance to soil-borne diseases in millet crops. As mycorrhizal association increases, rhizosphere pH increases because microbial activity increases. This causes a decrease in ammonium uptake, and it may be an important factor involved in disease resistance through allelopathy or other mechanisms. The specific mechanism is yet to be determined but there are many possibilities such as the production of ethylene by mycorrhizal fungi resulting in loss of nitrogen from leaves or suppression of rhizosphere pathogen population. It is also possible that rhizosphere microorganisms can also produce phytochemicals that exhibit an inhibitory effect on soil-borne pathogens. Some of these include phenolic compounds, flavonoids, cyanogenic glycosides, etc., which may reduce nutrient availability for pathogen growth. Some microbes have been known to release metabolites such as nitric oxide (NO) into the soil causing root growth inhibition, and similar mechanisms might exist for other microbes too. One study showed that NO produced by bacteria colonizing the millet rhizosphere resulted in greater plant growth than plants grown with non-inoculated soils. Though it would require more investigation to determine if NO was released specifically by bacteria associated with millet roots or if any other microbes were involved. However, there exist complex interactions among different microbes in the millet rhizosphere that help protect against soil-borne diseases (Lahlali et al. 2022). There has been some evidence to support these claims, but further research is required to confirm them. Another possibility could be that microbes in the millet rhizosphere could release volatile compounds into the soil causing changes in the local environment such as pH and nutrient availability for pathogens leading to their death.

8.2.3.2 Production of Growth Hormones

The effect of bacteria on the growth and yield of millets was studied, and it has been reported that a variety of growth hormones such as auxins, cytokinins, gibberellin, ethylene, and abscisic acid are produced by bacteria in the rhizosphere (Stefen et al. 2022). There are different responses of plants to hormone production by bacteria (Tyagi et al. 2022). The exudates and growth factors released from millets plants roots (Goswami and Deka 2022) stimulate bacterial population at the root zone resulting in the synthesis and release of various types of phytohormones into the surrounding medium/soil (Debnath et al. 2022). Millets such as pearl, foxtail, and person are cultivated around the world but their potential in terms of biocontrol and biocontrol agents is still unexplored (Gahukar and Reddy 2019).

Millets have the potential for growing biocontrol strains due to their rhizosphere effect (Shultana et al. 2022). When cultured in soil, certain microorganisms present on the root surface have significant impacts on the growth of adjacent plants (Patwardhan et al. 2022). Plant growth-promoting bacteria can be selected from the rhizosphere soil of millets for effective control of crop diseases through bio-fertilization strategies (Younas et al. 2022). It has been observed that interact with roots directly or indirectly with host plants by producing metabolites that stimulate physiological processes including photosynthesis and secondary metabolic pathways, leading to enhanced plant growth and yield (Younas et al. 2022).

Production of growth hormones was observed by using the bioassay-guided fractionation method. These growth hormones are beneficial in the initiation and development of root hairs which results in good growth and increases productivity in millet crops (Fadiji et al. 2022). In addition to the production of phytohormones such as indole acetic acid (IAA), auxin, and gibberellic acid (GA3), biosynthesis is also enhanced by increasing shoot length as well as the number at the later stage of millet plant development (Umapathi et al. 2022). Growth hormones produced in the rhizosphere increased to 70% at the flowering time with little variation in levels among all tested millets. Such control of hormone production during specific developmental phases provides a platform for the manipulation of endosperm quality and genetic improvement programs for economically important cereals, legumes, and oilseed crops through genetic engineering techniques or transgenic approaches. IAA plays an important role during the seed-filling phase; GA3 is involved mostly during the early stages while cytokinins (CKs) play a crucial role toward the maturity phase of plants mostly toward bud set and early seed development stages.

8.3 Biocontrol Induced by Rhizosphere in Millets

The rhizosphere, the region of soil in which the roots of a plant are present, plays an important role in millet growth (Pal et al. 2022; Gao et al. 2022). The rhizosphere is beneficial to millet because rhizosphere bacteria exhibit antagonistic activity against pathogenic bacteria from the soil and inhibit their growth by secreting bacteriocins, which are proteinaceous antibiotics used as natural pesticides in nature. Striga, Rhizoctonia, Fusarium, and other diseases also can attack millets (Dutilloy et al. 2022). Wherever host crop plants are denser, the incidence of the disease is high. So, it is important to check for the presence of these disease organisms in bulk and rhizosphere samples of a bulk sample before treatment with bio-control agents. Microbe-induced biocontrol measures in the millet rhizosphere are given in Fig 8.3.

The rhizosphere benefits millet by improving its growth (Zhou et al. 2023), reducing vulnerability to pathogens, and increasing photosynthetic efficiency (Khan et al. 2022).. Millets are from the grass family, and they are very harsh and

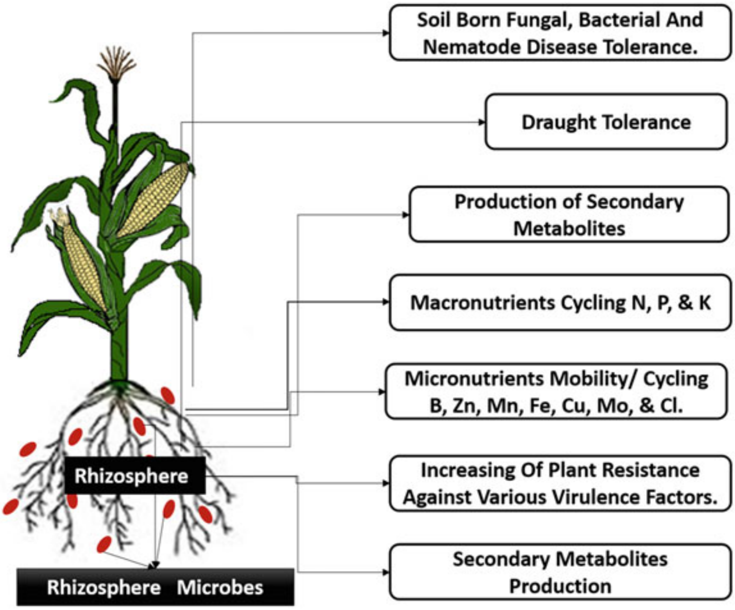

Fig. 8.3 Microbe-induced biocontrol measures in the millet rhizosphere

can face many extreme biotic and abiotic stress (Pati et al. 2022) upon it researchers found that most millets are resistant to many types of diseases and cause little damage (Rana et al. 2021). The reason is that in parts of their growth cycle, they produce chemicals called phenolics (Chandrasekara and Shahidi 2022), which inhibit or prevent microorganisms from growing and reproducing (Bhatt et al. 2022). They also contain volatile organic compounds (VOCs) that protect against fungal spores (Nerling et al. 2022) and other pathogens by reducing moisture availability and interrupting spore germination (Nzabanita et al. 2022). However, it is important to note that even if a pathogen is not directly damaging millet crops, it could still be affecting them indirectly by making them more susceptible to disease caused by other factors such as drought or cold (Velásquez et al. 2018).

A majority of rhizosphere microbes are PGPR and other microbes in the rhizosphere have positive effects on plants, but their benefit is mostly indirect (Bhatt et al. 2022). Rhizosphere bio-control processes are different from those in infected roots, but both of them play a role in the biocontrol of plant pathogens (Maitra et al. 2022). The rhizosphere biocontrol can control diseases independently of cultivar resistance and even without direct contact between the host and the pathogen (Afridi et al. 2022). Bacterial leaf blight and bacterial leaf spot caused by *Xanthomonas oryzae* PV and *Pseudomonas syringae* pv. Japonica (Narasimhamurthy et al. 2022) Oryzae is one of the major bacterial diseases of non-gramineous crops, including rice, wheat, and other major millets (Chanwala et al. 2022). Among factors that determine epidemic occurrence and intensity, host plant resistance is an important component (Manyasi et al. 2022; Sau et al. 2022). Meliloti does not produce siderophores, probably because pseudomonas fluorescens is also present in the rhizosphere and it produces siderophores. However, *P. fluorescens* failed to inhibit *P. graminis* due to the production of ammonia by *B. subtilis* released by *R. meliloti* into soil water after bacterial nodulation induced by inoculation with *Bradyrhizobium* strain CIAT 899 which significantly increases nitrogen concentration available for uptake by plants and delays senescence during grain filling period under drought stress conditions (Saeed et al. 2021), farmers have reported an increase in yields when growing millets with a mycorrhiza fungus, which suggests there may be another benefit from adding it to the soil (Schütz et al. 2022). Mycorrhizae colonize and grow on roots, making them longer and more effective at drawing water and nutrients out of the ground (Kumar et al. 2022).

Most crops are susceptible to virus diseases because of their dependence on leaf-and-stalk inoculation. Paddy, sorghum, finger millet pearl millet (Mbinda et al. 2021), maize, and soybean are severely affected by different types of viruses causing huge losses to the agriculture/farmer economy. Attempts were made to develop a method for controlling viral diseases by using bio-agents that include arbuscular mycorrhiza (am), rhizobacteria (rb), and fungi which promote disease resistance in host plants (Rao and Reddy 2020).

Using effective and quick control strategies against major pests is becoming a need of the hour, given present-day crop management techniques. It was hypothesized that rhizospheric bacteria, responsible for inducing systemic resistance in plants may also act on nematodes parasitizing the root system of cereals and millets through plant roots and therefore their number can be reduced (Subedi et al. 2020). Results revealed that the transmission capacity of inoculum via the rhizosphere effect significantly increased over its direct application route. It protected both mature and developing stage hosts against pathogenic nematodes (Poveda et al. 2020). More research needs to be conducted into how biocontrol can be used by farmers with the different types of crops and soils they use. Furthermore, cultivation should investigate making seeds that already have the right type of rhizospheric microbes inside them so they will grow more quickly. The type of soil being used must also be taken into consideration when planting seeds so they will flourish in their new surroundings without any problems encountered. Several advances of plant disease research describing causal organisms of soil-borne and other plant diseases commonly found in the millet rhizosphere are presented in Table 8.1.

Table 8.1 Advances in plant disease research in the millet rhizosphere

S. No.	Disease name	Organism	Category	Reference
1.	Bacterial leaf streak	*Xanthomonas campestris pv. Pennamericanum*	Bacteria	Qhobela and Claflin (1998)
2.	Bacterial leaf stripe	*Acidovorax avenae*	Bacteria	Murakami et al. (2022)
3.	Bipolaris leaf spot	*Bipolaris setariae*	Fungi	Haq et al. (2022)
4.	Cercospora leaf spot	*Cercospora penniseti*	Fungi	Nagaraja et al. (2017)
5.	Curvularia leaf spot	*Curvularia penniseti*	Fungi	Manzar et al. (2022)
6.	Downy mildew	*ScleROSpora graminicola*	Fungi	Lavanya et al. (2022)
7.	Downy mildew	*Plasmopara penniseti*	Fungi	Lavanya et al. (2022)
8.	Drecslera leaf spot	*Drechslera dematioidea*	Fungi	Arun et al. (2022)
9.	Ergot	*Claviceps fusiformis*	Fungi	Kumari et al. (2022)
10.	Exserohilum leaf blight	*Exserohilum ROStratum*	Fungi	Meenambal et al. (2022)
11.	False mildew	*Beniowskia sphaeroidea*	Fungi	Crouch et al. (2022)
12.	Head mold	*Various fungi*	Fungi	Luttrell (1954)
13.	Myrothecium leaf spot	*Myrothecium roridum*	Fungi	Mangandi et al. (2007)
14.	Phyllosticta leaf blight	*Phyllosticta penicillariae*	Fungi	Wang et al. (2022)
15.	Pyricularia leaf spot	*Pyricularia grisea*	Fungi	Kumar et al. (2022)
16.	Rust	*Puccinia substriata var. Indica*	Fungi	Singh and Nara (2023)
17.	Seedling blight	*Various fungi*	Fungi	Navale et al. (2022)
18.	Smut	*Moesziomyces penicillariae*	Fungi	Chakrabarty et al. (2011)
19.	Southern blight	*Sclerotium rolfsii*	Fungi	Garcia-Gonzalez et al. (2022)
20.	Top rot	*Fusarium moniliforme*	Fungi	Bramel-Cox and Claflin (1989)
21.	Zonate leaf spot	*Gleocercospora sorghi*	Fungi	Ashajyothi et al. (2022)
22.	Burrowing nematode	*Radopholus similis*	Nematode	Ssango et al. (2004)
23.	Cyst nematode	*Heterodera gambiensis*	Nematode	Silva et al. (2022)
24.	Dagger nematode	*Xiphinema Americanum*	Nematode	Heve et al. (2018)
25.	Lance nematode	*Hoplolaimus indicus*	Nematode	Jain (2009)
26.	Panagrolaimus nematode	*Panagrolaimus spp.*	Nematode	Jain (2009)

(continued)

Table 8.1 (continued)

S. No.	Disease name	Organism	Category	Reference
27.	Ring nematode	*Criconemella ornate*	Nematode	Singh and Kanwar (2019)
28.	Root knot nematode	*Meloidogyne incognita,* *Meloidogyne javonica* *Meloidogyne arenaria*	Nematode	Kumar and Dara (2021)
29.	Root lesion nematode	*Pratylenchus mulchandi* *Pratylenchus brachyurus* *Pratylenchus zeae*	Nematode	Kumar and Dara (2021)
30.	Stubby-root nematode	*Paratrichodorus minor*	Nematode	Heve et al. (2018)

8.4 Conclusion

Microbes are an important part of rhizospheric ecology. The microbes that live in millet rhizospheres produce a range of services. From nitrogen fixation and water uptake, to promoting growth and defending against phytopathogens, rhizospheric microbes provide a plethora of benefits for their host plants. In return, millet plants benefit from having microbiomes that not only promote plant health but also make them more competitive with other plants when it comes to accessing resources. Some of these microbes may even be involved in bringing about a healthy growth of millet. Understanding how microbial population helps a plant grow stronger and healthier has led to applications in farming for species such as millets. For example, in vitro studies have shown that adding rhizosphere fungi may help plants assimilate nutrients from the soil and improve their disease resistance. Similarly, rhizosphere bacteria could potentially help plants fight off diseases by producing antibiotics or competing with other microbial species. Overall, there is strong evidence that rhizosphere microbes play an active role in promoting plant growth; however, we still have a lot more work to do before we understand exactly what role they play and how we can make use of them more effectively.

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Chapter 9

Role of Phosphate Solubilizing Microbes on Phosphorous Availability and Yield Attributes of Millet

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Abstract Millets produce particularly well under poor nutrient conditions, including under low phosphorous (P) soils. The use of phosphate fertilizers associated with inoculation with P-solubilizing microorganisms (PSM) has proved to be a technology that meets the precepts of ecological intensification of agriculture, both by reducing the use of synthetics in these environments and by adding beneficial microorganisms. PSM are able to convert complexed insoluble phosphates to soluble phosphate forms, which can easily be assimilated by plants, through production of organic acids, siderophores, protons, and hydroxyl ions. In general, the use of PSM can be optimized with the appropriate combination of strains, plant genotypes, and environmental conditions. In this chapter, we highlighted the potential use of PSM collected in different types of soil to increase P uptake and millet yield. In Brazil, we showed the release and use of an inoculant containing two bacterial strains, isolated from agricultural areas in the country, capable of increasing P use efficiency and crop yield. We also described that the co-inoculation of N-fixer and P-solubilizer microorganisms can have beneficial effects on germination, seedling vigor, plant height, seed weight, and grain yield of pearl millet under controlled conditions. Arbuscular mycorrhizal fungi (AMF) were also reported as a promising strategy for increasing yield as they can enhance P uptake in millet allowing higher soil exploration beyond the rhizosphere depletion zone. In addition, AMF can solubilize P present in the soil by different mechanisms, such as production of phosphatases, organic acids, siderophores, and protons. Overall, this chapter approached the need to expand studies focusing on the use of PSM in millet crops associated with the prospection and characterization of more efficient PSM. Moreover, we suggested that more field studies under different edaphoclimatic conditions and using different millet genotypes should be performed to understand the interactions between bioinoculants and native microorganisms.

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_9

Keywords Phosphate solubilizing microorganisms (PSM) · Arbuscular mycorrhizal fungi (AMF) · Phosphorus fertilizers · Bioinoculants · Nutrient mobilization · Soil microbes

9.1 Introduction

The adaptability to acid and low fertile soils, which are significant growth limiting factors for most crops, is a recognized feature of millet. This adaptation is due to the root system architecture, which reaches deeper soil layers and increases the plant's capacity to extract nutrients from the soil. Therefore, different genetic materials and crop management methods are critical factors in optimizing millet production. In addition, millet has lower nutrient requirements and a higher response to fertilizer application than other crops, such as maize and soybean (Santos et al. 2010).

Synthetic fertilizers, especially the phosphate group, have ensured the expansion of agriculture since P in the soil is in suboptimal conditions, especially in tropical soils such as Latosols and Ultisols (Ramaekers et al. 2010; Cordell and White 2011; Bindraban et al. 2020). Mineral deposits corresponding to 0.12% of the Earth's crust are the primary P source (Samreen and Kausar 2019). According to their origins, the mineral deposits are divided into sedimentary, igneous, and biogenic. From an economic point of view, sedimentary and igneous deposits are more important than biogenic. Phosphates from marine sediments are found in the United States, Southeastern Mexico, Middle East, and Northwestern Sahara. Those from igneous deposits are present in South Africa, Russia, Finland, and Brazil, among other countries. Thus, the largest Brazilian reserves of phosphate rocks occur in igneous rocks. They account for 80% of the national production of phosphate and present average levels of soluble P_2O_5 in citric acid varying between 4% and 15%. These rocks have complex mineralogy and low uniformity, making their industrial use more difficult and more expensive. For this reason, the average price of Brazilian rock phosphate is higher than that practiced globally (Schueler et al. 2019). Furthermore, its efficiency is lower than reactive phosphates (Souza et al. 2014).

Currently, China, India, the United States, and Brazil are the biggest consumers of fertilizers, concentrating 58% of the world demand and directly influencing the dynamics of the market and the formation of prices (GlobalFert 2020). Dependence on phosphates fertilizer import leaves the agriculture sector vulnerable to fluctuations in the foreign market and raises production costs, reducing agribusiness competitiveness. Brazil has the most significant potential for expanding agricultural production in the world and should become, in the next 5 years, the world's largest grain exporter, surpassing the United States. The country imports 70% nitrogen, phosphate fertilizers, and over 95% potassium, to meet the national demand for agribusiness for nutrients (GlobalFert 2020).

A strategic approach to the integrated management of fertilizers consists of maximizing the release of phosphorus from the soil using root growth promoting microorganisms, which also improve the availability of this nutrient in the rhizosphere. Developing microbial inoculants opens a new perspective for increasing productivity and soil fertility, with the potential for partial or total replacement of synthetic fertilizers.

9.2 Phosphorus: An Essential Element for Plant Growth

Phosphorus (P) is one of the most limiting nutrients for plant growth. Although the total P of most soils is relatively high, the P available to plants is very deficient, especially in weathered tropical soils (Novais et al. 2007). Phosphorus deficiency can cause growth retardation and interference with photosynthesis, respiration, energy storage and transfer, cell division and growth of plant cells (White and Hammond 2008).

The efficiency in using P by plants is a complex character and is still not well understood. In maize and sorghum, P use efficiency under field conditions is more associated with the plant's ability to acquire P from the soil (acquisition efficiency) than with its ability to utilize P within the plant (internal use efficiency) (Parentoni and Souza Jr 2008). Plant tolerance to P-deficient soils is associated with different mechanisms, including associations with rhizosphere microorganisms and changes in root architecture (Niu et al. 2013; Malhotra et al. 2018; Barros et al. 2020).

Soil phosphorus is found in organic (Po) and inorganic (Pi) forms, which differ by the degree of stability or solubility, which leads to different availability to plant absorption. Pi occurs mainly in insoluble mineral complexes and precipitates (Rengel and Marschner 2005), while organic matter represents between 20 and 80% of the Po in soils (Richardson et al. 2009). There are mainly two types of ion phosphate reactions of fixation in soils: (a) sorption of phosphate on the surface of clay minerals and (b) precipitation of phosphate by free Al^{3+}, Fe^{3+} , and Ca^{2+} in the soil (Havlin et al. 1999). Soils with the highest capacity for P fixation occupy about 1 billion hectares in the tropics (Sanchez and Logan 1992). In general, only 0.1% of total soil P exists in a soluble form, readily available for immediate plant uptake (Zhou et al. 1992). P fixed in soils are quite stable, needing to be dissolved or biologically altered to increase P availability to plants. In some environments, plants compete with soil microorganisms to acquire P (Jain et al. 2007). In addition, P is considered one of the nutrients with a lower mobility in the soil (Raghothama 1999), with higher concentrations in the surface layers and low concentration in the lower layers (Vance et al. 2003), being transported to the roots by diffusion, this process occurs slowly (Rengel 2001).

P must be supplemented in most agricultural soils by the addition of synthetic chemical fertilizers. However, it is estimated that a large proportion of P added to the soil and not removed by crops (>70%) remains in the soil in forms not available to plants (Pavinato et al. 2020). Thus, implementing alternative environmentally sustainable and economically viable strategies to increase the P availability for plants is crucial. One feasible strategy is microbial inoculants with P solubilization and mineralization capacity.

9.3 Phosphate-solubilizing Microorganisms (PSM)

Insoluble P compounds in the soil can be solubilized by organic acids, phosphatases, and chelating agents produced by plants and microorganisms. Phosphate-solubilizing bacteria (PSB) and fungal (PSF) species can increase the solubilization of phosphate compounds (Son et al. 2006; Rafi et al. 2012). Considering the soil microbial population, PSB typically comprises between 1% and 50%. In comparison, PSF represents 0.1–0.5% of the total microbial population (Chen et al. 2006), highlighting bacteria of the genera *Bacillus, Pseudomonas, Azotobacter*, and *Burkholderia*, as well as fungi of the genera *Aspergillus* and *Penicillium* (Oliveira-Paiva et al. 2009; Gomes et al. 2014; Etesami and Maheshwari 2018; Kalayu 2019). In addition, the entomopathogenic fungi *Beauveria* and *Metarhizium*, commonly used as biopesticides, are also important as biofertilizers to promote the growth and development of plants, revealing possibilities for their multifunctional use (Kowalska et al. 2020) (Fig. 9.1).

Microorganisms play an important role in all three major components of the soil P cycle (dissolution-precipitation, sorption-desorption, and mineralization-immobilization). The main P solubilization mechanisms employed by microorganisms include: (1) release of organic acids, siderophores, protons, hydroxyl ions, CO_2; (2) release of extracellular enzymes (biochemical P mineralization), and (3) P release during substrate degradation (biological P mineralization) (Mcgill and Cole 1981). In addition, these microorganisms, in the presence of labile carbon, serve as a drain for P, quickly immobilizing it, even in soils with low P content (Sharma et al. 2013).

Microorganisms and plant roots transform organic P into inorganic P by synthesizing enzymes known as phosphatases. Phosphatases are enzymes that catalyze the hydrolysis of phosphoric acid esters and anhydrides and are classified based on optimal pH as neutral, acidic, and alkaline (Raghav et al. 2011). Phosphatase production is regulated by phosphoesters compounds available in the environment (Nalini et al. 2015). However, there are several subclasses such as hydroesterases, phosphomonoesterases, phosphodiesterases, hydrolases, and phytases. Phosphomonoesterases include acidic and alkaline phosphomonoesterases that hydrolyze monoesters, including carbohydrate mononucleotides and phosphates. The acid phosphomonoesterase activity decreases with the availability of organic P in the soil due to the increase in P mineralization. Thus, the reduction of organic P in the soil is closely related to the P concentration in the microorganisms' biomass. Phytate, particularly in cereal grains, is plants' main organic compound for P storage. Phytate is a highly stable compound, and its release requires the activity of specific enzymes, such as inositol phosphohydrolases, alternatively known as phytases. Phytases hydrolyze phytate gradually, resulting in products that phosphatases can further hydrolyze (Su et al. 2015).

Bacteria are the primary source of alkaline phosphomonoesterase activity in the soil, while acidic phosphomonoesterase and phytase can be derived from plants, fungi, and bacteria (Brookes et al. 1984; Rengel and Marschner 2005; George et al. 2002; Vance et al. 2003; Richardson et al. 2009; Neal et al. 2017). These enzymes

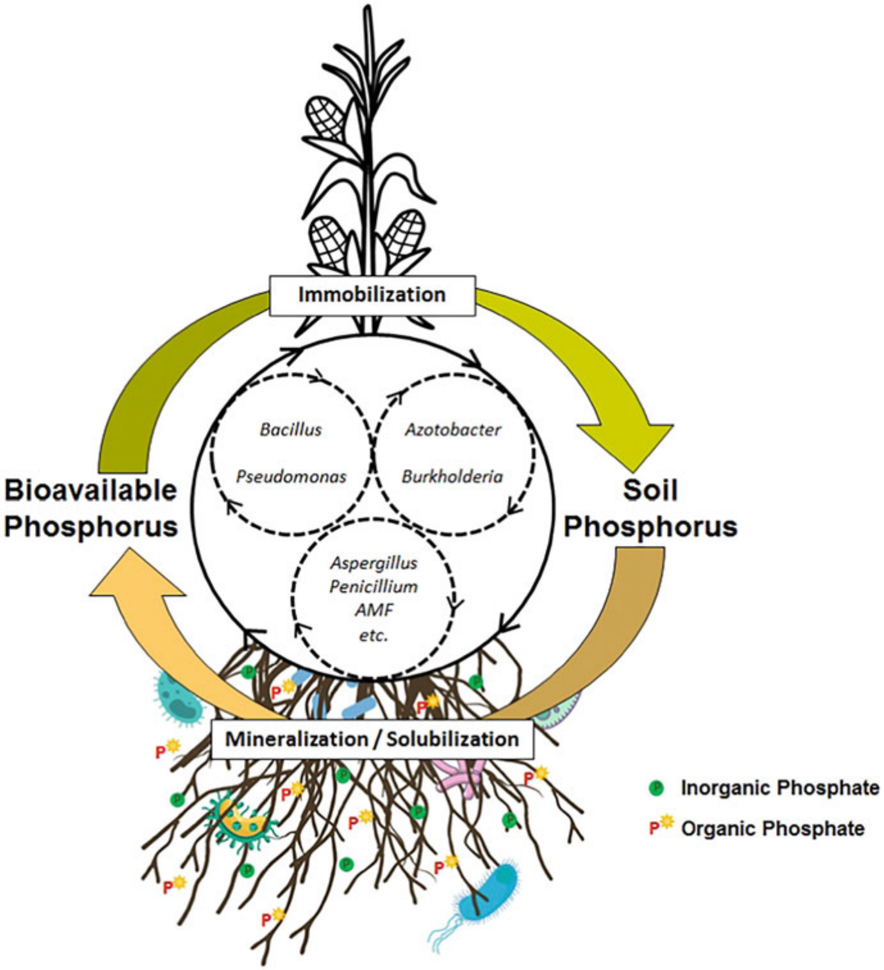

Fig. 9.1 Representation of phosphorus solubilization/mineralization and immobilization by microorganisms in the soil

are produced by over 70–80% of the soil microbial population, including bacteria such as *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Streptomyces*, and fungi like *Aspergillus*, *Penicillium*, and *Rhizopus* (Yokoyama et al. 2017).

Some genes of microorganisms, namely *pqq* and *bpp,* involved in the solubilization and P mineralization, respectively, have already been identified and isolated in different species of microorganisms (Rodríguez et al. 2006; Jorquera et al. 2011). Therefore, these genes could be exploited in selecting and developing more efficient strains for P solubilization or mineralization in agriculture.

9.3.1 Phosphate-solubilizing Bacteria as Inoculants for Agriculture

In Brazil, the Embrapa Maize and Sorghum team has been researching and selecting phosphate-solubilizing microorganisms (PSM) for almost 20 years (Oliveira-Paiva et al. 2009; Gomes et al. 2014; Sousa et al. 2021; Velloso et al. 2020). These studies culminated in developing the inoculant BiomaPhos®, released in 2019, in partnership with a private company. This inoculant contains two bacteria strains, *Bacillus subtilis* (CNPMS B2084) and *B. megaterium* (CNPMS B119), isolated from agricultural areas in the country, capable of increasing P use efficiency and crop yield. It has the potential to reduce the use of synthetic phosphate fertilizers.

The strain of *B. megaterium* (CNPMS B119) was isolated from the maize rhizosphere. It could solubilize rock and calcium phosphates and produce phosphatase, while the strain of *B. subtilcould*S B2084) is endophytic, solubilizes calcium and iron phosphates, presents high production of gluconic acid and phytase (Abreu et al. 2017; Oliveira-Paiva et al. 2009; Velloso et al. 2020). Furthermore, these strains have distinct growth-promoting properties, such as the production of IAA, siderophores, exopoly saccharides, and biofilm formation that stimulate the increase in root surface, especially of thinner roots (Sousa et al. 2021; Velloso et al. 2020). Bacteria of the genus *Bacillus* can form endospores, allowing them to adapt to extreme abiotic conditions such as temperature, pH, radiation, desiccation, ultraviolet light, or exposure to pesticides (Bahadir et al. 2018).

9.3.2 The Effect of P-solubilizing Bacteria on Millet

The search for technologies to improve P use efficiency has been continuously encouraged. Millet presents a short life cycle with fast development, producing particularly well under poor nutrient conditions, due to its high capacity to acquire nutrients from the soil. Moreover, millet is a crop that responds well to inoculation with plant growth promoting bacteria (PGPB), as exemplified below.

Based on multifunctional PGPB traits, *Acinetobacter*, *Streptomyces,* and *Penicillium* strains were selected and inoculated in great millet and/or foxtail millet under greenhouse or field conditions. These PGP drought-adaptive phosphorus solubilizers efficiently mitigated the adverse effects of drought by enhancing the accumulation of different osmolytes, such as glycine betaine, proline, sugars, and decreasing lipid peroxidation (Kour et al. 2020a, 2020b).

Examples of P solubilizing microorganisms inoculated in pearl millet (*Pennisetum glaucum*) seeds include the bacteria *Pseudomonas striata* and its application to soil fertilized with low-grade Mussoorie rock phosphate at 60 kg P_2O_5 ha^{-1} (RP60 + *P. striata*) (Gaind 2013). The results indicate that the inoculation improved the root and shoot biomass, increased the availability of soil P, and resulted in higher P uptake by straw and grain. Furthermore, the crop yield in the presence of RP60 + *P. striata* was comparable with a low dose of triple superphosphate at 30 kg P_2O_5 ha^{-1} (SP30 + *P. striata*). *P. striata* also produces tartaric acid, malic acid, citric acid, succinic, gluconic acid, and extracellular phytase. In addition, the capacity of phytate mineralization and solubilization of tricalcium, rock, ferric, and aluminum phosphates indicated that *P. striata* presents high potential as phosphobacteria.

Harinathan et al. (2016) investigated the effect of the inoculation of PSB *Bacillus* sp. strain C2 and *Pseudomonas* sp. strain C7 in the growth and development of pearl millet in pot experiments. The strains were selected due to high amount of phosphate solubilizing capacity in vitro. The authors observed an increase of fresh and dry weight of pearl millet when treated with isolate C2 compared to C7 and non-inoculated controls after 30 and 45 days of growth. In addition, isolate C2 treated plants recorded maximum total chlorophyll, IAA, and starch compared to C7 strain and control plantlets. The authors concluded that the inoculation of PSB proved the potential to increase plant height, numbers of tillers, and root and shoot length of pearl millet.

The establishment of two PSB strains, 44R and A6, in the rhizosphere of pearl millet was studied under pot experiment (Kundu et al. 2006). The authors observed that the main problem to follow and monitor the survival of the inoculated bacteria in the rhizosphere is to obtain a methodology to detect the introduced bacteria. In that study, they used two phosphates solubilizing bacterial and their *lac*Z marked transconjugants that could be easily detected by a quick and consistent method using chromogenic substrate. In addition, the experiment evaluated the capacity of the bacteria inoculated to increase plant biomass under pot conditions. Millet seeds were sown in a pot containing sandy loam soil fertilized with different doses of single superphosphate (SSP) and Mussoorie rock phosphate (MRP) mixed in the upper 5 cm layer of the soil. Approximately, 10^8 cells mL^{-1} of PSB were inoculated in pearl millet seeds, and sampling was conducted at 30 and 60 days after sown (DAS) for plant biomass, shoot total P content, and PSB count. The results showed that the introduced bacteria survived up to 60 DAS in the rhizosphere following treatments with MRP and SSP compared to the control. According to the authors, P availability in the soil amended also helped in the improvement of bacterial number in the pearl millet rhizosphere. P solubilizers help the plant by supplying this nutrient to the roots and the plants, which in turn provide exudates for the survival of the bacteria. The plant biomass was increased in both stages (30 and 60 DAS) with seed inoculation, but there was no significant difference among cultural treatments. Interesting, the total bacteria count was approximately 100 times more than the PSB indicating that 1% of the soil bacteria can solubilize P.

Isolates with high mineral phosphate-solubilizing (MPS) activity can help plants to tolerate stress like heavy metals. Six isolates with high MPS activity were selected and subjected to the analysis of MPS activity under various stress conditions of $ZnSO_4$, NaCl, and temperature (Misra et al. 2012). The results showed that the MPS activity of isolates of the genera *Pseudomonas*, *Citrobacter*, *Acinetobacter*, *Serratia*, and *Enterobacter* was very high (up to 459 mg/mL). A significant decline in the MPS activity was noted with increasing concentrations of NaCl in the medium, although the isolates showed MPS activity until 0.8 M NaCl. P solubilization was affected by increased concentration of $ZnSO_4$, occurring with 30–73% inhibition of the MPS activity in different isolates. However, the significant activity of P solubilization was observed even at 1.2 M of $ZnSO_4$. A sharp decline in MPS activity (20–75%) was noticed with increasing growth temperature, although isolate RP19 tolerates a temperature of 42 °C. Seed inoculation of pearl millet with all of the six selected isolates, with no stress, in soil supplemented with 0.1% (w/w) tricalcium phosphate resulted in significant improvement in plant growth with increases of 5–68% and 45–75% on the shoot and root length of inoculated plants, respectively. Additionally, up to 100% increase in lateral root formation and 64–88% increases in fresh weight were observed. The authors concluded that the increase of plant growth was due to the P mobilization from the tricalcium phosphate.

The effectiveness of the P solubilizing *Chryseobacterium* sp. PSR 10 strain was evaluated in finger millet (*Eleusine coracana* VLM-149) inoculated host plant (Veer and Goel 2015). Pot experiments were designed with sterilized and unsterilized soil systems under greenhouse conditions. PSR 10 inoculation promoted plant growth (root and shoot length and fresh and dry weight) and increased the agronomic plant growth parameters, total chlorophyll content and nitrate reductase activity in both soils. According to the authors, the results in the sterilized soils of PSR 10 inoculation were significantly smaller than in the unsterilized soils, except for the plant day weight, which showed a better increment in sterilized soil, but still higher than the control treatment. The inoculation increased root and shoot length, fresh and dry weight in unsterilized soil. The efficiency of this strain in promoting plant growth is related to its greater P solubilization activity in soil with low P availability. In addition, the authors observed an increase in the total chlorophyll content in the sterilized system and an increase of nitrate reductase activity of plant leaves in comparison to uninoculated control. Consequently, increased chlorophyll content enhanced photosynthesis and increased plant growth and yield.

Some authors isolated and characterized PSB from millet seeds and seedlings. For example, Misganaw et al. (2019) isolated endophytic bacteria from finger millet (*Eleusine coracana* subsp. *coracana*) of the genera *Pantoea*, *Pseudomonas*, *Enterobacter*, *Sphingobacterium*, *Microbacterium*, and *Curtobacterium*. Finger millet seeds were collected from six different sampling sites in Ethiopia and contained a high number of endophytic bacteria per gram of dry weight of seeds suggesting that a large population of endophytic bacteria reside inside the finger millet seeds. Furthermore, most of the isolates produced indole-3-acetic acid (IAA) and grew on a nitrogen-free medium indicating the production of ACC-deaminase enzyme, solubilized phosphate, and hydrogen cyanide. They also observed that different genera showed restricted occurrence to a specific geographic location and that the capacity of growth promotion is related to the location showing interesting practical implications for selecting the best microbial inoculants to improve plant growth and crop yield.

In the same way, endophytic bacteria isolated from the root of finger millet (*E. coracana*) were screened for different plant growth promoting activities (Bambharolia et al. 2020). The authors isolated 30 endophytic bacteria efficient in

ACC deaminase activity, IAA production activity, phosphate solubilization, and nitrogen fixing activity. The most efficient isolates were identified as *Bacillus subtilis* and *Achromobacter xylosoxidans*.

Another study of endophytic bacteria isolated from pearl millet (*P. glaucum*) grown in salt affected areas was described by Kushwaha et al. (2020b). They isolated 102 endophytic *Bacillus* strains from the root, leaf, and stem region of pearl millet, and based on salt tolerance (≥10%), these bacteria were reduced to 28 strains. Phenotypic and biochemical features, together with the 16S rDNA sequence showed that the strains were identified as *Bacillus albus*, *B. amyloliquefaciens*, *B. aryabhattai*, *B. halotolerans*, *B. haynesii*, *B. pacific*, *B. paramycoides*, *B. proteolyticus*, *B. pumilus*, *B. subtilis*, *B. siamensis*, *B. tequilensis*, *B. wiedmannii*, and *B. zhangzhouensis*. *B. amyloliquefaciens* EPP90 was considered a potent multi-stress-tolerant crop growth promoter based on the selection of *in vitro* traits, such as nutrient solubilization, heavy metal and polyethylene glycol tolerance, extracellular enzyme production, and phytopathogens antagonistic potential. This strain was inoculated in pearl millet (cv. NDFB-2) seeds in a pot experiment maintained in a greenhouse. The experiment was performed in natural sandy loam field soil and salt stress was created by irrigating pots with 200 mM NaCl solution every 3 days. The treatments were seeds inoculated with *B. amyloliquefaciens* EPP90 without NaCl; EPP90 with NaCl 200 mM; control without NaCl and control with NaCl 200 mM. The results indicated that pearl millet plants treated with EPP90 grown under saline (200 mm NaCl) showed superior shoot and root dry weight, shoot and root length, seed germination, and seedling vigor compared with non-inoculated control treated with NaCl 200 mM. Interestingly, 30 days after inoculation, strain EPP90 was successfully recovered from shoot, root, and leaf of endophytic treated seeds demonstrating that it persists for the entire growing season of millet.

Plant growth promoting and antifungal activity in endophytic *Bacillus* strains isolated from pearl millet was assessed by Kushwaha et al. (2020a). According to the authors, different studies have pointed to *Bacillus* as a significant endophytic microorganism in pearl millet offering stress tolerance, antagonistic potential against phytopathogen, and plant growth promotion traits. In this work, the authors identified 19 *Bacillus* strains isolated from root, stem, and leaf displaying antagonistic activity toward the phytopathogens *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium solani*. The strains include *B. amyloliquefaciens*, *B. subtilis* subsp. *subtilis*, and *B. cereus* were identified by morphological, biochemical, and molecular methods. This indicates that pearl millet contains a diversity of *Bacillus* endophytic highly promising antagonists, showing ≥50% fungal mycelium growth inhibition against all the tested phytopathogenic fungi. They also presented three genes (*ituD*, *bmyC*, and *srfA*) related to effective pathogen inhibition. Besides displaying antimicrobial capacities, these strains varied in phosphate, potassium, and zinc solubilizing properties and showed indole acetic acid and siderophores production. Among the strains isolated, six were phosphate solubilizers, seven K solubilizer, and five Zn solubilizers. In addition, these strains showed prominent halo zone of solubilization in the culture medium. In the greenhouse, under pathogen presence, three endophytes (EPP5, EPP62, and EPP65) were selected based on their potential antagonistic activity against fungi and PGP attributes. Seeds treated with antagonistic endophytic strains increased the plant biomass including root and shoot length when applied singly or in consortium mode in the treatments conduced in vivo. Interestingly, the combined application of EPP5, EPP62, and EPP65 has additive effects compared to single inoculation.

The effects of co-inoculation of N-fixer (*Azospirillum lipoferum* and *Rhizobium* sp.) and P-solubilizing microorganisms (*Bacillus megaterium* var *phosphaticum*) indicate the beneficial effect of co-culturing in the germination, seedling vigor, plant height, and seed weight, resulting in 6% increase in grain yield of pearl millet under greenhouse conditions (Poonguzhali et al. 2005). In addition, the enzymes phosphatase, nitrogenase, and urease were also significantly increased in the crop by co-inoculation compared to single inoculation. As nitrogen and phosphorus are two more essential fertilizers for crop production, the co-inoculation of a P solubilizer and a nitrogen fixer microorganism indicate a more significant benefit than both individually inoculated. In another study, a co-inoculation experiment was conducted with *Azospirillum lipoferum* and phosphate-solubilizing bacteria (PSB) in foxtail millet [*Setaria italic* (L.) Beauv] (cv. Chitra) (Rafi et al. 2012). Inoculation with two *Azospirillum* strains and one strain of PSB, individually and in combination, significantly increased plant height, shoot and root dry weight over control. Moreover, both the panicle and seed weight increased due to the simultaneous inoculation of the plants with *Azospirillum* and PSB.

9.3.3 The Effect of Mycorrhizae on Phosphorus Uptake in Millet

Among the mechanisms associated with plant P acquisition, arbuscular mycorrhizal fungi (AMF) is a promising strategy for increasing yield in millet (Misganaw et al. 2019). The hyphae constitute an extra radical mycelium that extends from the roots allowing plant to explore the soil beyond the rhizosphere depletion zone, enhancing thereby P uptake and yield (Smith and Read 2008). The AMF depends on the carbon and lipids supply from the plant, which in turn delivers mineral nutrients, especially P and nitrogen (N) to its host (Smith and Read 2008; Jiang et al. 2017; Luginbuehl et al. 2017), including other benefits such as protection against biotic and abiotic stresses (Smith et al. 2009). In addition, several AMF can also solubilize P present in the soil, either directly by exudation of enzymes and acids or indirectly through soil MSP community modification (Mackay et al. 2017).

The dependence of plants on AMF association to produce its maximum growth and yield at a certain level of soil fertility is referred as mycorrhizal dependency (MD). The MD is estimated by the difference between the plant growth increase in response to FMA compared to non-colonized plant (control). Kandhasamy et al. (2020) assessed the effect of two soil types on MD, shoot and root biomass and N and P content of finger millet genotypes cultivated under a greenhouse after 45 days of growth. The inoculation of AM fungi resulted in a significant shoot and root biomass increase in both soil types. One genotype was highly dependent on AM fungi and the others were AMF-dependent, varying in their MD.

The response of AMF inoculation on the growth of millet (*Pennisetum glaucum* L.), mung bean (*Vigna radiata*. L.), mash bean (*Vigna mungo* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* L.) was evaluated by Sharif et al. (2011). They conducted an experiment using the crops inoculated in the presence of indigenous mycorrhiza with the inoculum containing 40–50 AMF spores mixed with 20 g sterilized soil. Different crops presented higher nutrient uptake and yield when inoculated with AMF. An increase of about 20% in shoot and root dry matter was observed in inoculated millet compared to non-inoculated. Millet and mash beans crops showed AMF root infection of 35% and 32%, respectively, which resulted in an increase of 67% in N and iron (Fe) in millet and 166% in P uptake in mash beans.

Studies related to the role of naturally occurring AMF on early mycorrhization and colonized root length on P uptake and pearl millet performance are scarce (Beggi et al. 2016). To fill this gap of knowledge, these authors had grown eight pearl millet varieties contrasting in low-P efficiency in pots under low and high P conditions and harvested them 2, 4, 6, and 8 weeks after sowing. AMF colonization was positively correlated with dry matter production. Two weeks after sowing, the P-efficient genotypes differed in the percentage of AMF infection, and they were colonized almost twice as much as P-inefficient ones. P-efficient genotypes had greater total root length infected with AMF, higher percentage of AMF colonization, and increased P uptake than P-inefficient genotypes under low P.

9.3.4 Phosphate-solubilizing Bacteria and Millet in Brazilian Conditions

Four endophytic *Bacillus* strains inoculated in millet (*Pennisetum glaucum*) seeds were evaluated in Brazil (Ribeiro et al. 2018). These authors used Araxá rock phosphate or soluble triple superphosphate as a P source and the results indicated an increase in pearl millet growth, and nutrient uptake under low-P conditions. In addition, some *Bacillus* strains increased shoot and root dry weight and the nitrogen, phosphorus, and potassium (N, P, and K) content of plants cultivated in soil with no P added. Specifically, B2084 and B2088 strains, despite of solubilizing less iron phosphate (Fe-P) than B1923, showed positive performance on dry weight and accumulation of N, P, and K nutrients in the shoot while B1923 increased root and shoot biomass and root N and P. The authors concluded that the strains presented multiple PGP traits as Fe-P solubilizing, siderophores, and high levels of indole-acetic acid (IAA) production. Also, these strains presented potential as biofertilizers for commercial applications without P fertilization.

Moreover, 101 bacterial isolates obtained from the rhizosphere and endosphere of maize plants were evaluated for their potential to solubilize P in vitro and to promote millet (*P. glaucum*) growth under soil amended with rock phosphate (RP) and triple superphosphate (TSP) (Silva et al. 2021). Six bacteria were selected from in vitro tests, *Microbacterium* sp. UFMG61, *Klebsiella variicola* UFMG51, *Pseudomonas* sp. UFMG81, *Pantoea ananatis* UFMG54, *Bacilus megaterium* UFMG50, and *Ochrobactrum pseudogrignonense* CNPMS2088. *Microbacterium*, *Klebsiella* and *Pseudomonas* were tested in soils amended with TSP and RP at a final concentration of 300 mg P/dm^3 soil. The results indicated that the plant growth parameters were higher in soil with P soluble source (TSP) than in the soil with RP. However, some millet growth parameters evaluated (foliar area, plant height, root and shoot dry weight, and plant dry weight) and P absorption (root and shoot P) were higher in the soil fertilized with RP than TSP. Two bacterial strains, *B. megaterium* UFMG50 and *O. pseudogrignonense* CNPMS2088, performed better than the others in the RP assays, improving at least six physiological traits of the plants. The strains UFMG50 and CNPMS2088 combined with *P. ananatis* UFMG54 and *Pseudomonas* sp. UFMG81 significantly increased the soil P content, when compared with soil without inoculation. *O. pseudogrignonense* CNPMS2088 promoted increase in plant height, root, shoot and plant biomass and P in the shoot and plant. *B. megaterium* UFMG50 showed the best performance, increasing all the parameters evaluated when compared to the un-inoculated treatment: foliar area, plant height and root, shoot and plant biomass, and P content in the shoot, root, and plant. The complete genome sequencing of the six bacteria evaluated for millet growth showed that genes related to organic acids production (such as gluconic, 2-keto gluconic, acetic, lactic, glyoxylic, glycolic, and formic acids), phytohormones IAA (five genes), and cytokinins (four genes) are among the mechanisms contributing for millet growth. The authors concluded that endophytic bacteria were more efficient in RP solubilization than bacteria isolated from rhizospheric soil.

In another study, PSBs were inoculated in pearl millet (*P. glaucum*) in combination with alternative phosphate sources (granulated organomineral, branned organomineral fertilizers) and Bayóvar rock phosphate in two growth trials in the greenhouse: the first using clayey soil and the second with using sandy soil (Santos et al. 2022). The treatments comprised five P sources, with or without inoculation with CNPMS B119 (*Bacillus megaterium*) and CNPMS B2084 (*B. subtilis*) strains. The results showed that alternatives P sources, Bayóvar and inoculation with PSM stimulated the production of dry shoot mass in millet grown on sandy soils, which was not observed in clayey soil. No effect of PSM inoculation on nitrogen use efficiency (NUE), potassium use efficiency (KUE), and P use efficiency (PUE) were observed, while it was higher with the alternative P sources compared to triple superphosphate (TSP) treatments. However, between P source treatments and the two soil types, the PUE of inoculated treatments was higher in the alternative treatments compared to TSP. These results suggest a beneficial effect of PSB to increase PUE by millet plants. Plants receiving TSP were less efficient in absorbing P than those receiving granulated, branned organomineral, and Bayovar, suggesting a potential for alternative P sources in millet nutrition.

Another experiment, the inoculation of PSM (*Bacillus* sp. and *Burkholderia* sp.) in organomineral fertilizers from agricultural residues (in increasing doses of 30–90 g.pot^{-1}) mixed with rock phosphates on the growth of pearl millet was conducted in a pot experiment (Almeida et al. 2016). The experiment was conducted on two successive crops of millet. The inoculation of PSM sprayed in the furrow in the first crop resulted in higher growth of millet, greater nutrient extraction by the plants, and higher P availability in the soil. In the two successive crops, the dry shoot mass of the inoculated organomineral mixed with rock phosphates fertilizers was superior compared to non-inoculated treatments. On the other side, there was no difference in the root dry mass of the inoculated and non-inoculated treatments. In general, the accumulation of nitrogen (N), phosphorus (P), and potassium (K) by the millet shoot and root and the soluble P in the soil were higher in the inoculated organomineral fertilizers. The authors also evaluated the soil enzymatic activity (acid and alkaline phosphatase) and showed that alkaline activity was lower than acid phosphatase in both crops. The acid phosphatase activity in the first crop did not show a significant difference among the treatments while the alkaline phosphatase showed higher activity in the higher doses of organomineral fertilizers (60 and 90 g. pot^{-1}) in the first and second crops.

9.4 Conclusions

Over the last decade, biological products have become one of the fastest growing segments in agriculture. In this chapter, we highlighted the potential of using phosphate-solubilizing bacteria to increase P use efficiency and enhance millet production, as bioinoculant increases the biological component in agricultural production systems, promoting soil health, both reducing the use of synthetic fertilizers and adding beneficial microorganisms.

In general, the use of millet P-solubilizing microorganisms can be optimized with appropriate combinations of strain selection, environmental conditions, and plant genotypes. Therefore, additional efforts should be made in the development of better commercial inoculants and production systems that allow reducing the chemical fertilizers used to increase soil fertility and productivity of this crop.

To expand and consolidate the offer of bioinoculants in the market, future research should be done at (i) identifying and characterizing new microorganisms that are more efficient in the P solubilization; (ii) characterize mechanisms of action for a better understanding of the processes and selection of strains; (iii) define optimal conditions for the activity of inoculants, including interactions between different microorganisms native of the soil and other widely used bioinoculants; (iv) expand studies under field conditions to define doses and improve the understanding of responses to inoculation in different soil and climate conditions; (v) expand studies with different cultures to define affinity and doses; (vi) conduct long-term field experiments to understand the long-term behavior of inoculants and impacts on soil microbiological health, including crop rotation.

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Chapter 10
Impact of Rhizosphere Ecology on Nitrogen Fixation in Millets

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Abstract Millets are prime underutilized nutraceutical crops that have potential to substitute staple crops. The agro ecological characteristics and nutritional qualities of millets are superior to those of major cereals, which make them efficient crops to meet immediate food security needs. A plant's normal functioning is dependent on the condition of the soil, as it is a reservoir of water and nutrients. The rhizosphere is the area around a plant's roots, and there are complex relationships among the biotic and abiotic communities in this region. Microorganisms that comprise the rhizosphere microbiome include archaea, viruses, fungi, bacteria, and eukaryotic organisms which occupy a compact region of soil near the roots of plants. Symbiosis between plants and microbes has long been recognized as a helpful and important part of sustainable agriculture. Rhizospheric zones contain a wide variety of microbial populations. It is not just that different microbes interact, but they also have an impact on the host. The rhizosphere of millet is an important habitat for beneficial microbes, providing nutrition to plants and maintaining soil health. Rhizobacteria, nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, and phosphate solubilizers and other micro- and macro-organisms make up the microbial community of millet crops. The rhizosphere of millets was dominated by *Actinobacteria, Proteobacteria, Acidobacteria*, *Enterobacter*, *Pantoea*, *Klebsiella*, *and Arthrobacter. Among them species such as Chloroflexi Pseudomonas Pseudomonas fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae* were mostly present. Certain microorganisms are involved in the biological nitrogen fixation, using nitrogenase enzyme, these organisms convert atmospheric nitrogen into ammonia. A plant's rhizosphere harbors microorganisms that are capable of reducing atmospheric nitrogen to ammonia. In root zones, beneficial microbes are found to aid in the formation of root hairs, the increase of biomass, and a better growth rate. In this chapter, we will have a look at millets and the role of rhizospheric microbes associated with them.

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_10

Keywords Millets · Rhizosphere · Nitrogen fixation · Microorganisms · sustainable agriculture

10.1 Introduction

Plant resources are being depleted under the influence of climate change, rising population, and unrestricted use of natural resources (El-Beltagy and Madkour 2012). The need of the hour necessitates exploring new food alternatives other than the staple crops. There are a number of underutilized crops with great potential to ensure food security. Millets are prime underutilized nutraceutical crops that have potential to substitute staple crops (Muthamilarasan and Prasad 2021). The varieties of millet include proso millet, finger millet, barnyard millet, pearl millet, foxtail millet, kodo millet, browntop millet, sorghum, and little millet (Mushtaq et al. 2021). The agro ecological characteristics and nutritional qualities of millets are superior to those of major cereals, which make them efficient crops to meet immediate food security needs (Saleem et al. 2021). Millets are gluten free, highly nutritive with no compromise in taste and texture (Opole 2019). They provide ongoing income to marginal populations, do not require synthetic fertilizers, and produce low carbon footprints compared to major cereal crops (Muthamilarasan and Prasad 2021). Due to a lack of knowledge regarding their nutritional value, their consumption is still limited.

A plant's normal functioning is dependent on the condition of the soil, as it is a reservoir of water and nutrients. The rhizosphere is the area around a plant's roots and there are complex relationships among the biotic and abiotic communities in this region (Dutta and Podile 2010). Microorganisms that comprise the rhizosphere microbiome include archaea, viruses, fungi, bacteria, and eukaryotic organisms which occupy a compact region of soil near the roots of plants (Berg et al. 2020). A rhizosphere is characterized as an area in the soil that is affected by roots secretions and exudates, which are vital to plant health and growth and for microbes also. A number of soil metabolites are reported to be released including organic acids, siderophores, inorganic acids, sugars, amino acids, vitamins, polysaccharide mucilage, nucleosides, etc. (Badri et al. 2009). These soil microbes appear to be sensitive to a variety of chemical substances, including fertilizers and other chemical or physical changes that would selectively enrich certain microbes over others. The microbes in the rhizosphere can be both beneficial and harmful to plants. Soil health is improved by the beneficial microorganism as the detoxification and degradation of pollutants help in cycling nutrients, solubilize phosphates and silicates, fix nitrogen, and influence soil biology and chemical properties in a positive manner. In this way, the rhizosphere is crucial in maintaining the properties of soil and plant health (Khatoon et al. 2020).

10.2 Rhizosphere of Plants

The soil area adjacent to roots, ranging in distance from 1 to 3 mm provides a niche for plant root and microbial interactions. It is one of the most dynamic interfaces of earth. It is estimated that thousands of species of microbes are associated with plant roots. Per gram root may contain up to 10^{11} microbial cells with greater than 30,000 prokaryotic species. There are many genes in this microbial community that are significantly larger than plant genes. In fact, it is referred as the second genome of plant (Berendsen et al. 2012). In the rhizosphere, there are a variety of bacteria such as *Pseudomonas chlororaphis*, *Bacillus* spp., *Pseudomonas fluorescens*, *Chromobacterium violaceum*, *Rhizobium* sp., *Bradyrhizobium japonicum*, *Burkholderia*, *Arthrobacter*, *Paenibacillus*, *Acinetobacter*, *Azospirillum* sp., *Azotobacter* sp., and *Enterobacter* sp. (Barra Caracciolo and Terenzi 2021; Alawiye and Babalola 2019). As a result of advances in research on plant–microbe interactions, it has been shown that plants are capable of shaping the microbiome of their rhizosphere (Middleton et al. 2021). These results are made evident by the fact that different plant species host different microbial communities when grown on the same soil. These rhizospheric microbes aid plants and provide lot of benefits like protection from fungal pathogens by production of secondary metabolites that inhibit their growth, induction of systemic resistance to protect from phytopathogens, encourage plant growth, by promoting the outbreak of secondary roots, aid in biological nitrogen fixation, and production of plant growth promoting substances and cycling of minerals and nutrients (Colla et al. 2017; Igiehon and Babalola 2018).

10.3 Rhizosphere of Millets

Symbiosis between plants and microbes has long been recognized as a helpful and important part of sustainable agriculture (Ray et al. 2020). Rhizospheric zones contain a wide variety of microbial populations. It is not just that different microbes interact, but they also have an impact on the host (Berendsen et al. 2012). The rhizosphere of millet is an important habitat for beneficial microbes, providing nutrition to plants and maintaining soil health. Rhizobacteria, nitrogen-fixing bacteria, arbuscular mycorrhizal fungi, phosphate solubilizers, and other micro- and microorganisms make up the microbial community of millet crops (Choudhary et al. 2020). As a result of their association with millets, these rhizospheric microbes confer protection against various stresses. A number of mechanisms are also involved in this association, which aid millet plants in adapting to hostile environments as well as in enhancing their productivity, either directly or indirectly. The rhizosphere of proso, foxtail, and sorghum was found dominated by actinobacteria, proteobacteria, acidobacteria, and chloroflexi. In the same study, the microbial richness of proso millet was found more, whereas it was less in case of foxtail millet (Tian et al. 2022). *Streptomyces labedae*, *Streptomyces flavofuscus*, *Pseudomonas* *poae*, and *Pseudomonas fluorescens* were found to be the most promising isolates among 104 bacteria and 96 actinomycetes isolated from finger millet root rhizospheres (Kamal et al. 2015). Similarly, pearl millet rhizospheres were found to harbor 184 strains of bacteria, including *Rhizobiales* and *Bacillales*. Bacterias belonging to *Gaiellaceae* and *Sphingomonadaceae* families and *Bradyrhizobium*, *Mesorhizobium*, and *Trichoderma* genus were associated with the large rhizosheath phenotype in this plant (Ndour et al. 2017; Ndour et al. 2021). Based on the phylogenetic analysis, numerous strains of foxtail millet were found to belong to six genera which include *Pseudomonas*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Arthrobacter*, and *Ochrobactrum*. Among these the species identified include *Pseudomonas fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae* species (Niu et al. 2018). Similarly, from the foxtail millet rhizosphere, 624 genera belonging to 254 families of microbes were isolated. Investigation of the structure of the microbiota of foxtail millet roots revealed that it mainly consisted of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteri* (Jin et al. 2017).

10.4 Nitrogen Fixation

As a crucial component of the cell, nitrogen is present in proteins, amino acids, DNA, RNA, NAD, ATP, chlorophyll, etc. (Jespersen and Huang 2015). Despite being available in gaseous form, N_2 must be converted into usable forms before it can be used by plants. Nitrogen in oxidized (NO_x) or reduced (NH_3, and amines) forms is assimilated by plants (Lindström and Mousavi 2020). The nitrogen in atmospheric gases is converted into usable forms through industrial or biological processes. A plant's rhizosphere harbors microorganisms that are capable of reducing atmospheric nitrogen to ammonia (Nwachukwu and Babalola 2021). Certain microorganisms involved in the biological process such as *Azospirillum*, *Azotobacter*, *Rhizobium*, *Frankia*, *Methanogens* are involved in fixation of nitrogen (Mącik et al. 2020). Using nitrogenase enzyme, these organisms convert atmospheric nitrogen (N_2) into ammonia (NH_3). This enzyme assists in the breakdown of triple covalent bonds between nitrogen atoms, which allows plants to produce usable forms of nitrogen so that they can manufacture nitrogenous biomolecules (Brouder and Volenec 2020). ATP is required to fix nitrogen and it requires 16 moles of ATP for fixing each mole of N_2 (Cheng et al. 2021; Sampath et al. 2020). Nitrogenase is a two-component enzyme which composes of MoFe protein (also called dinitrogenase or component I) and the electron-transfer Fe protein (also called dinitrogenase reductase or component II), a reducing source and MgATP is required for catalysis. Fe protein and MoFe protein associate and dissociate in a catalytic cycle involving single electron transfer and MgATP hydrolysis, MoFe protein contains two metal clusters: the iron–molybdenum cofactor (FeMo-co), which provides the active site for substrate binding and reduction, and P-cluster, involved in electron transfer from the Fe protein to FeMoco (Mus et al. 2018). The nitrogen cycle begins with the conversion of nitrogen gas (N_2) to ammonia (Lindström and Mousavi 2020). The conversion of ammonia into nitrates is aided by nitrosomonas or nitrococcus. The formed nitrates are again converted into nitrates by nitrobacter. This process in which ammonia is converted into nitrates and nitrites is named nitrification (Robles-Porchas et al. 2020). Nitrites are usable forms of nitrogen and plants absorb these by roots. The absorbed forms are assimilated into the plant body. After the death and decay of plant, the organic nitrogen is converted back into ammonia and released into atmosphere by ammonification process (Troitsky et al. 2019).

10.5 Impact of Rhizosphere Ecology of Foxtail Millet on Nitrogen Fixation

Both symbiotic and non-symbiotic bacteria are able to put forth beneficial outcome on plants by certain plant growth regulators and providing usable forms of nitrogen. In foxtail root zones, beneficial microbes are found to aid in the formation of root hairs, the increase of biomass, improved nitrogen fixation, and a better growth rate in (Liu et al. 2019) (Table 10.1). Two hundred eighty-five *Azospirillum* isolates were examined for their nitrogen-fixing abilities to indicate that *Azospirillum* contributes significant amounts of nitrogen to foxtail millet. A notable difference was observed between the pure culture isolates obtained from the rhizosphere samples and those obtained from the non-rhizosphere samples in terms of nitrogen fixation ability. It is interesting that pure culture isolates obtained from the rhizosphere samples exhibited higher nitrogen-fixing ability compared to the isolates obtained from the non-rhizosphere samples. A significant increase in shoot dry weight, panicle weight, branching, root development, and length was found when *Azospirillum brasilense* was found in association with *Setaria italic* L. Nitrogen content of plants inoculated with *A. brasilense* was found to increase because of the fixation of N_2 by the bacteria. Based on the results of the study, it appears that *A. brasilense* may increase plant yield while saving nitrogen fertilizer in foxtail plants (Kapulnik et al. 1981). Similarly, in another study, *Azospirillum* species were collected from foxtail millet rhizosphere soil as well as non-rhizosphere soil. It was determined that 56 strains of *Azospirillum* spp. are capable of fixing nitrogen out of total of 100 strains of *Azospirillum*. Out of these strains, 20 fixed nitrogen more efficiently than the other strains. Each of these strains was identified as *Azospirillum lipoferum* (Raffi and Charyulu 2012).

In another study, *Azospirillum* isolates from the rhizosphere of foxtail millet were isolated and evaluated for nitrogen-fixing activities, and it was observed that *Azospirillum* populations were usually lower in non-rhizosphere samples than in rhizosphere samples. Furthermore, the isolates of *Azospirillum* not only had had different nitrogen-fixing capacities but rhizospheric isolates are better at fixing nitrogen than non-rhizosphere isolates (Vijayalakshmi and Swamy 2019). Studies on rhizobacteria were conducted to improve the productivity of foxtail millet and its nutritional content. Compared to the control, foxtail millet seeds treated with

Table 10.1 Effect of different rhizospheric microbes on plant morphological and biochemical parameters

Species	Rhizospheric bacteria inoculated/present	Benefit	References
Foxtail millet	*Azospirillum brasilense*	Improved nitrogen fixation, nitrogen content, shoot dry weight, panicle weight, branching, root development, and length	Kapulnik et al. (1981)
Foxtail millet	*Azospirillum lipoferum*	Increased plant yield and nitrogen fixation	Raffi and Charyulu (2012), Vijayalakshmi and Swamy (2019)
Foxtail millet	*Bacillus* sp. and *Rhizobium* sp.	More chlorophyll, increased nutrient uptake, and were higher in protein rise in nitrogen and phosphorus uptake	Khatri et al. (2016)
Foxtail millet	*Azospirillum brasilense*	Improved nitrogen content and activities of enzymes related to nitrogen fixing	Okon et al. (1983)
Kodo millet	*Actinobacteria* and *Proteobacteria*	Improved fixation of carbon, helps in uptake of nitrogen, biosynthesis of phosphorus, nitrogen, and sulfur synthesis	Prabha et al. (2019)
Finger millet	*Dyadobacter* sp	Promote growth, fix N_2, improved total chlorophyll, soil nitrate concentration, soil nifH, and nitrate reductase activities	Kumar et al. (2018)
Barnyard millet	*Pseudomonas, Trichoderma*, and *Acaulospora laevis*	Plant growth parameters and nutrient uptake were improved	Channabasava and Lakshman (2013)
Pearl millet	*Azospirillum brasilense*	Increased yield and nitrogen uptake	Tilak and Subba Rao (1987)
Pearl millet	*Azospirillum lipoferum* and *Azotobacter chroococcum*	Increased grain yield, nitrogenase activity, leaf nitrate reductase activity	Wani et al. (1988)

Bacillus sp. and *Rhizobium* sp. yielded significantly more chlorophyll, increased nutrient uptake, and were higher in protein. There was also a rise in nitrogen and phosphorus uptake, total dry weight, and grain yield. Nutrients are more likely to be absorbed by roots when roots have increased area and number of uptake sites, as well as when roots are more able to exploit the soil. Thus, in this case the increased uptake of nutrients may be due to increased surface area and more uptake sites in root (Khatri et al. 2016). A study was conducted to determine the effect of *Azospirillum brasilense* inoculation and nitrogen fixation on the yield of *S. italica*, and it was observed that root and shoot dry weight increased significantly. The nitrogen content and activities of enzymes related to nitrogen fixing also improved in comparison to control (Okon et al. 1983).

10.6 Impact of Rhizosphere Ecology of Kodo Millet on Nitrogen Fixation

Microbial diversity in the rhizosphere of Kodo millet was associated with functional capacities that supported the plants development and growth. A total of 65 taxonomically diverse phyla were identified, with *Actinobacteria* dominating the rhizobiome followed by *Proteobacteria*. In terms of metabolic function, the multifunctional rhizobiome carries out several metabolic functions, including fixation of carbon, helps in uptake of nitrogen, biosynthesis of phosphorus and sulfur synthesis, alleviation of stress, production of plant secondary metabolites, and disease defense. It is clear that the plant's growth, development, and survival are attributed to the presence of genes related to N, P, S, Fe, and aromatic compound metabolism, as well as phytohormone production. The increased transcripts in the rhizosphere of kodo millet which are associated with assimilation of ammonia, nitrate, and nitrite ammonification increases the nitrogen use efficiency of this plant. Moreover, the enzymes like glutamate synthase, glutamate-ammonia-ligase adenylyl transferase and the enzymes related to ammonium transport, nitrate ABC transporter, nitrate/nitrite transporter, and nitrite reductase were identified in the rhizospheric zone, indicating that much ammonia was assimilated in the rhizosphere in presence of rhizospheric micro biota. The reads corresponding to nitrate/nitrite ammonification (the process in which reduction of nitrate or nitrite to NH_4^+ takes place without nitrous oxide) were found dominant in the rhizosphere of kodo millet. Due to dominant microbial communities, all these processes may enrich the soil N content (Prabha et al. 2019).

10.7 Impact of Rhizosphere Ecology of Finger Millet and Barnyard on Nitrogen Fixation

An evaluation of the growth-promoting potential of the psychrotolerant bacterium *Dyadobacter sp.* against finger millet was conducted and it was found that this bacterium was capable to promote plant growth and fix N_2. Besides, nitrate reductase activity and total chlorophyll was improved with respective controls. It was found that soil pH, soil nitrate concentration, soil nifH, and plant leaf nitrate reductase activities are positively correlated (Kumar et al. 2018). Similarly, using inoculation and assessing the impact of *Glomus mossease, Azospirillium brasilense*, and PSB on finger millet height, dry root and shoot weight, root colonization, spore counts, P, and N uptake, it was found that the growth parameters of AM fungi were moderately improved following single inoculation as well as combined inoculation with *Azospirillium brasilense* or PSB. However, the highest growth parameters were found with triple inoculation of AM fungi, *Azospirillium brasilense*, and PSB (Ramakrishnan and Bhuvaneswari 2014). In another study, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Trichoderma*, and *Acaulospora laevis* were used to cultivate barnyard millet, and it was found that the plant growth parameters and nutrient uptake were improved. The treated plants showed improved responses with the maximum better results after dual inoculation of *Acaulospora laevis* and *Azotobacter* (Channabasava and Lakshman 2013). Moreover, in a recent investigation on the role of two C4-dicarboxylate transporters of *A. brasilense* Sp7 and their capacity to develop and fix nitrogen on dicarboxyates revealed that *A. brasilense* Sp7 dicarboxylate transporters play a significant role in the colonization and proliferation of finger millet roots (Singh et al. 2019).

10.8 Impact of Rhizosphere Ecology of Pearl Millet on Nitrogen Fixation

From the microscopic observations of the root system of pearl millet (*Pennisetum americanum* (L.) Leeke), an extensive colonization by *Azospirillum* species was observed. Under different agro climatic conditions, pearl millet yield and nitrogen uptake by plants increased after seed inoculation with *A. brasilense* (Tilak and Subba Rao 1987). Pearl millet yields were significantly enhanced after inoculating rhizospheric microbes and a greater improvement in plant morphological parameters was observed with treatment than with control plants. In this study, it was found that combinations of diazotrophic bacteria *Pseudomonas fluorescens*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Acetobacter diazotrophicus*, and *Trichoderma viride* considerably boosted the height, dry weight, ear length, grain, and yield of pearl millet. In addition to nitrogen fixation and phosphate solubilization by microorganisms, the increase in plant height may also be due to increased nitrogen and phosphorus uptake by the plants (Singh et al. 2016). Similarly, in another study, pearl millet was studied after being inoculated with N_2-fixing bacteria *Azospirillum lipoferum* and *Azotobacter chroococcum* and the increase in grain yield was observed. Repeated inoculations with *Azotobacter chroococcum* and *Azospirillum lipoferum* also increased grain yield by 14.4% and 9.8%, respectively. Furthermore, the nitrogenase activity, leaf nitrate reductase activity, and efficiency of N-assimilation were increased after inoculation (Wani et al. 1988). When pearl millet seeds were inoculated with *Azotobacter*, significantly higher plant heights, accumulations of dry matter, and total tillers are observed at different growth stages of growth. As a result of *Azotobacter* inoculation, increased growth may be because more efficient strains of *Azotobacter* are present in the rhizosphere, so more atmospheric nitrogen is utilized. Possibly, the roots absorbed nitrogen from soil near the root zone, which was fixed by *Azotobacter* (Togas et al. 2017). In another study, *Azospirillum brasilense* was inoculated into *Pennisetum americanum* L. in the field to determine plant yields and acetylene reductions. As a result of inoculation, dry weight and total N of plants increased significantly (Bouton et al. 1979). A study on plant growth-promoting endophytic bacteria isolated from pearl millet showed that most cultures possessed P-solubilization, ACC-deaminase activity, IAA, and siderophore production, whereas one culture showed N_2 fixing potential under free-living conditions (Manjunatha et al. 2016)

10.9 Conclusion

As a fundamental component of amino acids, nitrogen contributes to protein synthesis. In the atmosphere, this element makes up 78% of the air. It is mostly non-reactive and practically inert (N_2). Today, the majority of agriculture practices are dependent on nitrogen fertilizers. The increased use of N fertilization in agriculture is associated with pollution which has become a global problem. Plants fix nitrogen with the help of rhizospheric microbes and use the usable forms for growth and development. There is a positive relation between rhizospheric biota and fixing of nitrogen. It is possible to use this association to improve nitrogen fixation in plants which in turn can reduce the use and need of synthetic nitrogen fertilizers, thereby reducing pollution.

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Chapter 11
Synergistic Effects of Arbuscular Mycorrhizal Fungi and PGPR on Yield Improvements in Millets

N. Umashankar, H. R. Raveendra, P. S. Benherlal, and K. B. Bhagyashree

Abstract The contribution of soil microflora in maintenance of soil fertility and plant health is significant. The mycorrhizal fungi are one of the key factors in microbial population. By establishing a symbiotic relationship with host plant, they positively influence the plant growth and nutrition. Plant growth promoting rhizobacteria (PGPR) helps in mycorrhizal association with the plants by increasing spore germination and hyphal permeability in host plant roots. These bacteria promote crop growth through production of plant hormones, activating host defense system. Millets are considered as major cereal crops with many nutritional benefits and ability to grow under marginal lands. In order to enhance the potentiality of these millets, combined application of vesicular arbuscular mycorrhiza (VAM) and PGPR is an eco-friendly approach. So, here interaction effects of VAM and PGPR in various millets cultivation are discussed with respect to increase in nutrient availability, management of biotic and abiotic stresses.

Keywords Arbuscular mycorrhiza · Plant growth promoting rhizobacteria · Millets · Synergism · Plant nutrition

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_11

11.1 Introduction

In the developing world, millets are considered as major cereal crops. They are important in semiarid tropical areas like Asia and Africa, as human food and livestock feed (Alavi et al. 2019). They represent small grain group, grown in marginal land area. Finger millet, pearl millet, kodo millet, barnyard millet, proso millet, foxtail millet, and little millet are normally grouped as millets and to this group, pearl millet is an exception because of its morphological characteristics (Govindaraj et al. 2020).

Millets are having high nutritional value compared to common cereal crops and more content of essential amino acids and methionine in their protein profile (Nithiyanantham et al. 2019). They offer huge health benefits with many micronutrients and phytochemicals. In pearl millet, biochemical profiling revealed about 7% fats, 2% crude fibers, 63%starch, 13% protein and 92% dry matter, soluble and insoluble dietary fibers, and antioxidant enzymes with resistant starch in large amount.

Foxtail millet, with the presence of lysine, the essential amino acid, is regarded as a supplemental source of protein. Finger millet being rich with polyphenols, phytochemicals (Dayakar Rao et al. 2017), calcium, methionine, tryptophan, fiber, 2% minerals, 4% crude fibers, 9% protein and 81% carbohydrate and amino acids like valine, threonine, and lysine make millets as efficient source for utility in food industries (Singh et al. 2018).

Despite their benefits, millets yield is limited by many stresses like environmental and climate changes (Ullah et al. 2019). Productivity of millets is affected by serious effect of heat and drought stress (Yadav et al. 2020). These stresses at seedling and reproductive stage are reported to cause yield loss of 60% and 40% in pearl millet and teff, respectively (Abraha et al. 2015). To prevail over the effects of climate change and related stresses, in order to enhance the yield, there is necessity for stress-tolerant and high-yielding varieties and are also many ways to enhance stress tolerance and productivity in these crops. For example, PGPRs application has been employed to improve yield and stress tolerance in wheat (Nawaz et al. 2020) and paddy (Joshi et al. 2020), so it is needed to be tested in minor crops.

11.2 Arbuscular Mycorrhizal Fungi and Plant Nutrition

The term "mycorrhiza" is originally from Greek words, *mykes*-fungus and *rhiza*-root. A situation was first reported by Frank (1885) where "certain tree species consistently don't feed themselves individually in the soil but they establish a symbiosis with a fungal mycelium which takes over the entire nourishment of the tree from the soil." Based on its morphological characteristics, mycorrhizal fungi can be grouped into seven classes, viz., arbuscular mycorrhiza (AM), ericoid

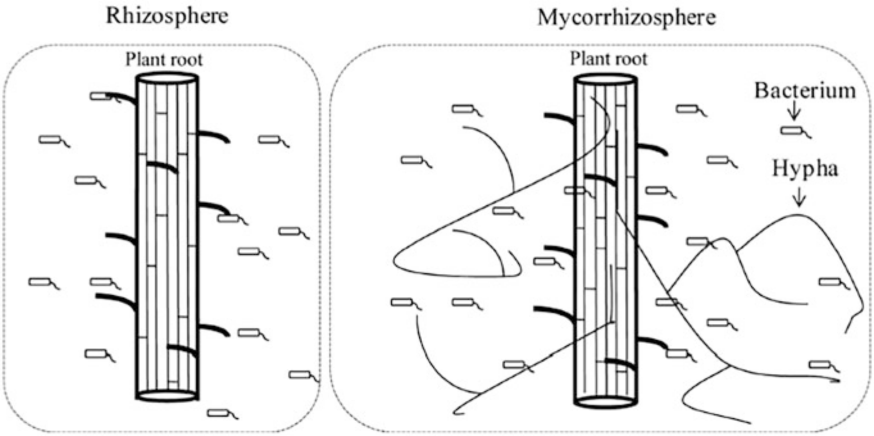

Fig. 11.1 Zone of coverage by rhizosphere and mycorrhizosphere in the soil around the root. Mycorrhizosphere region increases microbial activity and helps in increased nutrient absorption compared to the rhizosphere region

mycorrhiza, ectomycorrhiza, orchid mycorrhiza, monotropoid mycorrhiza, arbutoid mycorrhiza, and ectendomycorrhiza.

Arbuscular mycorrhizal fungi (AMF) are a key functional group of the soil-microflora that can contribute to crop productivity and ecosystem sustainability. AMF symbiosis occurs among a wide variety of plants, ranging over 250,000 species, but only 150–200 species of AMF have been distinguished on the basis of morphology with DNA-based studies suggesting even more (Santos et al. 2006). AMF can interact with different bacterial species. These interactions occur in the root zone and fungal hyphae, commonly referred to as “mycorrhizosphere” (Rambelli 1973). Compared to the rhizosphere region of the plant the mycorrhizosphere has increased the area of the rhizosphere by extending the mycelium of the fungus (Fig. 11.1). The mycorrhizosphere helps in enhanced nutrient absorption, stability of soil and water retention efficiency, biocontrol ability, improved secondary metabolite synthesis, tolerance to abiotic stress, phytoremediation, and phosphate mobilization.

These AMF form vesicles, arbuscules, and hyphae in the associated roots and also produce spores and hyphae in the rhizosphere. The development of a hyphal network by the fungus, which is connected with plant roots, provides plants a greater access to soil surface area, which will result in improved growth (Eroglu et al. 2020). It boosts plant nutrition by enhancing the availability and translocation of different nutrients. Glomalin, a proteinaceous compound secreted by the fungus, helps in soil aggregation and stimulates nutrient cycling, improves soil quality and ultimately, plant health (Mardukhi et al. 2011).

Stoffel et al. (2020) evaluated the agronomic efficiency of a commercial mycorrhizal inoculant (Rootella BR) based on *Rhizophagus intraradices* at sowing (with and without application) and three phosphate fertilization treatments (0, 50, and

100% of the recommendation) in six Brazilian states (RS, SC, PR, MT, GO, and MG). The mycorrhizal inoculant significantly increased the biomass of crops, mainly in the treatment with 50% P. The inoculation increased grain yield of corn and soybean by an average of 54% and 25% per hectare, respectively. The application of the inoculant provided a greater enhancement in the P uptake by plants, especially in soils that originally had low or medium levels of available phosphorus (P). In conclusion, the mycorrhizal inoculant has agronomic efficiency for both corn and soybean even under different soil-climatic conditions.

Although mycorrhization does not compensate the P demand completely, symbiosis reduces the metabolic limitation of photosynthesis in conditions of P scarcity, due to physiological and molecular aspects in the expression of P-transporter genes (Bulgarelli et al. 2020; Pudake et al. 2017). In addition, research into the combined application of chemical and biological fertilization is among the suggested methods for providing cultivated plants with adequate amounts of nutrients. Microbial inoculants, including plant growth-promoting rhizobacteria and AMF, are promising components of such management systems to enhance the efficiency of fertilizer use (Battini et al. 2017).

AMF inoculation increased phosphorus uptake in wheat cultivars by up to 30%. Plant tissue phosphorus increased in inoculated plants, potentially due to inoculation-induced changes in microbial community composition and/or nutrient cycling in the rhizosphere (Elliott et al. 2020). Zhang et al. (2019) quantitatively analyzed the potential role of AMF in increasing grain production from seven cereal crops with exceptional importance for human nutrition worldwide: corn, wheat, rice, barley, sorghum, millet, and oats and found that field inoculation of AMF led to a 16% increase (overall effect) based on the field dataset.

Three major roles AMF play in intercropping systems: (1) mediation of plant interspecific transfer of C, N, P, and water resources and facilitative interactions; (2) control of parasitic weeds and plant pathogens; and (3) remediation of heavy metal(loid)-contaminated soil.

The facilitative effects of AM fungi on N-fixation by legumes and subsequent N transfer to companion crops have been demonstrated by a number of studies using 15N labeling. Ingraffia et al. (2019) showed that AM fungi accounted for 20% of increases in the N fixed by faba bean (*Vicia faba*) and in the N transferred to the intercropped wheat (*Triticum turgidum*).

11.3 Plant Growth Promoting Rhizobacteria and Plant Nutrition

Plant growth is a function of an interaction between plants and its immediate environment. For roots, environment is soil or planting medium, which supply the nutrients and give structural support. The term "rhizosphere" was introduced in 1904 by the German scientist Hiltner to denote that region of the soil which is influenced by plant roots. Improved plant growth and crop yield can be achieved by the beneficial microbes which are also termed as plant growth-promoting rhizobacteria (PGPR). The term plant growth-promoting rhizobacteria-PGPR is coined in the late 1970s (Kloepper et al. 1989).

Plant growth promoting rhizobacteria (PGPR) are of two categories based on their mode of action. (1) Biocontrol PGPRs, which will indirectly promote the plant growth, (2) PGPRs, which directly affect plant growth, seed germination (Glick et al. 1998). PGPR directly facilitates the plant growth through phytohormone production and by enhancing nutrition. By suppressing the growth of pathogens via antibiosis, parasitism, competition within the root zone, and activating host defense responses, PGPR indirectly benefits the plant growth.

PGPR are classified into extracellular (ePGPR), i.e., existing in the rhizospheric soil or in the intercellular space among root cortex cells, and intracellular (iPGPR), existing inside root cells, mostly in nodular structures. The extracellular PGPR include Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas, and Serratia (Figueiredo et al. 2010). The intracellular PGPR includes Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, and Rhizobium of the family Rhizobiaceae (Santoyo et al. 2016).

Many studies have reported different mechanisms of action of PGPR and their applications in agriculture (Ramakrishna et al. 2019). PGPR promote crop growth by production of siderophores, phytohormones, phosphorus solubilization, nitrogen-fixing (Olanrewaju et al. 2017), and lowering ethylene levels in plants through 1-amino-cyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ethylene.

11.4 Association Mechanisms of AMF and PGPR

Alteration in the composition of root exudates is the most eminent physiological change that occurs during AM root colonization of plants (Azaizeh et al. 1995). This chemical alteration in the rhizosphere of mycorrhizal plants is responsible for the bacterial community change, resulting in the mycorrhizosphere effect (Linderman 1988). PGPRs are reported to play a significant role in the establishment of AM symbiosis with host plant growth (Marschner and Timonen 2005). Bacteria may be found adhering to the AM hyphae (Bianciotto et al. 1996a) as well as embedded within the AM spore walls (Walley and Germida 1996) (Fig. 11.2). Bacteria adhering to the AM mycelium get benefited by feeding on hyphal exudates and/or use the mycelium as a vehicle for colonization of the rhizosphere (Bianciotto et al. 1996a).

PGPR are known to enhance AM fungal growth, by supporting the spore germination and mycelia extension of AMF (Xavier and Germida 2003). Hildebrandt et al. (2002) reported that *Paenibacillus validus* under in vitro conditions supported the mycelial growth of *G. intraradices* in the absence of a host root, through the production of sugars such as raffinose. Nutrient exchange occurs

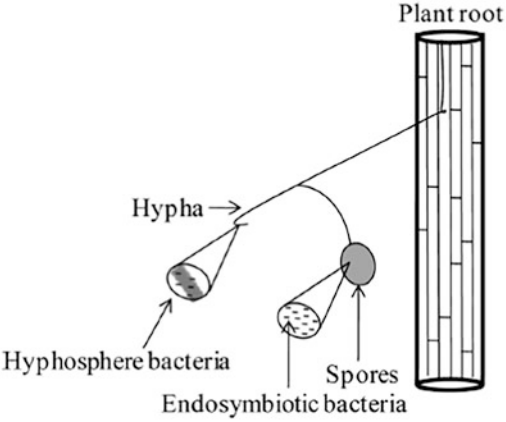

Fig. 11.2 Schematic view of bacteria associated with arbuscular mycorrhizal fungi. Mycorrhiza helper bacteria are associated in the hyphosphere (region around hyphae) while endosymbiotic bacteria are harbored inside the spores

between PGPR and AM fungi by close contact (Artursson et al. 2006), and these bacteria are considered to be mycorrhiza helper bacteria (MHB) (Garbaye 1994). MHB stimulates AM propagule germination, hyphal growth, and root-colonization (Mayo et al. 1986; Vosatka and Gryndler 1999; Xavier and Germida 2003) Further studies by Ludwig-Muller and Guther (2007) showed an increased auxin level in mycorrhizal plants suggested that these hormones could be a signal for the AM colonization process (Meixner et al. 2005).

The widespread AMF, are unique in hosting bacteria in their cytoplasm, and these intracellular structures are found to have similarity with bacteria and bacteria-like organisms (BLOs) (Mosse 1970). Ultrastructural observation of field collected AMF isolates clearly divulged the presence of bacteria in the spores. Morphological observations (electron and confocal microscopy) and molecular analyses were used to identify BLOs and their symbiotic relationship with AMF (Bianciotto et al. 1996a). *Gigaspora margarita* BEG 34 spores showed the presence of large number of BLOs detected by staining with fluorescent dyes specific for bacteria and capable of differentiating between live and dead bacteria (Bianciotto et al. 1996a). Bonfante et al. (1994) based on ultrastructural observations performed on high-pressure freezing/freeze-substituted samples showed large number of rod-shaped BLOs in the germinating spores. On the basis of the 16S rDNA sequences, the bacterial endosymbionts living in the fungus *G. margarita* (BEG 34) were identified and reported to belong to the genus *Burkholderia* (Bianciotto et al. 1996b). Minerdi et al. (2001) reported that the endosymbionts of AMF have *nif* genes, which are responsible for nitrogen fixation and are also capable of nutrient exchange.

AM fungi are among the most important and influential soil microbes, significantly affecting the growth of plants and other soil microorganisms. The soil environment around the plant roots and AM hyphae, where AM fungi and soil bacteria are interactive, is called “mycorrhizosphere” (Linderman 1988).

11.5 Synergistic Action of PGPR and Arbuscular Mycorrhizal Fungi in Millets Production

Plant growth promoting rhizobacteria (PGPR) have an immediate impact on improving profitability based on higher crop yield. Some PGPR produce 1-aminocyclopropane-1-carboxylic (ACC) deaminase (EC 4.1.99.4), which controls ethylene production by diverting ACC into α-ketobutyrate and ammonia. Arbuscular mycorrhizal fungi (AMF) show a symbiotic relationship with plants, which helps in efficient uptake of mineral nutrients and water and also provide protection to the plants against biotic and abiotic stresses. The dual inoculation of PGPR and AMF enhances nutrient uptake and productivity of several crops compared to a single inoculation in both normal and stressed environments. Positively interacting PGPR + AMF combination is an efficient and cost-effective approach for sustainable agriculture, this suggests a key role of ACC deaminase of PGPR in favoring the establishment of AM symbiosis through the modulation of ethylene synthesis in cucumber (Gamalero et al. 2008).

Application of microbial bio-inoculants like AMF and rhizobacterial communities (biofertilizers and biostimulants) has gained importance as a result of adverse effects of excess use of chemicals and also the positive interaction between rhizosphere microflora and plants (Gosling et al. 2006; Srivastava et al. 2016; Alori et al. 2017). With the synergistic effects to improve crop health in agriculture, PSB (phosphate solubilizing bacteria), PGPR, and AMF consortia have received prior weightage (Antoun 2012). Pseudomonads and Azotobacter are widely used to promote crop growth by increasing plant nutrients accessibility (Soleimanzadeh and Ghooshchi 2013; Dey et al. 2017; David et al. 2018), synthesis of phytohormones, phosphate solubilization, siderophores production and by production of antibiotics and lytic enzymes, which disintegrate the fungal cell wall and toxins (David et al. 2018) and promotes mycorrhizal formation and function by acting as mycorrhizal helper bacteria (Pesrez-de-Luque et al. 2017).

The dual inoculation of an AM fungal strain and a rhizobium strain led to significant increase of N uptake in co-cultivated soybean (*Glycine max*) and maize and the mycelium-derived N transfer from soybean to maize than any of single inoculation, it suggests the synergistic effect between AM fungi and rhizobia (Meng et al. 2015).

The influence of AM fungus on growth and yield response of Naga King chilli individually and along with rhizobacterial inoculants was evaluated in sterilized and non-sterilized soils under greenhouse conditions, and it was found that greater shoot and root lengths, collar diameter, biomass and leaf number in microbial inoculated Naga King chilli seedlings than uninoculated controls under SS and NS soil conditions. Under SS soil conditions, increase in shoot length of chilli plants was observed under combined (AM + PSB + PGPR) application (Surendirakumar et al. 2019).

Effect of five identified bacterial cultures and one mycorrhizal culture were evaluated for nutrient uptake like phosphorus, nitrogen, potassium, and other morphological and biochemical effect on cultivation of pearl millet and also analyzed for flavonoids, phenols, and super oxide dismutase (SOD). All the bacterial cultures and mycorrhiza showed increased agronomical traits and enhancement in nutrient uptake like potassium, nitrogen, and phosphorus. Increased level of phenols, flavonoids, and SOD confirmed microorganism effect on plant growth promotion. PS-05 and mycorrhiza were most effective in plant growth promoting activities (Singh et al. 2017).

Another important element of integrated soil fertility management is plant intercropping, the technique of cultivating two or more crops simultaneously in the same field (Brooker et al. 2015). To improve crop productivity and soil fertility in a sustainable way, "biofertilization" and intercropping may be combined (Wezel et al. 2014).

Saharan et al. (2018) tested the profitability for finger millet in intercropping with pigeon pea in the presence of "biofertilization" with arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR), under drought conditions using compartmented microcosms and revealed that biofertilization strongly increased nitrogen and phosphorus uptake by both plants, both under well-watered and drought conditions. In the presence of AMF, both plants also acquired 15N and 33P, offered in a labelling compartment accessible to fungal hyphae but not to roots. It suggests that "biofertilization" with AMF alleviates the negative effects of drought condition on finger millet, indicating that the CMN connecting pigeon pea and finger millet exert clearly a positive influence in this simulated intercropping system (Schütz et al. 2022).

An intercropping and biofertilization combination is able to boost crop yields under low-input conditions. Mathimaran et al. (2020) reported that dual inoculation of AMF+PGPR in finger millet and pigeon pea intercropping resulted in increased grain yields compared to single inoculation, grain yields of both crops in the system increased up to +128% with biofertilizer application.

Employment of plant growth promoting bacteria (PGPB) and mycorrhiza could serve as environment-friendly alternatives for the use of excessive NPK fertilizers and producing biofuel. Nuclear magnetic resonance (NMR) analysis detected 28 metabolites in foxtail shoot with most of them upregulated in ecto/endomycorrhiza group and combined with PGPB. The inoculation with both PGPB and endomycorrhiza gave the best results with reference to total sugar yield which indicates that PGPB and endomycorrhiza combination is well suited for enhancing biomass and boosting sugar yield, which are useful attributes for utilizing foxtail millet as a biofuel source (Dhawi et al. 2018).

Potential role of *Pseudomonas fluorescens* and mycorrhizal fungi (AMF) in reducing cadmium toxicity in the Arizona cypress seedlings was studied both individually and in combination in a factorial experiment with three factors comprising AMF inoculation (*Rhizophagus irregularis* or *Funneliformis mosseae* or a mixture of both species, and non-inoculated controls), bacterial inoculation (*P. fluorescens* or non-inoculated), and five levels of Cd (0, 5, 10, 15, and 20 mg kg^{-1}) and decreased growth of Arizona cypress was observed when exposed to high levels of Cd contamination. However, co-inoculation plants with all mycorrhizae and *P. fluorescens* grown under the highest levels of Cd contamination showed increased photosynthetic pigments. Furthermore, AMF and *P. fluorescens* increased ascorbate peroxidase activity, which is responsible for the elimination of H_2O_2 when plants exposed to Cd stress, which suggests that either co-inoculation with AMF and *P. fluorescens* or individual inoculation with AMF could potentially ameliorate harmful effects of Cd on Arizona cypress growth (Aalipour et al. 2021).

In a study on effects of applications with AMF and an *Enterobacter radicincitans* strain, shoot yield and nutrient uptake (P and N) of faba bean (*Vicia faba*) and soil characteristics (basal respiration, microbial biomass, and the number of P solubilizing bacteria) with the single and combined effects of AMF and *E. radicincitans*, efficacy of PGPR and AMF with or without mineral fertilizer (P, K, and Mg) application. The effect of AMF was comparable to the positive effect of mineral nutrient application. However, AMF application alone or with mineral nutrient supply increased the soil respiration and soil microbial biomass. The studies showed the potential of AMF applications for plant growth and nutrition mainly under nutrient-deficient conditions (Almethyeb et al. 2013).

In Tunliu, field experiment was conducted with foxtail millet (Setaria italica) to evaluate the impacts of solid waste-based soil conditioner (SWSC) and arbuscular mycorrhizal fungi (AMF) on millet safety and crop production. The application of AMF and SWSC inhibits plants from absorbing heavy metals from soil and reduces the $TF_{root/soil}$ of the millet. SWSC+AMF application inhibited the transfer of heavy metals from the roots to upper part of ground and reduced the $TF_{shoot/root}$ of the millet (Shen et al. 2022).

The coexistence of PGPR and AMF in the rhizosphere is very beneficial for the growth and development of most plants. This synergistic effect is a result of positive interactions between PGPR and mycorrhizal fungi that help promote the growth of each other, which ultimately benefits the plant (Hashem et al. 2016). Co-inoculation of Rhizobium with AMF resulted in significant enhancement of yield, nodulation, leghemoglobin, nitrogenase activity, IAA synthesis, and nutrient uptake of alfalfa subjected to salinity stress (Ashrafi et al. 2014). Inoculation with AMF in soybean improved various attributes as observed in alfalfa, but also conferred protection against membrane damage by reducing hydrogen peroxide and lipid peroxidation (Hashem et al. 2019).

Some AMF species produce metabolites such as organic acids, volatile compounds (ethylene), and non-volatile compounds that are able to attract specific bacteria (Younesi and Moradi 2014), and these bacteria are known to enhance colonization of AMF and are referred to as mycorrhiza-helper bacteria (MHB). Phosphate absorption is enhanced by AMF colonization, where PGPR solubilize phosphate in soil (Nanjundappa et al. 2019).

Dual inoculation of AMF and antagonistic and growth-promoting yeast *Brettanomyces naardensis* was effective in reducing the disease incidence of root rot and charcoal rot, caused by Macrophomina phaseolina, to 11.11% and 5.55%, respectively, and it also increased the growth parameters like plant height, dry weight, and leaf number, and it approved the induction of the immune system in sunflower *via* decreasing malondialdehyde content (MDA), and the double bioagents offer a promising biocontrol strategy in crop protection as a biofungicide and biofertilizer together in one bioformulation (Nafadya et al. 2019).

Mohameda et al. (2019) evaluated the potentiality of mycorrhizae, Bacillus subtilis, and Pseudomonas fluorescence inoculations in both single and combined application for management of Sclerotium rolfsii infection in common beans and also as inoculants for enhancing crop nutritional status, and it was reported that activities of defense enzymes like chitinase, peroxidase, and polyphenol oxidase were enhanced upon microbial inoculations.

Irankhah et al. (2021) reported the effect of PGPR and AMF on diosgenin content as a major bioactive constituent. The leaf proline content was the highest in plant subjected to drought stress with dual inoculation (*Glomus intraradices* and *Pseudomonas putida*), whereas the highest total soluble proteins belonged to plants with triple inoculation (*P. putida*, *Bradirhizobium japonicum,* and *G. intraradices*). Bacterial and fungal inoculation decreased peroxidase activity both in stress and non-stress condition.

11.6 Conclusion

Arbuscular mycorrhizae are ubiquitous and for better understanding of the AMF role in rhizosphere and their interactions with PGPR and host plant, one must understand the mycorrhizosphere region. The basal mechanisms behind the interactions on the mycorrhizosphere are poorly understood, so proposed mechanisms of interactions still need experimental confirmation. More insight into these mechanisms will enable optimization of the effective use of AMF in combination with their bacterial partners as a tool for increasing crop yields. The dual inoculation of PGPR and AMF enhances nutrient uptake and productivity of several crops compared to a single inoculation in both normal and stressed environments.

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Chapter 12
Millet's Rhizosphere Metagenomics for the Understanding of Rhizobiome Multifunctionalities

Rohini Mattoo and Suman B M

Abstract Millets are nutritionally rich crops, constituting an important part of the diverse food crops grown and consumed in most states of India and its surrounding regions. The different types of millets have different nutritional properties, and the nutrient profile varies depending on the soil conditions they are grown under. The millet rhizosphere harbours diverse and beneficial microbial communities playing myriad roles. The important roles of the microbes include supporting plant growth, pest and pathogen resistance, acclimatising the plants to harsh climatic conditions and anthropogenic pressures. The millet rhizobiome is unique since it may confer to drought resistance, participate in biogeochemical cycles of nitrogen, carbon, phosphorus, potassium, sulphur, etc., allowing the plants to adapt well in arid and nutrient-deficient soils. Furthermore, microbial diversity, abundance, and their multiple functions are essential for reductions in greenhouse gases (GHG) from agricultural soils, water retention, decomposition activities, increasing soil organic matter, weathering, etc. Advancing interdisciplinary tools for deciphering microbial communities including high throughput metagenome sequencing, advanced computational tools, etc. are increasingly being used to decipher and elucidate the microbial communities in the rhizosphere. Metagenomics has helped unravel many bacterial communities that are dominant in the millet rhizosphere, paving way for future characterization of their potential multifunctionalities. In this chapter, we have given an account of the metagenomics performed for different millets and the multifunctionalities associated with these future climate-adaptable crops.

Keywords Metagenomics · Multifunctional microbes · Rhizobiome · Finger millet · High throughput sequencing

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_12

12.1 Introduction

Millets adapt to varying climatic conditions and are important food security crops. Today, millet varieties developed in India have spread to other countries and are widely used as food and fodder (reviewed in Chap. 11). In order to strengthen food security measures, India has developed millet varieties (154 varieties from 2014 to 2021), which could find a global market for their growth and consumption. Like rice, millets have been cultivated in India since time immemorial, as is evidenced in the oldest texts in the world. Also, the earliest evidence of domestication has been found in the Indus Valley Civilization and through paleobotanical studies. It has been validated that millets were grown in all states in India, even in the upper regions of the Himalayan mountains and states that include Jammu and Kashmir, Ladakh, Arunachal Pradesh, Assam, Himachal Pradesh, Manipur, Meghalaya, Nagaland, Sikkim, Uttarakhand and West Bengal (Bhat et al. 2019). The total area under millets in India is currently estimated to be around 14 million hectares, with the states of Karnataka, Rajasthan, and Maharashtra being the largest producers. More than 65% of agriculture fields in India are dependent on monsoons, and most millets are sustainably grown under these rain-fed conditions. Rejuvenation and enhancement of mixed millet farming, along with rice, pulses, oil seeds, legumes and fruits, paves a path to ensure security of food, fodder, the economy, and the environment.

According to the ICAR (Indian Council of Agriculture Research, India), millets are being grown even in the cooler climates of Himalayan states although the land under agriculture must be increased in order to meet the demands of a growing population. Many barren areas could be regenerated with organic amendments and tended to support the natural farming of millets. Post the green revolution, predominantly the high yielding varieties of rice and wheat have been produced and grown extensively resulting in neglect of other local crops. Similarly, research activities need to be directed towards maintaining the nutritive composition of millets. Today, India, having achieved grain sufficiency, must now focus on expanding millets amongst other local crops to all the states and mainstreaming crops neglected earlier to achieve food security and nutrition sufficiency.

Millets have been conferred with the popular name ‘famine crop’ due to many reasons. The resistance to environmental conditions such as drought, growth in deficient soils, and their ability to endure the attack of pests under storage conditions for at least 10 years (Rodríguez et al. 2020) make millets superior to many climate-sensitive crops. It has been speculated that, along with other factors, the inherent genetic architecture, especially the abiotic-stress determinants of millets (Singh et al. 2021) allow them to withstand higher temperatures and survive under drought-prone conditions. The C4 mechanism of millets increases the concentration of CO_2 which suppresses photorespiration and increases the activity of RuBisCo. As a result, at elevated CO_2 and warm conditions, the rate of photosynthesis is high, thereby conferring immediate water use efficiency and nitrogen use efficiency on millets (Bandyopadhyay et al. 2017). It has been observed that RuBisCO enzyme in plants capable of C4 photosynthesis functions efficiently during increasing CO_2 levels and hence millets undergo better photosynthetic capacity in warmer climates, conferring efficient water and nitrogen use that are up to four-fold greater in the C3 plants (Sage and Zhu 2011). Interestingly, because of this efficient mechanism, the requirement of just 257 g of water for 1g of biomass for fox millets could be utilized to expand the cultivation of millets as compared to wheat, which requires 510 g and maize, which requires 470 g of water (Li and Brutnell 2011).

At the molecular level, response to environmental stress has been expressed and studied via various factors (Saleem et al. 2021). Lipid transfer gene (SiLTP) expressed in foxtail millet improved tolerance by increasing proline and sugar contents (Pan et al 2016). The association of the plasma membrane proteolipid genes with certain proteins confers abiotic stress tolerance by maintaining homeostasis (Yeshvekar et al. 2017). Drought-tolerant QTL (quantitative trait locus) in pearl millet was found to limit Na+ accumulation in leaves during salt stress (Sharma et al. 2011). ACC deaminase and EPS-producing bacteria like *Pseudomonas fluorescens, Enterobacter hormaechei*, and *Pseudomonas migulae* in the rhizosphere of foxtail millet could alleviate drought stress and improve seed germination and growth (Niu et al. 2018). The activity of the enzyme ACC deaminase helps plants to withstand stress by reducing levels of the stress hormone ethylene, while EPS might provide a microenvironment that helps in water retention, thus protecting bacteria from drying. Millets like foxtail and proso contain non- crystalline minerals in their cells called phytoliths, which occlude carbon, thus serving as important long-term reservoirs of terrestrial carbon which play major roles in carbon sequestration and the global carbon cycle (Zuo and Lü 2011) in the soil habitats.

The soil habitats include the plant rhizosphere, which is home to diverse microorganisms, 90% of which continue to remain unidentified and whose functional significance remains unknown (Raynaud and Nunan 2014). The rhizosphere is the most diverse habitat on Earth (Ling et al. 2022). They support plant growth by assisting in the mineralization of valuable elements in the soil and facilitating their uptake by plant roots (Wardle et al. 2004). Apart from their roles in nutrient cycling, presence of beneficial microbial populations in soil have shown to prevent pathogen attacks on the plant (Singh et al. 1999) and improve the productivity of food crops, and boost the plant's defence mechanisms (Weller et al. 2002). *Gemmatimonadetes* are soil bacteria which are involved in carbon turnover (Fan et al. 2021). The carbon cycle in the soil functions harmoniously due to the roles of many microorganisms including methanogens and methanotrophs such as *Methylobacter* and *Methylosinus* (Iguchi et al. 2015). Organic matter and biomass important for plant productivity are generated through the activities of phototrophic, chemo-autotrophic microorganisms which can fix atmospheric carbon dioxide (CO_2) followed by auto and heterotrophic microorganisms which then facilitate the liberation of the carbon back into the atmosphere (Singh et al. 2010). *Firmicutes* and *Actinobacteria* found in the rhizosphere (Nacke et al. 2011) have been suggested to play an important role in the biogeochemical cycles (Nacke et al. 2011) thereby contributing to effective rhizosphere functioning. Furthermore, beneficial microbial functions modulate greenhouse gas (GHG) emissions and reductions, retention, and uptake of water in soils, cycling and generation of organic matter, decomposition activities, and significant removal of heavy metals in soil (Gurung and Mattoo 2021). Therefore, it is not only challenging, but genomic and functional aspects of microbial growth in soil, especially in the rhizosphere, for sustainable crop production form a necessary part of research activities, especially on food security crops. For example, the metagenome sequencing of the rhizosphere of citrus has yielded important insights as to how the microbiome of soil rhizosphere is important not only for enhancing citrus yields but also for plant health (Xu et al. 2018). The rhizosphere is thus a valuable platform to understand the complex and myriad interactions amongst the soil, micro/macrobiota, plants and climate. The microorganisms in the endosphere, rhizoplane, and rhizosphere vary due to the type of nutrients, soil type, geographical regions, and others.

The above ground plant surface, the phyllosphere, influences microbial communities in the rhizosphere and vice versa, thereby expanding our knowledge of the factors important for microbial abundance at plant–soil–microbe interface. The rhizosphere, the soil region in close proximity to the roots, is hence a challenging hotspot for studying multifunctional interactions between plant roots and microorganisms in soil. The millet rhizosphere provides a unique platform to study the multifunctional rhizobiome due to the plants' unique ability to withstand varying environmental conditions such as drought, low rainfall conditions, and deficient soils.

As millets possess many important traits of agricultural importance, studies on the microbiome and natural methods to enhance their production would be of great benefit in the future. The microorganisms are involved in key functions in better crop and grain productivity, although more direct methods are needed to elucidate their roles in these along with nutrient uptake, mineral availability, tolerance to disease, and adaptation to climate change. It has also been observed that root exudates released by plants serve as an important source of carbon and feed for rhizosphere microbes and macro-organisms thus altering soil properties and function. Detailed studies and characterization of millet microorganisms for their functions remain largely unknown. Thus, modern methods, such as metagenome sequencing coupled with computational tools to determine microbial abundance, interactions, and sustainable practises, for enhancing millet productivity are challenging and much needed.

As depicted in Fig. 12.1, a combination of techniques involving high throughput techniques of metatranscriptomics, metaproteomics, metabolomics, stable isotope probing, G + C content, FAME, RFLP, TRFLP, DNA microarray, and PCR targeting 16S rDNA would be beneficial in characterizing the millet rhizobiome functions. Technological advancements in microbiological tools and solutions have spearheaded a new direction in unravelling microbes from diverse habitats such as agroecosystems, the atmosphere, glaciers, oceans, etc. and empowered scientists to unravel these complex populations of microbes at a faster pace compared to decades ago. The improvements in current technologies include omitting DNA cloning and vectors, hosts which ease DNA library assembly, and lessen DNA contamination.

The microbiology of the millet rhizosphere offers a formidable challenge since more than 90% of the soil microbes cannot be cultured on laboratory media (Kent

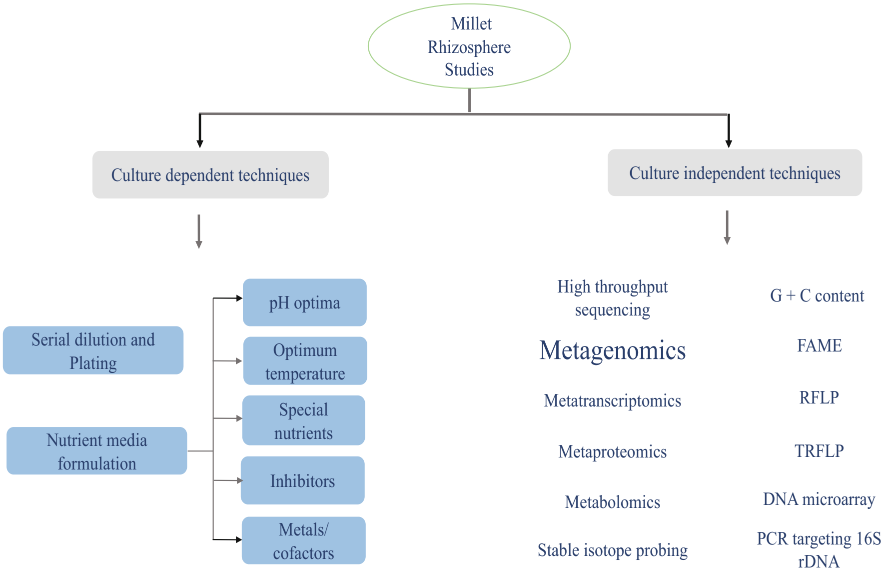

Fig. 12.1 The figure shows the available methods that could be employed in order to decode the millet rhizosphere. The combination of culture dependent techniques and culture-independent techniques along with bioinformatics, help to understand which are the beneficial rhizosphere bacteria and what are their major roles in soil root interface. Integrating metagenomic techniques such as different high throughput techniques along with metatranscriptomics, metaproteomics, metabolomics, stable isotope probing, G + C content, FAME, RFLP, TRFLP, DNA microarray, and PCR targeting 16S rDNA would be useful in characterizing the rhizosphere of millets, which is yet largely unknown

and Triplett 2002; Doornbos et al. 2012). Therefore, metagenomic tools allow mining of unknown genomes that influence plant yields, which can help engineer the rhizosphere and in designing microbial formulations in sustainable agricultural technologies (Mattoo and Gowda 2022). Exposure and unravelling the microorganisms in the millet rhizosphere would shed light on drought resistance, pest tolerance, and higher nutritive values which are important to engineer the soils and crop health and yield. We discuss the major metagenomic studies conducted on millets thus far, along with the major bacteria and their possible functions. Most of the millets in India are known by their local names, and these have been used in the chapter, and the major millets commonly grown have been tabulated previously. Also, Table 12.1 summarizes the millets whose rhizosphere has been studied using metagenome studies. Many interesting and common features have been observed, which offer immense potential and pave the way for future studies.

Table 12.1 Millets on whose rhizosphere metagenome sequencing methods have been carried out

Sl. No.	Millet	High throughput sequencing method	Dominant microorganisms	Reference
1.	Finger millet (Eleusine coracana)	Illumina Miseq	Planctomycetes Proteobacteria Bacteria unclassified	Mattoo et al. (2021))
2.	Kodo millet (Paspalum scrobiculatum)	Illumina Hiseq	Actinobacteria Proteobacteria Chloroflexi	Prabha et al. (2019))
3.	Pearl millet (Pennisetum glaucum)	Ion Torrent	Proteobacteria Firmicutes Actinobacteria	Ndour et al. (2021)
4.	Proso/broom-corn millet (Panicum miliaceum)	Illumina Miseq	Actinobacteria Acidobacteria Proteobacteria	Tian et al. (2022)
5.	Sorghum (Sorghum bicolor)	Pyrosequencing	Cyanobacteria Bacteroidetes Proteobacteria	Kumar et al. (2021)
6.	Foxtail millet (Setaria italic)	Illumina Miseq	Proteobacteria Acidobacteria Actinobacteria	Han et al. (2017)

12.2 Metagenomics Studies on Millets

12.2.1 Ragi/Finger Millet

Ragi or finger millet as a C4 crop, possesses high tolerance to abiotic and biotic stresses (Talwar et al. 2020; Chandra et al. 2020). It can be easily cultivated in diverse climates and soils (Chandra et al. 2016) and preserved for at least 5–6 years. The finger millet grains contain rich proteins, minerals including iron, and vitamins (Chethan and Malleshi 2007; Chandra et al. 2016), and their calcium content is reported to be 10 times higher than major cereal crops such as wheat, rice, and maize (Chandra et al. 2016). Research studies have shown that due to the lower glycemic index, ragi is more beneficial than rice for people with diabetes (Shobana et al. 2013). Therefore, in order to enhance the yield of ragi using sustainable methods and avoiding chemical inputs, research studies are necessary in order to gain more knowledge on the rhizosphere of finger millet.

In the study by Mattoo et al. 2021, metagenomic sequencing by Illumina revealed taxonomically rich communities associated with the rhizosphere of chemically fertilized (NPK 100:50:50) and bio-inoculated (nitrogen fixer-*Azotobacter chroococcum*; phosphorus solubilizer-*Bacillus megaterium*; potassium solubilizer-*Frateuria aurantia*; Plant Growth-Promoting Rhizobacteria-*Pseudomonas fluorescens*; fungal bio-control agent *Trichoderma viride*) finger millets prior to the rainy or monsoon season. The rhizosphere of bioinoculated millets was dominated by members of Acidobacteria that showed beneficial traits like carbon usage, nitrogen assimilation, production of antimicrobial compounds, and drought resistance. Planctomycetes and Rhizobiales, which were also abundant in the ragi rhizosphere, are predominantly involved in denitrification and nitrogen fixation, respectively. Actinobacteria were enriched in the ragi rhizosphere treated with bioinoculants, and these consist of various members involved in production of secondary metabolites, anticancer and antifungal compounds. On the other hand, the rhizosphere of chemically fertilized millets consisted of members of Bacteroidetes and ZB2 involved in the degradation of compounds and fermentation, indicating an environment containing complex compounds. Synthetic treatment suppressed the growth of the beneficial bacteria leading to the predomination of pathogenic bacteria like Flavobacterium, and the anoxic conditions favoured the growth of bacteria belonging to Firmicutes and OD1 (Mattoo et al. 2021). In the finger millet studies, it was observed that treating the soil with chemical fertilizer led to no further enrichment of populations of microorganisms that are important for soil health and crop productivity while the treatment led to an increase in the abundance of those that serve as opportunistic disease producers (*Flavobacterium succinans* and *Flavobacterium columnare*). The chemical treatment was observed to contain a different profile compared to the control and bioinoculant treatments as it suppressed microbes involved in functions of carbon and nitrogen cycles, etc., while the biofertilizers treatment, when applied is involved in multifunctional, beneficial activities in the rhizosphere.

Surprisingly, many operational taxonomic units (OTUs) that were absent in ragi rhizosphere without any treatment were discovered, and these included species of *Noicardioides*, *Larkinella* belonging to Bacteriodetes, *Mucilaginibacter* and *Novosphingobium*, which may perhaps be the core microorganisms involved closely with Ragi plants and responsible for the mutual benefit to the host. Many of these organisms are reported to be involved in many functions, such as the production of important bioactive compounds which may have pharmaceutical or commercial value (Lazzarini et al. 2000; van Bergeijk et al. 2020). *Larkinella* (Ludwig et al. 2012; Xu et al. 2017) would perhaps be involved in the nitrogen cycle in ragi rhizosphere where they play roles in nitrate to nitrite conversion. Importantly, *Mucilaginibacter* found in the ragi rhizosphere, (also enhanced in bioinoculant treatment) are chemo-organotrophs causing cellulose decomposition in soil (López-Mondéjar et al. 2016) hence important in the recycling of nutrients in the rhizosphere of Ragi. Another important organism identified through metagenomics of Ragi (Mattoo et al) is *Novosphingobium* which may perhaps play multifunctional roles in salt tolerance, signal communication, and degradation of compounds that are aromatic (López-Mondéjar et al. 2016). Other genera identified in the Ragi rhizosphere whose roles are not yet known include E2, BB34, and OTUs 0593 and 0748. Close association of these important and beneficial microorganisms highlights that these microbes may also play roles in suppressing pathogenic ones and conferring the plants with salt tolerance, medicinal value, and climate adaptation (Fig. 12.1). Further studies are needed to decipher the functions of these directly, which may enrich plant growth and enhance the yield of Ragi using sustainable technologies, other combinations of bioinoculants, and also including soil amendments.

12.2.2 *Kodo Millet*

Kodo millet, is another important millet, having originated in India where the climate favours its cultivation and production. Although its cultivation is not as widespread as that of wheat or rice, it is a significant crop capable of thriving even on poor soils. Kodo millets can be stored for long periods of time and comprise more than 11% protein, more than 1% fat, more than 1.5% carbohydrates, iron (up to 39.6 ppm), calcium (27%) and antioxidant free-radical scavengers (Prabha et al. 2019) varying due to the richness of soil used in their cultivation. Because these millets contain at least 9% fibre as opposed to rice and wheat (0.2% and 1.2% fibre), they constitute an important part of the diet of people with metabolic diseases such as diabetes, and hence these grains have been thought to be medicinal by those who consume them regularly.

Kodo millet has a high drought tolerance and can effectively produce good yields even in arid and semi-arid regions of India. As a food crop, it is grown in many parts of India, such as Gujarat, Karnataka and parts of Tamil Nadu although Madhya Pradesh ranks first in its cultivation. The phytochemical properties and nutritional parameters have been summarized (Bunkar et al. 2021).

Metagenomic analysis of the Kodo rhizosphere concluded that the rhizosphere displayed high microbial diversity (205 classes were analysed). The highest abundance of actinobacteria at the level of class was 42.22% of the total reads, while the others Alphaproteobacteria, Betaproteobacteria, Ktedonobacteria, Acidobacteria, Gammaproteobacteria, Deltaproteobacteria, Clostridia Planctomycetacia, Bacilli, Unclassified, Spartobacteria, Sphingobacteria, Thermomicrobia, Chloroflexi, Verrucomicrobiae, and Cytophagia which made up (12.57%), (4.45%), (4.42%), (3.61%), 3.48%), (3.05%), 2.33%), (2.32%), (2.29%), (1.92%), (1.90%), (1.6%), (1.23%), (1.18%), (1.12%), (1.02%) of the classes were also dominant. The studies also showed that the metabolic multifunctionalities were related to physiological pathways, nutrient cycling/acquisition, secondary metabolism, carbon cycle, xenobiotic degradation, stress-defence response, bioremediation, etc. The authors also concluded that the microbial communities in the Kodo rhizosphere could assist in plant growth, development, and crop survival under difficult conditions of the environment; hence, they form a part of the rich gene pool for the secondary metabolite production pathways such as those involved in the formation of bacteriocins or antibacterial peptides, resistance towards antimicrobial products, and detoxification of metals and compounds (xenobiotic). The authors also discovered genes connected to the metabolism of aromatic compounds, phosphorus, nitrogen, iron, sulphur, and phytohormones which support plants under dry periods. The presence of Actinobacteria like *Streptomyces*, that produce antimicrobial compounds, reveals that Kodo millet rhizosphere microbiome possesses metabolic activities that confer biotic stress resistance, while Frankia involved in nitrogen fixation, supports the nitrogen requirements of plants during low nitrogen conditions (Prabha et al. 2019).

These results pave the way for future studies which would provide a detailed account of the microbial populations thriving in the kodo rhizosphere using metagenome studies coupled with other advanced culture-dependent techniques to directly decipher the multifunctionalities in the rhizosphere with regard to climate change and food security.

12.2.3 Navane/Kangni Foxtail Millet

Kangni, or foxtail millet, is also one of the oldest crops grown widely in almost all Indian states, including the Himalayan regions, and consumed widely. Similar to other millets, it is widely grown in arid areas and can easily adapt to adverse environmental and climatic conditions. Kangni millet is a low-water-requiring crop and has a maturation period of 65–70 days. Being drought tolerant, it can easily grow at higher elevations, even as high as 600ft. It has been grown in mixed cropping practices, and along with sorghum, can be sown as an alternate millet on black cotton soils in India. It can be grown even on rich and alluvial soils with high yields. Foxtail millet grains are nutritionally dense and endowed with highly important nutrients (Sharma and Niranjan 2018). One of the challenges in foxtail millet production has been the smut disease. Research studies on the metagenomics of Kangni rhizosphere have provided good leads on the rhizobiome of a smut disease-resistant Kangni cultivar. The bacterial community compost of a smut-resistant cultivar was used as a model to understand the rhizosphere bacterial community linked to smut disease suppression and also to explore microbes to be employed as biocontrol agents for pathogen control. In the study conducted by Han et al. (2017) variations amongst three cultivars of foxtail millet were found, which attributed differences in root exudation. The study showed that when the plants were treated with *Ustilago crameri* (the causative agent of smut disease), a higher population of Actinobacteria was seen in smut-resistant cultivar, Jigu20 rhizosphere in contrast to Jingu21 and Changnong35 which were smut susceptible.

Illumina sequencing also showed that there were differences in the rhizosphere soils between smut disease-resistant cultivars (Jigu20) and those that were smut susceptible (Jingu21 and Changnong 35) which led to the recruitment of different microbes when treated with *Ustilago crameri.* The results conclusively observed that Streptomyces and Bradyrhizobium could play big roles in the suppression of the foxtail millet smut disease (Han et al. 2017). Therefore, beneficial species of *Streptomyces* and *Bradyrhizobium*, which are not pathogenic and native to the soil, could be incorporated in the consortia of bioinoculants, used to treat seeds in order to prevent smut disease as the plants mature.

12.2.4 Jowar/Sorghum

Sorghum, commonly known as Jowar, in most regions is among the most produced crops in the world after rice, wheat, maize, and barley. Jowar is grown in many regions of India and the world, and similar to other millets, has been recognized as an important food security crop. The pyrosequencing of sorghum rhizosphere samples obtained from different geographical locations showed differences in bacterial communities due to different soil properties like soil pH, nutrient content, and cultivation practises. The majority of core-microbiome taxa were Cyanobacteria, Bacteroidetes, and Proteobacteria. Cyanobacterium was found to be most dominant in soils with low pH and was dominant, because of the soil conditions which were nutrient-rich (Kumar et al. 2021).

Metagenomics analysis and amplicon sequences revealed an abundance of nitrogen fixing bacteria, *Bradyrhizobium*, in sorghum roots (Hara et al. 2019). Another study used large scale field experiments and metagenomic studies to study microbial features associated with conditions of drought in the sorghum rhizosphere. The study observed 55 dominant bacterial genomes, including four genomes of Actinobacteria from the class Thermoleophilia. The authors have predicted and found that enrichment of Actinobacteria due to drought could be because of the reduction in available iron due to the higher soil aerobic conditions and lower phyto-siderophores important for the growth of microbes. Further results of the study on drought, iron uptake, and microbiome by metagenomics have helped advance our understanding of the rhizosphere of sorghum, paving the way to demonstrate the importance of metagenomics coupled with bioinformatics for discovering vital plant–microbe relationships.

12.2.5 Proso Millet/Broomcorn Millet/Barri/Chena

Barri or Chena, is also one of the oldest grains known to people in India and China and grows well in arid and semiarid regions. They are rich in vitamins (B-complex, folic acid, and niacin), minerals (Ca, Zn, Fe andP) and essential amino acids. This millet is gluten free and, similar to other millets, has a low glycaemic index making it suitable for people with metabolic diseases. The study by Na et al. (2019) characterized the bacterial diversity of two broomcorn millet cultivars under drought conditions using Illumina MiSeq technology. Key observations of the study on the two cultivars of broomcorn millet grown in China were: first, the main driver of the alpha diversity was the stage of the broomcorn millet and the bacterial rhizosphere structure; and second, the development stage of millet affected the bacterial composition. It was observed that as the millet matured, there was a decrease in the richness and evenness of the bacterial populations, and the rhizosphere bacteria were robust during drought. The abundance of bacterial populations of Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes,

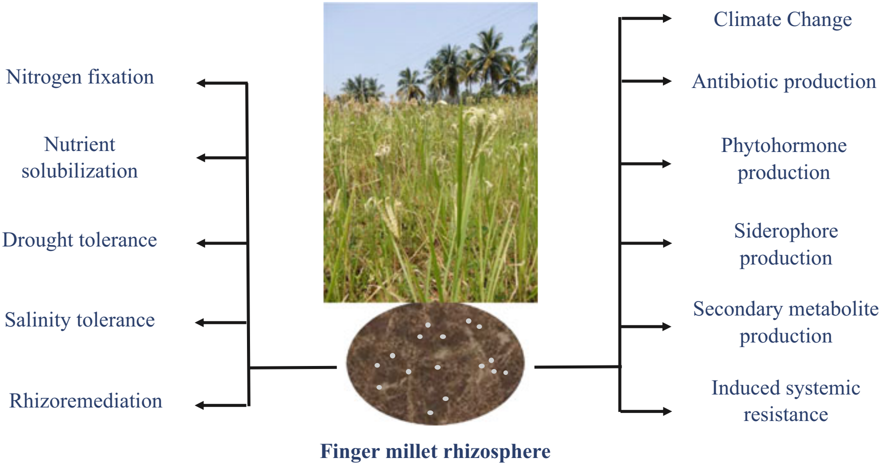

Fig. 12.2 The figure illustrates the contribution of metagenomics of the millet rhizosphere (e.g., of ragi/finger millet) in the discovery of multifunctionalities of microbial populations. These bacterial communities are involved in nitrogen fixation, nutrient solubilization, drought tolerance, salinity tolerance, rhizoremediation, climate Change, antibiotic production, phytohormone production, siderophore production, secondary metabolite production, induced systemic resistance. These rhizophere communities include a wide variety of microorganisms, and hence high throughput genome sequencing could hold the key to further elucidation of these microorganisms, which have multifunctional and beneficial roles

Firmicutes, Verrucomicrobia, and Planctomycetes was (33.8%), (21.0%), (10.7%), (8.2%), (6.3%), (5.9%), (3.5%), (2.9%), (2.7%), respectively, and these comprised the core groups of the broomcorn millet rhizosphere. The flowering stage harbored Proteobacteria, AD3, WPS-2, Chlorobi, GN04, TM6, Spirochaetes, OP3, Chlamydiae, Elusimicrobia, WS3, Euryarchaeota, Deferribacteres, Parvarchaeota, Fusobacteria, Tenericutes, OD1, and Synergistetes; while the grain filling stages had the highest abundance of Actinobacteria Bacteroidetes and Firmicutes. Interestingly, it was also recorded that Acidobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes, Cyanobacteria, Verrucomicrobia, etc. were abundant during the jointing phase, but there was decrease in their abundance as the plants matured (Na et al. 2019). These studies also observed that drought did not significantly affect microbial diversity or structure but differential responses to drying were seen between the cultivars, suggesting that the host played an important role. This study paves the way towards understanding the multifunctionalities of the broomcorn millet rhizosphere by linking the bacterial abundance and roles that aid plants in their growth and maturation in low nutrient and stressed soil conditions. Figure 12.2 illustrates the multifunctionalities of rhizobacteria associated with millets. Beneficial microorganisms play significant roles in promoting millet growth and development through various mechanisms such as tolerance to stress, nutrient solubilization, production of growth hormones, and biocontrol of pathogens. Figure 12.3 highlights the bacteria that are dominant in the millet rhizosphere and their multifunctional

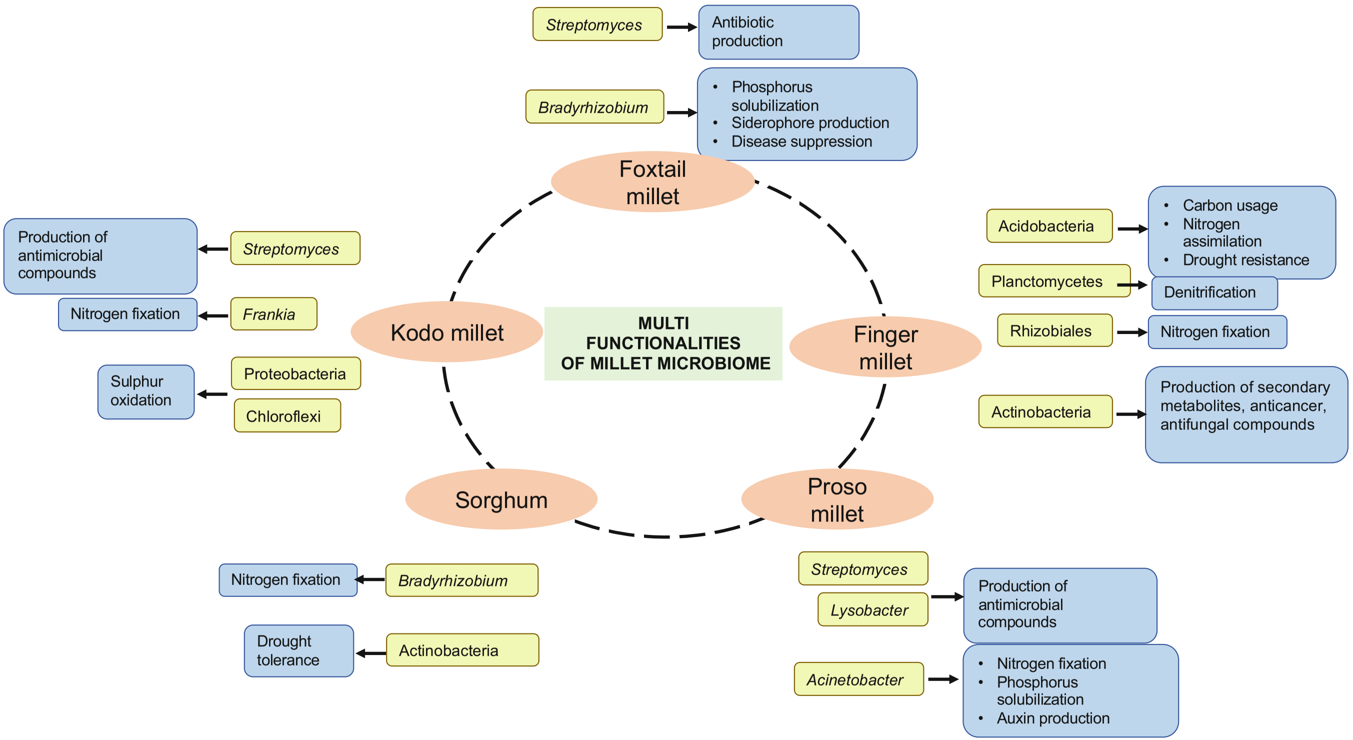

Fig. 12.3 A summary of the common functional groups of bacteria in millets whose rhizobiome structures have been studied. Proteobacteria are commonly associated with all the millet rhizospheres. Most millets have drought-tolerant properties and recruit beneficial microorganisms, which play important roles in the biogeochemical cycles of nitrogen, sulphur, carbon, phosphorus, etc. The microbial roles extend to the production of antimicrobial compounds, auxins, siderophore production, secondary metabolites, disease suppression, denitrification, etc. More insight using different methods would help decipher the microbial roles in the rhizosphere of millet in a detailed manner

roles. It is noteworthy how bacteria such as Planktomycetes and Actinobacteria, dominate the soils involving themselves in myriad roles beneficial to soil, plants, and other microbes.

12.3 Other Studies on Multifunctional Microbes Associated with Millet Rhizosphere

12.3.1 Disease and Pest Resistance in Millets

Other useful studies apart from metagenomics have shown that *Penicillium chrysogenum*, a fungus present in the rhizosphere, is involved in upregulating the expression of defence-related genes LOX, POX, and TXT to provide resistance against *Sclerospora graminicola*, the causative agent of downy mildew in pearl millet (Murali et al. 2013). Another group has shown that edaphic factors of nitrogen, soil pH, and total nitrogen also affect microbial populations in the Proso millet, where Proteobacteria, Actinobacteria, and Acidobacteria play major roles in the rhizosphere (Tian et al. 2021).

The metagenome sequencing of bajra/pearl millet to know the multifunctional bacterial populations has not been carried out yet. One study was conducted on the rhizosheath size of bajra, which is the ratio of soil dry mass associated with the root-to-root tissue dry mass (RAT/RT) and how it influenced the bacterial diversity in the bajra rhizosphere. The study analysed bacterial and fungal populations using metabarcoding of 16S rDNA and ITS regions. These results concluded that a high ratio stimulated the presence of Proteobacteria and Rhizobiales, while a low ratio led to the stimulation of Bacilliales (Ndour et al. 2017).

Furthermore, it has been found that Trichoderma sp., when they colonise the roots of millets, influence the plants to produce IAA, siderophores, influence solubilization of phosphate, and also stimulate enzyme activities such as cellulose, chitinase, and protease, which prove effective in the control of downy mildew disease (Nandini et al. 2021). *Penicillium chrysogenum* and *Bacillus pumilus* present in the rhizosphere of millets have also been found to be important in the defence against other pathogens (Raj et al. 2012; Murali et al. 2013). Future metagenomics studies are needed to study the multifunctionalities with respect to pathogen control in millets.

12.3.2 Rhizosphere of Millets and Drought-tolerant Crops

The rhizosphere of millets provides a nutrient-rich environment, since it recruits beneficial bacteria that not only promote their growth and confer stress tolerance but also perform other functions such as driving the biogeochemical cycles and production of antibiotics, etc. The soil microbial community structure is also driven by the developmental stage of host plants (Na et al. 2019) amongst other factors. Interestingly, plant growth promoting bacteria belonging to *Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, and others* have been commonly observed in the rhizosphere of millets and major crops such as wheat and maize (Yang et al. 2017; Latif et al. 2020; Tian et al. 2022). Similar studies on millets and other crops have proved that drought induces enrichment of *Actinobacteria* and *Chloroflexi* within the rhizosphere, contributing to the plant's survival in extreme conditions (Santos-Medellín et al. 2017; Simmons et al. 2020).

Desiccation-resistant bacteria belonging to *Firmicutes* are common to the rhizosphere of millets (Mattoo et al. 2021) and upland rice (Pang et al. 2020; Ndour et al. 2021).This is probably due to the drought adaptation characteristics of the roots favouring the growth of thick-walled and sporulating Firmicutes (Pang et al. 2020).

Dryland wheat harbours *Pseudomonas* with PGP traits that improve phosphorus uptake during salinity and drought stress (Karimzadeh et al. 2021). The same organism was also isolated from the rhizosphere of millets. Exudation of specific carbon compounds by sorghum leads to enrichment of *Pseudomonas* in the rhizosphere, which confers biotic and abiotic tolerance (Chiniquy et al. 2021). Species of *Pseudomonas* recovered from the rhizosphere of finger millet have been demonstrated to possess potential traits such as phosphate solubilization, production of hydrolytic enzymes, and biofilm formation that help the plant cope with biotic and abiotic stress (Sekar and Prabavathy 2014).

Other important observations include the difference in methane-emitting bacteria between paddy and millet fields. Methanogens such as *Methanocella, Methanobacterium, Methanosarcina*, and *Methanosaeta* are observed in high numbers in the paddy rhizosphere, hence contributing to methane levels and greenhouse gases (Edwards et al. 2015). Interestingly, millets do not need waterlogged soil conditions and possess a lower abundance of methanogens, hence contributing to lower methane and greenhouse gas emissions, making them important crops in mitigating climate change.

12.3.3 Microbes Associated with drought Tolerance and Salinity Stress

Drought-adaptive bacteria in the rhizosphere of millets play a crucial role in tolerating high temperatures and coping with harsh conditions along with growth-promoting traits. ACC deaminase activity is an important stress-tolerant trait that reduces the amount of ethylene produced due to water scarcity. Higher amounts of ethylene disturb normal physiological and biochemical processes. *Streptomyces laurentii* sp. and Penicillium sp. isolated from rhizosphere of sorghum, showed ACC deaminase activity in addition to solubilization of nutrients such as phosphate (Kour et al. 2020). ACC deaminase producing bacteria like *Pseudomonas fluorescens*, *Enterobacter hormaechei*, and *Pseudomonas migulae*, associated with the foxtail millet rhizosphere produce EPS (Extracellular polymeric substances) that provides a microenvironment for water retention thus protecting bacteria from drying (Niu et al. 2018)

Pseudomonas strain SPF-33 alleviated salt stress by increasing the enzyme activity and proline content and strain SPF-37 increased germination, leaf water content, and total chlorophyll under salinity stress (Mahadik and Kumudini 2020). *Arthrobacter siccitolerans* 4J27 belonging to the halo-tolerant group Actinobacteria was isolated from the rhizosphere of finger millet (Niu et al. 2018).

12.4 Conclusions

The higher abundance of bacteria with beneficial traits in millets indicates that millets provide a favourable environment for positive associations. Members of *Acidobacteria* are involved in carbon usage, nitrogen assimilation, and iron uptake, thus enabling them to withstand low nutrient conditions (Mattoo et al. 2021). Millets that usually grow in nitrogen-deficient soil host bacteria like *Plantomycetes*, *Proteobacteria* (especially *Rhizobiales)*, and *Cyanobacteria* that contribute to nitrogen cycling via denitrification and symbiotic nitrogen fixation, providing essential nutrients and metabolites to the host (Dwivedi et al. 2016). *Chloroflexi* thrives in environments with limited carbon and nutrient availability (Tian et al. 2022). The presence of Actinobacteria in microbial communities producing antimicrobial compounds indicates that the millet microbiome possesses metabolic activities that are capable of withstanding biotic stress (Prabha et al. 2019). Similarly, *Proteobacteria*, known for their drought resistance traits are enriched in the rhizosphere of millets promoting their growth during drought stress and water deficient conditions (Xu et al. 2019). *Firmicutes* like *Bacillus* also play a key role in desiccation resistance due to their ability to form endospores (Marasco et al. 2012). The environment in which millets are grown contains complex compounds that are degraded by *Bacteroidetes* and *Actinobacteria* via production of extracellular enzymes that breakdown lignin, polysaccharides, and several compounds (Kopecky et al. 2011).

12.5 Future Aspects

Metagenomics coupled with other techniques, such as stable isotope profiling, studying metabolism, would be important in elucidating microbial communities in the millet rhizosphere. Integrating the classical culture dependent techniques with modern tools would be necessary for the characterization of the unknown microbial populations and their interactions in the rhizosphere region of millets. Majority of the studies have focused on bacteria; however, elucidation of viruses, fungi, algae, macroflora in the millet rhizosphere is also vital and needs to be carried out in future. These organisms are constantly interacting with each other to perform multiple functions and seem highly complex, but these are essential in shaping the soil environment. In future, studies on the shifts in microbial diversity and abundance due to anthropogenic activities and climate change are essential to understand ecosystem balance in the rhizosphere of millets.

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Chapter 13
Understanding of Belowground Biochemical Communication in Millets Through Metabolomics

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Abstract After the green revolution, the consumption of rice and wheat became popular, but grain production became an area of concern. Now, the importance of millet was understood, particularly in developing countries of Asia and Africa. The novel technology termed as 'metabolomics' played a major role in improving the quality of crop and enhancing its yield. Metabolomics is the dominant driver of phytochemical analysis in the present times. This tool has helped in finding the quality and quantity of root exudates which determines the phenotypic fate of the cells, tissue, and the plant in whole crop. Metabolomics differs from traditional approaches of phytochemical analysis fundamentally, such as it is based on rational analysis, supported by scientific data, assessing all metabolites that can be measured without any pre-selection. Rhizosphere comprises varied populations wherein the plant roots compete in order to survive. For securing optimum water, essential nutrients and space, plants undergo inter-species competitions, as well as with other microorganisms present in soil. This competition for survival takes place through biochemical interactions in between roots and microbes, and among roots of different plants. Certain root secretions called as 'root exudates' are considered to initiate such metabolite communication and command these interactions. These exudates or secondary metabolites play a key role in underground communications between plant roots and beneficial microbes. The ability of plants to communicate simultaneously with other plants and other microbes as well as their potential to alter metabolic processes under stress conditions is an unexplored area. It is of utmost importance to gain more experimental evidence and conduct studies to fully understand the process of underground communication under biotic and abiotic stress conditions as well as nutritional benefits of millets, to improve its quality and production, in order to promote sustainable agriculture and meet the ever-growing

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_13

demand of food, especially in developing countries like India. Here, we have given an insight into the biochemical interaction between millet plants, studied using a holistic, data-driven approach, termed as metabolomics.

Keywords Biochemical · Environmental stress · Metabolomics · Millet crops

13.1 Introduction

Millets are cultivated mainly in areas prone to rainfall by small landholders and tribal farmers. They have been cultivated in India since time immemorial and are one of the ancient crops, categorised as major and minor millets. Post green revolution era, emphasis was given to production of fine cereals and grain production. The consumption of rice and wheat outweighed millet production, only to later understand its importance, particularly in developing countries like India. The improvement of crop quality and agricultural productivity has been a major concern, and it has been lately monitored using tools such as omics, termed as '**Metabolomics**'. It is important to know about resistance of pathogens, stress tolerance, robust ecotypes, and metabolic assisted breeding of crops (Shulaev et al. 2008). Approximately 200,000 compounds of diverse metabolites are part of the plant kingdom with about 10,000 secondary metabolites estimated in different plants. All the known metabolites vary in their properties such as morphological, biochemical, and functional properties which are important in plant biology (Foito and Stewart 2018). Large number of metabolite synthesis affects the plant growth and development under adverse environmental conditions. The interactions of plants with the environment are studied by environmental metabolomics. The main phenomenon is to explain the biochemical nature such as disease occurrence, effect of parasite load, and infection. There are two types of metabolites: primary and secondary metabolites, whose profiling aids in understanding the biochemical processes of plant metabolism (Sung et al. 2015). There are some tools which can detect, identify, assess, and evaluate them such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography mass-spectroscopy (LC-MS), and non-destructive nuclear magnetic resonance spectroscopy (NMR) (Che-Othman et al. 2020). Primary metabolites play a significant role in the development of plants through the synthesis of lipids, amino acids, and sugar. Flavonoids, atropine, phytic acids, and carotenoids are secondary metabolites which are produced by primary metabolism. Unlike primary metabolites, they do not directly participate in the reproduction, development, and normal growth of an organism and are described as organic compounds. These metabolites are formed in the stationary phase, which means they do not engage in the developmental phase. Absence of these metabolites leads to long-term deficiency of organism's survival rather than its immediate death. Secondary metabolites are based on the premise of improving plant production through the treatment of signal molecules, elicitors, and biotic disturbances. These metabolites aid in plants' defence mechanism against biotic and abiotic factors (Stamp 2003). The actual cellular dynamics of a plant system under stress conditions can be analysed holistically using metabolomics, which lacks in classical analytical methods, making it an unique tool. Plant metabolomics is a relatively young area of science and is being applied in various fields of plant biology (Fukushima et al. 2009). With the developing interest in millets among the cultivators and consumers, and limited experimental evidence, an initiative has been made to gather knowledge of belowground communication in millets under the influence of stresses and their nutritional values.

13.2 Types of Millet Crops

Millet crops are small, annual C4 grasses, which usually develop fully into both food and fodder (Singh et al. 2021). Small millet crops are rich in nutrients which are usually grown by the farmers of Asia and Africa. All millet crops belong to the family Poaceae of the monocotyledon plants. In 2013, millet grain production was 762,712 metric tonnes, with India leading the way with the annual output of 334,500 tonnes (43.85%). Following are the important millet crops: *Eleusine coracana* (Finger millet), *Setaria italica* (Foxtail millet), *Paspalum scrobiculatum* (Kodo millet), *Panicum miliaceum* (Proso millet), *Echinochloa* spp. (Barnyard millet) (Fig. 13.1).

In India, finger millet is usually termed as mandua or ragi and contains the properties of staple food that provide major portions of protein and calories to the low-income groups of people in developing countries. According to estimates, finger millet accounts for 10% of the 30 million tonnes of millet production worldwide. It has been reported that finger millet has shown extensive growth in South Asia and Africa under a variety of agro-climatic conditions (Kumar et al. 2016). Millet is a vital but underutilised crop in semi-arid and tropical regions of the globe. Due to its larger resistance towards pests, diseases, enhanced signal perception, smart adaptation to varied environmental conditions, and good yield against water accumulation, tolerance to drought, low input requirement throughout growth and comprises of important crops for human consumption in future due to ever growing world population and scarcity of water resources as a hardy plant. Finger millet is easy to grow under stressful conditions without sacrificing net yield. There is immense potential to turn millet into higher nutritive value food and beverage in developing countries. In addition, as it does not contain gluten, it is recommended for stomach patients (Chandrasekara and Shahidi 2010).

Foxtail millet is one of the ancient, cultivated millet crops, and it needs warm weather and an insignificantly small amount of water for fast maturing throughout dry months of the year. Foxtail millet comes second in total millet crop production worldwide. It is usually called foxtail millet or Hungarian/Russian/German/Chinese millet. It is a consequential millet crop tailored to dry as well as arid areas of India, China, North Africa, Asia, and Americas. Lately, the Joint order Institute (JGI) Department of Energy, BGI and USA, China, has sequenced its genome. Foxtail millet is one of the smallest amongst panic grasses and its genome size is like rice. Foxtail millet contains crude fat (5–8%), higher grain protein (14–16%), and higher

Pearl millet: The seeds or the grains are small, round, and golden yellow

Barnyard millet : low in calories, rich in dietary fibre, good source of iron

Little millet : Rich in antioxidants, gluten free, helps in lower cholesterol

Foxtail millet: Tiny seeds around 2mm in size, covered in a thin, crispy hull, light yellowish-brown in colour. Grown in semi-arid regions

Proso millet : Contains high lecithin which supports the neutral health system. Rich minerals, vitamins and essential amino acids.

Buckwheat millet: excellent source of minerals i.e.,copper, manganese, magnesium and phosphorus

Kodu millet : Easy to digest, high protein content, low fat and high fibre content

Sorghum millet: a nutrient-rich cereal grain, low in fat, high in protein, contain fiber, B vitamins, and micronutrients

Finger millet: Millet is a gluten-free grain that's rich in antioxidants, soluble fiber, and protein.

Fig. 13.1 Different types of millet crops and their nutritional importance mineral content than finger millet (Dwivedi et al. 2012). Considering foxtail millet biological advantages, it contains a larger proportion of digestible proteins than wheat and rice as it contains seven essential amino acids of the eight. Foxtail millet bran contains 9.4% crude oil enriched with oleic acid (13.0%) and linoleic acid (66.5%) and cereals contains edible fibres 2.5 more than rice (Dwivedi et al. 2012; Liang et al. 2010), and rich in fibre (42.56%) (Amadou et al. 2011). It is very helpful in reducing glycosylated haemoglobin, serum lipids, and blood glucose levels in type 2 diabetic patients. Germ foxtail millet seeds, particularly golden seeds, have excellent medicinal properties being used for curing indigestion, celiac disease, poor digestion, and stagnant food in the stomach (Thathola et al. 2011).

Kodo millet (*Paspalum scrobiculatum*) is also known as Indian crown grass or kodo. In countries like India, Philippines, West Africa, Thailand, and Indonesia; it is one of the most ancient grain millets that are cultivated. Kodo is a monocot crop that is robust and adaptable to drought, restricted to semi-arid areas, and is grown over 907,800 hectare of land each year, producing around 310,710 tonnes (Neelam et al. 2013). These millet crop grains have carbs (66.6 g per 100 g of grains), iron (25.86–39.6 ppm), minimal fat (3.6/100 g), as well as high-value protein (11%), antioxidant free-radical and calcium (27/100 mg) (Deshpande et al. 2015). Kodo plants have therapeutic qualities including anti-diabetic and anti-rheumatic effects, the ability to heal wounds, and a calming impact (Neelam et al. 2013; Deshpande et al. 2015). They are shown to be useful in preventing heart attacks, migraines, blood pressure, diabetic heart diseases, and atherosclerosis. The peak gallstone development is prevented by millets' high fibre content. Consuming whole grains promotes health promoting heart disease prevention and insulin resistance illness, ischemic stroke, diabetes, obesity, and asthma in children, cancer, and early mortality (Balasubramanian et al. 2013). Due to these advantages, millets can be utilized as a filler and functional food in nutraceuticals. Consequently, they are also known as 'nutri-cereals'. Kodo millet could be a healthier alternative to rice or wheat. The protein content in kodo millet is 8% with glutelin being the predominant protein portion. In contrast to the low fibre content containing rice and wheat (0.2%), kodo millet has higher fibre content (9%). Like other millets, kodo millet has 353 kcal and 66.6 g of carbs per 100 g of grain. Additionally, it has 2.6% minerals and 1.4% fat, with the range of iron content in kodo millet being from 25.86 to 39.60 ppm (Deshpande et al. 2015). It contains the least phosphorus amongst all the millets. Kodo millets are not only a major source of nutrition but they also have a lot of antioxidants, polyphenols, tannins, phytic acids, and phosphorus. These anti-elements interact with micronutrients such as zinc, calcium, and iron to produce complexes that decrease the solubility and bioavailability of these nutrients (Deshpande et al. 2015).

Panicum miliaceum L., often known as proso millet, is a significant cereal and an essential part of human nutrition, particularly in underdeveloped nations. The crop may be grown in a variety of soil types and in challenging environments since it is salt-, alkali-, cold-, and drought-tolerant (Wang et al. 2005). Its grains are mostly consumed as decorticated food. The nutritional value of proso millet, such as its starch and crude protein levels, has traditionally been used to assess its quality.

Increased intake of proso millet and its derivatives is linked to a lower risk of chronic illnesses, including high blood cholesterol, cardiovascular disease, type II diabetes, and liver damage, according to epidemiological research.

One of the ancient millet crops known as barnyard millet (*Echinochloa* species) is cultivated around the world in warm, temperate climates. It is significant in Asian countries, notably in Korea, China, Japan, and India. Barnyard millet is the fourth most produced minor millet and aids in food security to food deprived populations throughout the world. In terms of area (0.146 m/ha) and output (0.147 mt), India is the world's largest producer of barnyard millet, with an average productivity of 1034 kg/ha as recorded over the past 3 years (IIMR 2018). Most of the barnyard millet is grown for human purpose; however, it is also consumed by animals. Two of the most often used varieties of barnyard millet are *Echinochloa esculenta* (Japanese barnyard millet) and *Echinochloa frumentacea* (Indian barnyard millet), which are both cultivated as wild species (Sood et al. 2015). It has a short life span with high resistance against various biotic and abiotic challenges. Along with these agronomic benefits, grains of these millets are prized for their reduced cost and superior nutritional value when compared to main cereals such as maize, rice, and wheat. It contains micronutrients including zinc (Zn) and iron (Fe) that are linked to several health advantages as it is a rich source of fibre, carbs, protein (Singh et al. 2010; Saleh et al. 2013; Deshpande et al. 2015; Ugare et al. 2014). These qualities of barnyard millet make it a great supplement millet crop for farmers and an alternative crop when the rain affects the areas where rice and other major crops are grown (Saleh et al. 2013).

13.3 Biochemical and Physiological Changes in Millets During Abiotic Stress

Drought, extreme temperature, nutrient deficiencies, heavy metals, and salinity are classified as abiotic stresses. These factors can be a challenging factor to crop production worldwide (Vickers 2017). These stressful abiotic conditions usually lead to growth of reactive oxygen species (ROS), which causes inhibition of photosynthesis and extreme cell damage (Baxte et al. 2014). With the increase in population, agricultural lands are facing the consequences such as lack of water, land, and climate change. These problems can be solved by using plants that are naturally resistant to stress. The naturally resistant plant species ensure a stable production, global health security, and food security. These NSRPs (sub-crop) are genetically modified to increase their yield (Zhang et al. 2018). Millets, commonly known as small grain, are not well explored and researched for its production; however, this can be achieved by the development of genetic resources for their production (Lata 2015). Millets are agriculturally important crops as they are operant to biological, heat, drought, salt stresses, and can also survive in smaller areas (Tadele 2016).

Millet can survive in poor soil and low rainfall. Pearl millet is one of the most drought and heat tolerant millets and grows best in well-drained sandy or sandy loam soil. Finger millet is adapted to different humidity and temperature range and is grown on red–brown lateritic soils that have sufficient water-holding capacity and good drainage (Saxena et al. 2018). Foxtail millet usually grows on arid black cotton soils, and also grows on loamy, alluvial, or clay soil. Extreme drought- tolerant kodo millet is grown in hard gravel soil where other crops are not capable of growing. Fonio is cultivated on plateau savannas with humid conditions and slightly heavier soils. Finger millet is one of the most stress-resistant crops amongst millets under stress conditions such as poor soil, high temperature, and low humidity. As a result, it is used to improve other economically significant crops. Millet contains some important regulatory proteins and genes that are in charge of its adaptive traits, and also used to develop stress-resistant crops. Genetic engineered millet crops with desired properties can be engineered by inactivation with desirable stress tolerance properties or overexpressing transcription factor genes. These genes are identified by genome-wide expression characterisation (Odhong et al. 2019). Pearl millet cultivars have been described to have better capacity for drought stress tolerance than corn, which has better rate of photosynthesis; related water content (RWC); up-regulated expression of PIP2:3, CBF transcribes, and repressed action of Rub Sc in drought-resistant leaves (Iwuala et al. 2019). Amongst small millets, it was found that barnyard grass has the highest drought tolerance, followed by crabgrass and small millet when they are in the stage of reproductive developmental morphology. These free-range millet crops perform better particularly in reproductive ears, shoot number, grain weight, ear weight, straw yield, and grain yield (Ashok et al. 2018). In addition, pearl millet is highly nutritional and has been cited as an excellent solution to water stress conditions due to its drought stress tolerance (Sun et al. 2015). The salinity of the soil has rendered agricultural land worthless and dangerously affected the growth of many millet crops. Sodium and chlorine ions present excessively in substandard quality water and cause nutritional disturbance, ion specific toxicity, and osmotic damage in crops leading to salt stress (Fernandez and Ebert 2005). Finger millet is moderately salt tolerant, has a decline in (1) shoot dry weight, leaf count, leaf chlorophyll content, and leaf surface area and an increase in (2) electrolyte leakage, leaf chlorosis, leaf succulence, lignification of xylem vessels, destruction of chloroplasts severe tissue damage, proline content, and H_2O_2 increased under salinity (Satish et al. 2016). In addition, salt stress in millet leads to reduced germination rates, chlorophyll content, root and shoot growth, leaf K^+ concentration, and relative leaf water content, as well as increased salt and chlorosis and malondialdehyde levels. Also, salinisation and alkalinisation resulted in reduced plant dry weight, net assimilation rate (NAR), relative growth rate (RGR), leaf area ratio (LAR), relative water content, and nitrogen in foxtail and proso millet. Tolerant accessions of proso millet have been reported to have extremely high chlorophyll-*a* content under saline conditions. Chlorophyll-*a* content is usually related to salt tolerance in proso millet (*Panicum miliaceum*) (Sabir et al. 2009). Another type of abiotic stress is high temperature or thermal stress which affects the productivity of food crops. Studies state that under high temperature, roots play a critical role in the survival of plants due to their lower optimal temperature range for growth, their lower potential for acclimatisation to extreme conditions, and their greater sensitivity to variations in soil temperatures compared to other exposed parts of the plant. In reaction to high temperatures, plant roots and leaves undergo several structural and functional changes. These alterations result in molecular, cellular, and phenotypic changes, such as metabolic and membrane properties, hardness of the cell wall, and shortening of the roots (Qadir et al. 2019). The findings gave insights into increasing soil temperature tolerance to boost production by exposing genotype-specific pathways for stress adaptation. According to the studies, tolerance of millet plant roots in terms of thermal stress could be associated with some changes in metabolites and its physiological behaviour. It showed that rather than translating into grain production, the metabolites produced under stress conditions were utilised for growth maintenance and other survival functions.

Drought stress is the condition that leads to severe water scarcity and has severely affected plant growth leading to damage of many biomolecules and lipid peroxidation (Bhatt et al. 2011). Ethylene (ET) and abscisic acid (ABA) are two main phytohormones commonly involved in water tolerance and stress signalling. Both jasmonic acid (JA) and salicylic acid (SA) improves plant tolerance to drought, heat stress, and salinity (Saxena et al. 2019). Due to water stress, the brown and black finger millet reduces photosynthesis, chlorophyll, and relative water content and increases proline content; the brown millet crop shows a higher tolerance than black millet crop (Khatoon and Singh 2016). Oxidation due to drought stress in finger millet resulted in increased proline, drooping shoots, curled leaves, malondialdehyde (MDA) levels, significant increases in H_2O_2, impaired membrane integrity, and electrolyte leakage. Drought increases the activity of antioxidant enzymes such as glutathione reductase (GR), superoxide dismutase (SOD), catalase (SAND), glutathione peroxidase (GPX), and ascorbate peroxidase (APX). Increased concentrations of the herbicide ‘atrazine’ caused physiological impairment in pearl millet seedlings by inhibiting photosynthesis. In metal stress of millet crop, the nickel toxicity in finger and pearl millet results in the inhibition of shoot length, seed germination, decreased root and shoot dry weight, and increase in proline content about four to five times (Gupta et al. 2017) (Fig. 13.2).

13.4 Biochemical and Physiological Changes in Millets During Biotic Stress

A plethora of pests and pathogens can invade plants and induce biotic stresses. Fungi act as parasites in plants, such as necrotrophic and biotrophic. Necrotrophic fungi kill the plants by secreting toxins inside them whereas biotrophic fungi feed on the viable cells. They are a potential source of many known plant infections such as cankers, vascular wilts, and leaf spots (Sobiczewski et al. 2017). Certain soil-borne diseases primarily caused by nematodes are seen which leads to wilting, delayed

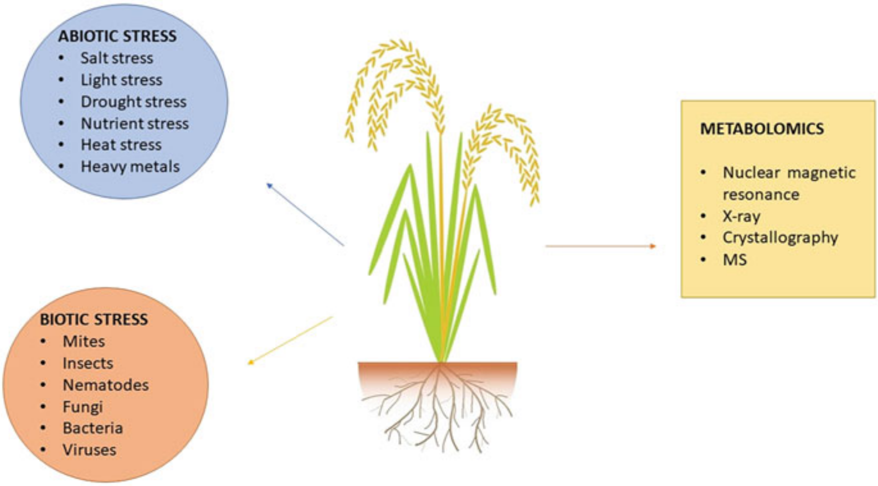

Fig. 13.2 Types of abiotic, biotic stress, and metabolic profiling by different techniques

growth, and deprivation in nutrient content (Osman et al. 2020). Likewise, local and systemic damage are caused by viruses promoting chlorosis and stunted growth. In contrast, insects and mites either feed on plants by piercing and sucking or lay eggs on them resulting in impairment of host cells. To fight against these stress factors, plants have developed an extensive defence system (Saijo and Loo 2020). The first line of defence in plants includes cuticles, wax, and trichomes which are passive physical barriers and aids in preventing the entry of the pathogens. On the other hand, they can also produce chemical compounds for their defence process. Plants stimulate defence machinery against the biotic stresses with the help of two levels of pathogen recognition. The first level of recognition encompasses PRRs (Pattern Recognition Receptors) and PAMPs (Pathogen Associated Molecular Patterns), termed as PAMPs-triggered immunity or PTI. In the second level of pathogen recognition, the plant resistance proteins (R) are involved that recognise specific receptors from a pathogen, also called as Avr proteins (Abdul Malik et al. 2020). Considerably, this is an effective mechanism of defence and encircles effector-triggered immunity, or ETI, triggering programmed cell death and hypersensitive responses. Upon exposure to biotic stress, the earliest plant response and signalling processes are the perturbations in the cytosolic concentrations of calcium (Ca^{2+}). The calcium ion signals are the basis of plant immune signalling pathways (Aldon et al. 2018). Depending on the Ca^{2+} signatures, the plant immune response varies. The calcium ion channels are activated by PTI and ETI, upon exposure to biotic stresses and results in an increment in the cytoplasmic concentrations of Ca^{2+} ions, post-translational activation of mitogen-activated protein kinase, and reactive oxygen species are produced (Jonak et al. 2002). It is evident that there will not be any defence mechanism developed in plants if they are never exposed to pathogens or biotic stresses. Therefore, both the plant and pathogen co-evolve and develop their own defence mechanisms over time. The role of nitric oxide has been found critical in stimulating the defence response during biotic stress (Wood et al. 2000). In addition to this, NO also plays a vital role in mediating the NADPH generating enzymes through regulating certain modifications after the translational process, as there is significant involvement of NADPH in proliferation, detoxification, growth of the cells, and intra-cellular redox state (Sharma et al. 2020). Salicylic acid or the ethylene pathways are the major pathways that confer the immune system in plants. Salicylic acid is the driving factor in plant defence response against biotrophic pathogens, but it has been observed to show antagonistic effect against the ethylene pathways which is a key factor in providing defence against necrotrophic pathogens and abiotic stresses (Glazebrook et al. 1997). In response to pathogenic attack, the concentration of salicylic acid increases due to the fluctuations in the cellular redox status. The role of NO in mediating both the pathways during plant immune response towards biotic stresses has been explained in much detail by Mur et al. (2013). However, extensive research is much needed to fully understand the mediation and governance of the underground biochemical communication and signalling networks during biotic stresses.

Plants communicate amongst one or more species in nature using complex mechanisms, which they have evolutionarily developed (Karban 2008). Plant behaviour is affected by the information perceived from the surroundings, which are optimised to enhance agricultural productivity (Novoplansky 2016). This information can be physical or chemical such as the organic compounds produced from roots and shoots of neighbouring plants (Chen et al. 2012). Underground communication is dominantly carried out by the wide range of organic compounds secreted from the roots (Penuelas et al. 2014). In the presence of biotic stresses, plants can get warning signals through their microbiome and inter-plant signalling molecules (Vahabi et al. 2018). The plants and the associated microorganisms are considered as holobionts or super organisms (Saikkonen et al. 2020). Holobiome consist of all the living organisms, metabolites, molecules, and genetic materials produced by them (Berg et al. 2020). The microbial community present in a specified habitat aids the holobiome to endure the biotic stresses. During biotic stresses, the presence and increment in the population of specific microbes alters in the microbiome (Carrion et al. 2019). The specificity of biochemical communication is determined by the ability to recognise or exchange molecular signals between the plants and pathogens. For the belowground communication to take place, there must be an exchange of signalling molecules, which are produced either by the plant or the microbe or both during their exposure to each other which results in biochemical, physiological, and molecular responses which determine the fate of their interaction. Out of millions of microbes present in the rhizosphere, only the compatible one can affect the host defence responses, being a potential pathogen through establishing successful communication, thus altering plant growth and health. The belowground communication can be either inter/intra species which is driven by signalling volatile organic compounds or quorum sensing or between kingdoms through root exudates, exchanging signalling molecules, volatile organic compounds, and bacterial QS signals (Bukhat et al. 2020). Certain chemical signals are released from plants which attract specific microbes, for instance, fumaric acid found in banana root attracts *Bacillus subtilis* N11 resulting in the formation of biofilm (Zhang et al. 2014). The nod genes in bacteria produce lipo-chitooligosaccharides, responsible for nodules formation in leguminous plant, also assists in the interaction between arbuscular mycorrhizal fungi plants by mimicking the QS molecules of bacteria and influences bacterial metabolisms (Hassan and Mathesius 2012). Moreover, rhizobacteria utilises plant root exudates like ACC and tryptophan as precursors of growth regulators of auxin and ethylene, respectively (Zahar Haichar et al. 2014). Silicon is abundantly present in the rhizosphere, making it an essential element to consider while focusing on the belowground communication. It was observed that silicon not only aids in increment of biomass but also provides resistance to plants against biotic stresses, thus giving protection to the plants (Perez-Labrada et al. 2020). Cell wall thickness is one prominent characteristic of plants accumulating high levels of silicon (Imperiali et al. 2017). Biotic factors such as insects and other microbes mediate the emission of volatile substances from the plants by altering the concentration of some VOCs or stimulating de novo VOC formation. The kind of VOC emitted is based upon the plants' genotype and the biotic stresses responsible for triggering its synthesis. These triggers could be chewing or sucking by pathogens, necrotrophic or biotrophic, etc. and elicit distinct VOCs, including their amount, quality, and the time duration of their emission (Sharifi et al. 2018). The characteristics of emitted VOCs are mostly determined by different trigger factors such as stress-induced plant volatiles (SIPVs) and microbe-induced plant volatiles (MIPVs) (Sharifi et al. 2018). Therefore, biological or chemical factors can modulate plant defence, and subsequently, the VOCs, when exposed to stresses. Thus, infestation of pathogens or biotic stresses can show considerable alteration in the emission of VOCs and their profile while simultaneously cross-communicating with sibling species or other species (Penaflor and Bento 2019). There are certain exudates released by the roots which act as signalling molecules between plants and are also sources of carbon and nitrogen for the microbes present in the rhizosphere. These exudates are often modified when stimulated by biotic stresses, which in turn is utilised as info chemicals by neighbouring plants (Rahman et al. 2019). Jasmonic acid and salicylic acid are some of the root exudates that act as signals between plants and induce defence response in them (Li et al. 2020). The info chemicals or signalling molecules accumulate around the roots of the producer plants and are enhanced by the receivers so as to amplify their zone of effectiveness. Few mycorrhizae and dodder facilitate these info chemicals in their diffusion up to several metres; however, the intensity of response in the receiver plant decreases as the distance increases. Studies have shown receiver plants can act as nodes in the signalling process between plants by propagating signals (Wenig et al. 2019). Receiver plants perceive volatile compounds on their epidermal cells which are trapped in the wax layer and released on exposure to biotic stresses (Camacho-Coronel et al. 2020). The receiver plant's proteome, transcriptome, and metabolome alter when the VOCs are absorbed (van Dam and Bouwmeester 2016). The gene expression and metabolic processes might not be necessarily affected due to the VOCs, but it conditions the receiver plant to effectively defend the pathogens that might invade in later times (Quintana-Rodriguez et al. 2015). During communication between plants, the volatile organic compound changes the physiology of the receiver plants and modifies the holobiome of others. On perseverance of the VOCs, plants show killing effects on fungi and bacteria up to several days (Camacho-Coronel et al. 2020). The emission of these info chemicals above-ground or below-ground might act in favour of or against herbivores and other pathogenic nematodes (D'alessandro et al. 2014). Root exudates play a significant role in triggering resistance in plants through diffusion and informing the neighbouring roots. For instance, SA, a root exudate, signals the neighbouring plants to modify their root microbiome using SAR signals (Kong et al. 2021) (Fig. 13.2).

13.5 Metabolomic Analysis of Interaction Between Millets and Rhizosphere

The quantity and quality of plant metabolomes determines their responses to different stress stimuli, physiological conditions, and genome sequence thus bridging the genotype and phenotype of the plant. Metabolomic analysis significantly contributes to the understanding of plants' response to varied stress conditions by studying the by-products or metabolites. These metabolites are considered to be the conclusive response during changes in the plant environment (Arora et al. 2018). The specialised metabolites act as messengers aiding in interactions between the millet plants and the rhizosphere, which is studied using metabolomics. There have been several metabolomic studies conducted that have shown significant belowground communication in between plants and the microbes, and also amongst different plants. Some important biotic interactions in plants are with PGPB, mycorrhiza, Trichoderma, etc., which depend greatly on the root exudates secreted by the plants. For instance, a study conducted by Zhang and colleagues showed that cucumber roots exudates containing citric acid attracted *Bacillus amyloliquefaciens* SQR9 and resulted in formation of biofilms (Zhang et al. 2014). Another variety of compounds called flavonoids found in these metabolites have been found to induce bacterial nod genes, thus inducing root nodule formation (Mhlongo et al. 2018).

13.6 Conclusion

Biochemical communication evolved millions of years ago. Scientists believe that about 450 million years ago this communication had developed between plants and microbes and also amongst different species. In this chapter, we discussed how millets communicate under the influence of both abiotic and biotic stresses, their mechanism to withstand such challenging environmental factors, as well as their nutritional advantages. Millets grains are rich source of several minerals such as zinc, iron, calcium, dietary vitamins, energy, several minerals insoluble dietary fibre and phytochemicals with antioxidant properties and are considered as 'Nutri-cereals' (Bouis 2000). Human population is growing rapidly, thus there is significant growth in the demand for food, making it critical to develop new techniques for improving crop yield and quality. The study of belowground communication is important to understand which microbes are beneficial for plant growth, the optimum environmental conditions for better yield, and nutraceutical properties influenced by such factors. This chapter is an initiative to draw attention towards the importance of understanding belowground communication in millets during stresses, to improve plant growth and protect soil ecosystems.

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Chapter 14

Prospects of Gene Editing Techniques in Manipulating the Rhizosphere Microbiome for Millets Productivity

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Abstract Millets are a group of important drought-resistant "nutri-cereals" commonly cultivated in arid and semi-arid areas. A renewed focus on increasing their production and highlighting nutritional benefits is critical for promoting diverse diets and ensuring climate and food-nutritional security. Although millets are comparatively more climate-resilient than other cereals, their growth and production are frequently hindered by prolonged exposure to several abiotic and biotic stresses. In this line, improved millet varieties can be developed through various simple yet precise genome editing techniques. Targeted editing of the plant genomes not only expands our knowledge of the fundamental basis of plant physiology but also provides an opportunity for improving productivity and quality of crops. In addition, the rhizospheric plant–microbe interactions can also be explored toward formulating sustainable agricultural practices under challenging environments. The rhizosphere is plausibly the most complex interface facilitating the dynamic interactions between a plethora of microbial entities and plant roots. The microbial assemblages of millets consist of many plants' growth-promoting rhizobacteria such as N_2-fixers, mineral (phosphate and zinc) solubilizers, anti-pathogenic bacteria, arbuscular mycorrhizal fungi, etc. The association of this microbial population with millet plants confers

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_14

direct or indirect resistance to several abiotic (drought, salinity, heat, cold, oxidation, etc.) and biotic stresses (insects attacks, soil-borne phytopathogens), also modulates rhizoexudation, root architecture, plant biometry, and phenology. As such, elucidating the microbial diversity, deciphering and managing their biological functions will help in harnessing plant–microbe cross-talks toward increasing ecosystem services, plant plasticity, and productivity under environmental perturbations. Advances in gene editing techniques such as CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/Cas9), ZFN (zinc finger nucleases), TALENS (transcription activator-like effector nucleases), base editing, prime editing, etc. allow us to untangle the web of plant–microbe interaction as well as to improve nutritional qualities and stress tolerance of crops. Since studies on rhizosphere microbiota structure associated with millets are scanty, most of our understanding in genome-editing techniques has been derived from non-millet plants. In this chapter, we focused on how mechanistic understanding of various genome-editing technologies can be leveraged for the manipulation of susceptible genes and increase the plant's fitness under diverse ecosystems.

Keywords Rhizosphere engineering · Gene editing · CRISPR · ZFN · TALENS

14.1 Introduction

Millets are one of the oldest food crops known to humans, possibly the first cereal grain used for domestic purposes. They are highly nutritious, non-glutinous, non-acid-forming, least allergenic, and easily digestible (Michaelraj and Shanmugam 2013). The most commonly cultivated millets are Finger millet [*Eleusine coracana* (L.) Gaertn.], Foxtail millet [*Setaria italica* (L.) P. Beauvois], Pearl millet [*Cenchrus americanus* (L.) Morrone.], Kodo millet (*Paspalum scrobiculatum* L.), Little millet (*Panicum sumatrense* Roth ex Roem. and Schult.), Proso millet (*Panicum miliaceum* L.), and Barnyard millet [*Echinochloa crus-galli* (L.) P. Beauvois]. Millets contain high amounts of minerals such as Fe, Mg, P, and K. Finger millet or Ragi is particularly rich in calcium, which is about 10 times that of paddy rice or wheat. India is the largest producer of several kinds of millets; however, its production is challenged by several factors including limited water availability (especially in developing countries), reduction in arable land (soil degradation), accelerated urbanization, and high crop failure due to the occurrence of diseases and pests. Therefore, to ensure a better and sustainable food supply for the ever-growing population, there is an urgent need of formulating efficient agricultural practices that integrate sustainability in farming practices leading to improved crop yield with minimal soil- and environmental degradation. As such, agricultural management practices for different agro-climatic conditions, the development of high-yielding stress-tolerant millets, and the application of plant growth-promoting rhizobacteria (PGPR) have garnered much scientific attention in recent times.

The rhizosphere, a narrow soil zone closely attached to the roots, provides a dynamic interface of microbial interactions between plant roots and soil (Dubey et al. 2019). The two different compartments in the rhizosphere, i.e., the ectorhizosphere and the endorhizosphere are densely populated by a plethora of belowground microbes and small soil-inhabiting lives (Dubey et al. 2019; Lakshmanan et al. 2014). Of the total microbial diversity associated with plants, most microorganisms (especially, endophytes) have been reported to exhibit positive interactions and promote plant survival and fitness (Egamberdieva et al. 2017; Mahmud et al. 2021). The presence of plant-growth-promoting endophytes such as *Microbacterium trichothecenolyticumn*, *Brevibacillus choshinensis*, *Endobacter medicaginis*, *Micromonospora* spp., etc. has been detected from a wide range of host plants (Ramírez-Bahena et al. 2014; Benidire et al. 2017). In a natural environment, plant health and survival critically depend on the physicochemical signaling between the plant roots and soil microbial populace. The endophytic microbial communities significantly promote plant health by improving macro- and micronutrient acquisition, phytohormone production, niche portioning in the root environment, pathogen biocontrol, and plant defense stimulation.

Soil microbes play a key role in the formation of soil structure, suppression of soil-borne pathogens, solubilization, and acquisition of nutrients and water (Moreira et al. 2022). Designing the rhizosphere with beneficial microbes holds promises for promoting plant growth under challenging environments. Several strategies of biological tools, technologies, and approaches have been adopted for manipulating the plant-associated microbiome. The most effective way to manipulate the microbiome is through bio-inoculation with plant growth-promoting rhizobacteria (PGPR) and Arbuscular mycorrhizal (AM) fungi (Hakim et al. 2021; Kerbab et al. 2021; Kumar and Dubey 2020). Bioinoculants have the potential to form associations with other residential microbiomes as well as stimulate microbe–microbe and plant–microbe interactions in native rhizosphere soils. Shaping the rhizosphere of plants may help in increasing the productivity of millets and other plants as well. Successful engineering of the rhizosphere microbiome requires a systemic approach. Recent advancement in synthetic biological tools and gene editing approaches has now made it possible to engineer microbiomes in a specific path with enhanced soil biological functions (Basu et al. 2018; Jaiswal et al. 2019; Rai et al. 2020; Shelake et al. 2019). Gene editing techniques involving zinc finger nuclease, transcription activator-like effector nucleases, CRISPR-Cas9 system, and speed breeding hold promises for developing engineered crops with better stress tolerance properties (Mao et al. 2019). The last few years have seen a remarkable change in plant cell and molecular biology. Sequencing and annotating the entire plant's genome sequences with databases containing information on a multitude of genes and their expression have been assembled (Le Nguyen et al. 2019; Ran et al. 2020). Although modern genetics and genomics studies have revolutionized our understanding of the genetic traits in most of the major food crops, such techniques are yet to make a significant contribution to millets. In this chapter, we discuss the gene editing techniques which have been successfully adopted in manipulating the rhizosphere microbiome for millet production.

14.2 Gene Manipulation in Microbes by Genetic Engineering

The effects of limited water availability in major millet growing regions are often confounded with mineral N and P-deficiency and the occurrence of opportunistic soil-borne pathogens. The plants under such poor soil fertility conditions show stunted root growth, lesser photosynthesis ability, low aboveground biomass, impaired panicle, grain size, etc. (Maharajan et al. 2019). In such circumstances, rhizosphere engineering with multifaceted stress-adaptive microorganisms shows agriculturally important traits such as N_2-fixation, mineral (P and Zn) solubilization, ACC deaminase, phytohormones (auxin, gibberellin, cytokinin), siderophore production, and biocontrol activities seems to be an effective approach for ensuring better plant and soil health. The transport regulation of different plant nutrients and minerals are important factors for the proper growth of millet plants. Bacteria belonging to the genera, viz*.*, *Azorhizobium*, *Sinorhizobium*, *Bradyrhizobium,* and *Mesorhizobium* are known as natural N_2-fixers associated with leguminous plants while Frankia and Cyanobacteria are often found attached to the non-leguminous plants (Mitter et al. 2021). The *nif* genes required for N_2-fixation are also present in PGPR strains like *Pseudomonas* sp. Although the symbiotic relationships are exclusive to plant types as evident through studies in several agriculturally important crops like rice, maize, and wheat (Mahmud et al. 2020; Ryu et al. 2020), there are ample scope for the creation of N_2-fixating potential recombinant symbionts that specifically target millet crops. Multiple studies reported the successful transfer of the *nif* gene (encoding the nitrogenase) between *Klebsiella pneumoniae* and *Pseudomonas putida* (Postgate and Kent 1987), *Klebsiella pneumoniae* and *Escherichia coli* (Dixon and Postgate 1971), as well as from *Pseudomonas stutzeri* A1501 to PGPR strain *P. protegens* Pf-5 (Setten et al. 2013) confirmed that N_2-fixation genes can be relocated from symbiotic to non-symbiotic bacteria through mobile genetic elements. A similar study reported the cloning of the entire seven operons (corresponding to the *nif* gene cluster) from *Klebsiella pneumoniae* to *E. coli* by replacing the native σ54 dependent promoters with T7 RNA polymerase-LacI expression system. The mutants expressing the *nif* genes exhibited 100% nitrogenase activity (Wang et al. 2013). Genetically modified plant-growth-promoting microorganisms can also be used as a genetic stock for use as donor or recipient of candidate genes. These microbes find applicability in agriculture and allied sectors such as food processing, environmental management, etc. (Ali et al. 2020).

14.3 Mechanisms of Genome Editing Systems

Novel genome editing tools, also known as Genome Editing with Engineered Nuclease (GEEN) technologies, allow proper cleavage (by the action of restriction endonucleases) and rejoining (ligase) of DNA molecules in specified sites to successfully modify the hereditary material of cells. However, these enzymes are efficient in cutting and pasting DNA molecules in small genomes such as bacterial and viral genomes, rather than in larger and complex genomes. Thus, efforts were made to resolve the issue by creating methods for the editing of complex genomes which are associated with the designing of "artificial enzymes" as oligonucleotides (short nucleotide sequences) that could selectively bind to specific sequences in the structure of the target DNA and have chemical groups capable of cleaving DNA (Knorre and Vlasov 1985). To address the challenge, a targeted approach was adopted with the design of chimeric nucleases which are complex proteins containing one or two structural units, one of which catalyzes the cleavage of DNA and the second is capable of selectively binding to specific nucleotide sequences of the target molecule, providing the nuclease action to the selected site. At present, genome editing techniques such as CRISPR/Cas9, ZFNs, TALENS, etc. are the most-preferred approaches for editing and manipulating any organism's genome.

14.3.1 CRISPR/Cas9 Gene-Editing System

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) is a promising genome editing system that has emerged recently with its intricate ability to selectively bind to specific DNA sites and regulate downstream gene activities (Barrangou and Doudna 2016; Gao 2018). The CRISPR/Cas9 editing system was originally adopted from a prokaryotic RNA-mediated type II used for their defense against invading viruses. A precursor CRISPR RNA (pre-crRNAs) is processed by a transactivating crRNA (tracrRNA) that is complementary to the repeat sequences of the pre-crRNA and triggers maturation by RNase III in the presence of the Cas9 protein (Fig. 14.1a). The Cas9 nuclease cleaves the protospacer-bearing DNA at a position complementary to the mature crRNA. For proper recognition, a proto-spacer adjacent motif (PAM) is required, which is located 20 bp downstream of the target sequence. The tracrRNA triggers RNase III-mediated precrRNA processing which ultimately activates the crRNA-guided DNA cleavage by Cas9. This cleavage of foreign double-stranded DNA is site-specific and produces blunt ends three base pairs upstream of the PAM. However, cleavage in the non-complementary DNA strand occurs at one or multiple sites in a region 3–8 bp upstream of the PAM. The HNH and RuvC domains of Cas9 cleave the complementary and non-complementary strands, respectively (Xu et al. 2017).

CRISPR/Cas9 system is a successful genome editing method that has been implemented in more than 20 crop plants now (Chen et al. 2019; Liu et al. 2021; Ricroch et al. 2017). This system has the ability to significantly improve crop yield as well as to ameliorate several abiotic and biotic stresses in plants. The CRISPR-Cas9 approach can be used to reshape the plant genome and root architecture in millet plants and enhance their survival in drought conditions (Pati et al. 2022). The

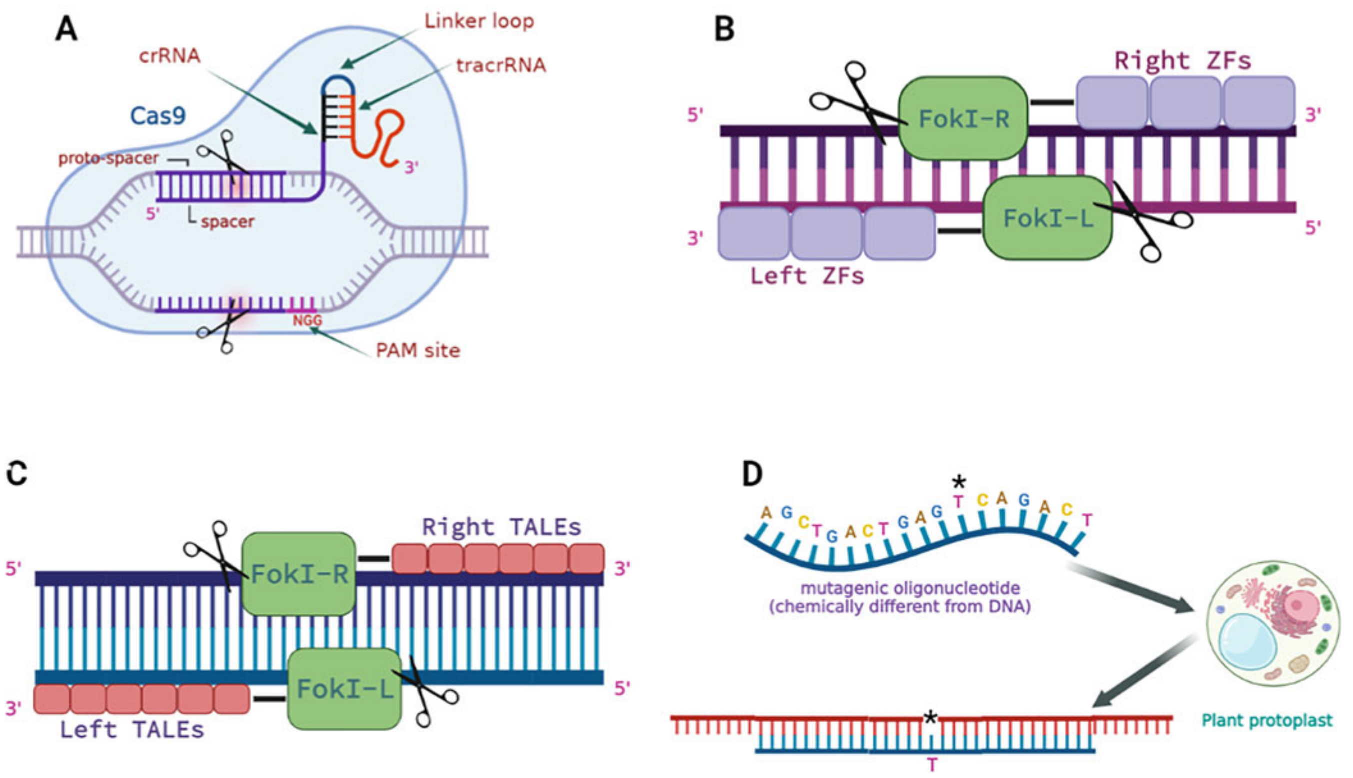

Fig. 14.1 Schematics showing the gene editing tools. (**a**) CRISPR/Cas9 editing system; (**b**) zinc finger nuclease (ZFN), (**c**) TALENS or transcription activator-like effector nucleases; and (**d**) Oligonucleotide-Directed Mutagenesis (ODM) *(This Figure is original in its content and was created with a licensed Biorender software and should not invoke any copyright issues)*

ability of the CRISPR/Cas9 system was evaluated in the protoplasts of several monocot and dicot plants including foxtail millet (Lin et al. 2018). The phytoene desaturase gene of foxtail millet was targeted by the plasmid pCAMBIA1300-35s-Cas9-OsU3-SiPDS, and the deletion of 43 bases as well as insertion of a single base was detected by sequencing. Another study on CRISPR/Cas9-mediated genome editing in foxtail millet reported the generation of double haploid (DH) lines for the *S. italica* Matrilineal (SiMTL) gene. The exonic region of SiMTL was targeted with two different gRNAs and was expressed under the control of the OsU3 promoter, with Cas9 kept under the control of the Uq promoter of maize (Cheng et al. 2021).

Several studies have reported the application of the CRISPR/Cas9 technique for knocking out candidate genes toward enhancing plant tolerance to abiotic stresses such as drought, salinity, cold, etc. (Biswas et al. 2021; Ma and Liang 2021). In addition, the gene editing technique can also be implemented to ameliorate yield loss resulting from several biotic stresses (Ali et al. 2015; Ghosh and Dey 2022; Tashkandi et al. 2018). In recent years, the genome editing (and non-editing) opportunities offered by the CRISPR/Cas9 technique have empowered the development of desirable agronomic traits and revolutionized microbial engineering efforts. Functional validation of candidate genes through inactivation is a tedious and time-consuming process. The reliability and scalability of the CRISPR interference (CRISPRi) system can be leveraged to identify the network of microbial genes involved in a particular trait (Singh and Ramakrishna 2021). In a study, the CRISPRi system was used to investigate the gene networks for bacterial biofilm production by *Pseudomonas fluorescens* SBW25. The system efficiently inactivated target genes encoding the GacA/S two-component system and the cyclic di-GMP signaling messenger regulatory proteins. The mutants exhibited swarming and biofilm phenotypes similar to classical gene inactivation (Noirot-Gros et al. 2019).

The CRISPR/Cas9-mediated manipulation of non-model organisms has also expanded our understanding of the multi-dimensional aspects of plant–microbe interaction. Plant-associated microbiota play important role in plant growth promotion as well as helping plants overcome various abiotic and biotic stress conditions. Nurturing the active microbiota as the plant's second genome holds promises of formulating an efficient strategy for sustainable crop production. The CRISPR-Cas9 system was used to unfold the plant–microbe interaction mechanisms of two plant-growth-promoting rhizobacterial isolates, *Bacillus subtilis* HS3 and *Bacillus mycoides* EC18. This study revealed fengycin and surfactin to be involved in the antimicrobial activity of *B. subtilis* HS3. In the case of *B. mycoides* EC18, ΔasbA (petropectin-deficient) mutant increased the biomass and total chlorophyll content of *Brassica rapa*. Overall, the CRISPR-Cas9 system developed for the two environmental isolates was successful in deciphering the interactions between *Bacillus* and plant (Yi et al. 2018). Therefore, it is opined that this genome editing technique can be used to study various aspects of plant–microbe interactions in millets grown under water-limited conditions to lower the risks of opportunistic soil-borne infection.

14.3.2 Other CRISPR-Based Gene Editing Systems: Prime Editing and Base Editing

The CRISPR/Cas9 system tends to edit multiple bases in the targeted region, which is a major limitation in introducing single-nucleotide polymorphisms (SNPs). Base editing is a simple and precise technique that overcomes such limitations for introducing single-nucleotide variants (SNVs) into DNA or RNA in living cells (Tsanova et al. 2021). It is a newer and alternative genome-editing approach that integrates CRISPR (a catalytically disabled nuclease) with other enzymes (often a nucleobase deaminase or nickase Cas9) for installing point mutations (A>G or C>T) without any double-stranded DNA breaks or undesired editing byproducts (Davies 2019). In fact, the two base editors, i.e., cytidine base editor-CBE (C>T) and adenine base editor-ABE (A>G), are widely used for introducing high-throughput single and multiple nucleotide modifications (Wang et al. 2020). A CBE system (precisely, CRISPR/Cas9-xyr5APOBEC1 based editing system) was used to substitute C with T in the NRT1.1B gene that encodes for a nitrogen transporter protein in rice. The developed lines showed 1.4–11.5% improved nitrogen use efficiency compared to the control (Hu et al. 2015). Similarly, a plant-compatible ABE system was applied to introduce single amino acid substitution in the FT protein for producing *Arabidopsis thaliana* and *Brassica napus* plants with late-flowering traits (Kang et al. 2018).

A typical prime editing system contains two components, a contiguous extension of the edit-containing guide RNA template (known as prime editing guide RNA, pegRNA) and a modified reverse-transcriptase (M-MLV RT) integrated with the C-terminus of Cas9 nickase. The Cas9 targets three bases upstream to the protospacer adjacent motif (PAM) site and cuts the non-complementary strand of the DNA region without any double-strand break. The 3′-OH overhang in the DNA flap binds to the primer binding site located in the pegRNA and serves as a primer for the M-MLV RT that extends the 3′-end as per the edit sequence (Scholefield and Harrison 2021). The prime editor technique was used to engineer herbicide resistance in rice plants by targeting rice Acetolactate synthase genes (OsALS-1 and OsALS-2) (Xu et al. 2020). The study recorded G>T and C>T substitutions with editing efficiencies of 1.1% and 1.1%, respectively. Similarly, a prime editor Sp-PE3 was used to introduce G>A substitutions (17.1% editing efficiency) in the same gene, i.e., *ALS* at the rice callus stage (Hua et al. 2019). The application of prime editors remains limited in plants due to the fact that the base replacements and indels introduced by these editors are not limited to the PAM and can extend to a broad range of positions (+1 to +33) yielding undesired editing byproducts (Xu et al. 2020).

14.3.3 Zinc Finger Nuclease (ZFN)

In addition to a deeper understanding of the dynamics of genome structure and function, modern biotechnology has been gifted with ZFN tools that allow for the controlled alteration of DNA sequences within plant genomes. ZFNs consisting of both DNA binding and nuclease domains have been designed to recognize specific DNA sequences and thereby enable targeted cleavage (Urnov et al. 2010). The ability to cleave specific DNA sequences and promote different mechanisms of DNA repair enables various types of genomic modification methods starting from single-nucleotide mutations to large sequence deletions, rearrangements, and/or even integrations (Curtin et al. 2012). Novel phenotypes can also be created by modifying genetic information in a precise and specific manner. With an expanding human population, the need to engineer more complex traits in crops, such as enhanced yield and stress tolerance will require more sophisticated and precise approaches. Genome editing via designed nucleases represents one of the critical enabling capabilities for future crop improvement. ZFNs consist of zinc finger protein domains that are capable of sequence-specific DNA binding, fused to a nuclease domain for DNA cleavage (Fig. 14.1b). DNA binding results from a tethered array of 4–6 zinc-finger protein domains; each domain recognizes approximately 3 bp of DNA. The ZFNs are most effectively assembled from an archive of two-finger modules (each module recognizes specific 6-bp DNA sequences) whereby domain junctions within each module are optimized for sequence recognition (Urnov et al. 2010). The catalytic domain of the type II restriction enzyme FokI has been used for DNA cleavage (Kim et al. 1996). Since the catalytic domain must dimerize to cleave DNA, two adjacent ZFN pairs orient themselves with appropriate spacing at the target site.

ZFNs have been designed to bind and cleave virtually any stretch of DNA sequence, thus allowing for the creation of DNA double-strand breaks (DSBs) at any specific loci. DSB repair generally uses homologous sequences as templates for synthesizing the damaged strand (Moynahan and Jasin 2010). Such homology-directed repair (HDR) uses sister chromatids, homologous chromosomes, or other related DNA. However, alternative repair pathways involve the nonhomologous end joining (NHEJ) of broken ends (Lieber 2010). ZFN genome editing technique is predicated on the ability to create sequence specific DSBs and thus exploits the cell's DNA repair machinery to produce desired genetic outcomes. In plants generally, the DNA repair mechanism is performed by NHEJ, where broken ends are simply re-ligated, but the repair process itself can be error-prone, resulting in small insertions, deletions, and/or rearrangements (Gorbunova and Levy 1999). ZFNs, on the other hand, are designed to bind and cleave a specific genomic locus that can be introduced and expressed, resulting in NHEJ-mediated sequence alteration.

In plants, especially in *Arabidopsis thaliana*, and a few other crop species, ZFN expression was achieved by delivering coding sequences under the control of a viral promoter by *Agrobacterium*-mediated transformation (Osakabe et al. 2010; Zhang et al. 2010). However, direct DNA transformation and viral delivery have also been successful in plants (Alghuthaymi et al. 2021; Rohatgi et al. 2022; Varanda et al. 2021). A ZFN system was designed to cleave within the ABA-INSENSITIVE-4 (ABI4) gene, which is driven by a heat-shock protein promoter. After heat induction, mutations in ABI4 were observed in somatic cells at frequencies up to 3%. The homozygous mutant T3 progeny displayed the expected loss-of-function phenotype for this gene, i.e., ABA and glucose insensitivity (Osakabe et al. 2010).

A gene encoding a ZFN targeting two soybeans paralogous DICER-LIKE (DCL4b) genes, DCL4a and DCL4b, under the control of an estrogen-inducible promoter was delivered through *Agrobacterium* in the presence of estrogen. Sequence analysis of three recovered T0 plants revealed that one of the plants had an adenine-base insertion at the DCL4a locus and another had two-base thymine and adenine insertion into DCL4b. Both plants appeared to be heterozygous for mutation. The plant with the dcl4a mutation exhibited phenotypic abnormalities, including aborted seeds. The dcl4b plant appeared normal and produced T1 progeny in which the dcl4b mutation segregated 1:2:1 as expected (Curtin et al. 2011). Thus, these studies have shown that the expression of genes encoding ZFNs can generate heritable mutations at targeted endogenous loci. ZFNs were used to make targeted indels in soybean and to introduce specific mutations and transgene insertions that confer herbicide resistance in tobacco and corn, respectively (Shukla et al. 2009; Townsend et al. 2009). ZFNs also successfully targeted the inactivation of endogenous genes in *Arabidopsis* (Osakabe et al. 2010; Zhang et al. 2010), high-frequency modification of tobacco genes (Townsend et al. 2009), and precisely targeted addition of herbicide-tolerance gene, as well as insertional disruption of a target locus in maize (Shukla et al. 2009).

14.3.4 Transcription Activator-Like Effector Nucleases (TALENS)

In recent times, an alternative to ZFNs for genome editing called the transcription activator-like effector nucleases (TALENs) has rapidly emerged for introducing targeted DSBs. TALENs are very similar to ZFNs and comprise a non-specific FokI nuclease domain fused to a customizable DNA-binding domain which is composed of highly conserved repeats derived from transcription activator-like effectors (TALEs). TALEs are proteins expressed by *Xanthomonas* to alter the transcription of genes in host plant cells. They are composed of a central domain responsible for DNA binding, a nuclear localization signal, and a domain that activates the target gene transcription (Sun and Zhao 2013). The DNA-binding domain consists of monomers; each of them binds one nucleotide in the target nucleotide sequence (Fig. 14.1c). The monomers present are tandem repeats of about 34 amino acid residues, two of which are located at positions 12 and 13 and are highly variable and are responsible for the recognition of a specific nucleotide (Boch et al. 2009; Moscou and Bogdanove 2009). Lamb et al. (2013) reported that before the 5′-end of a sequence bound by a TALE monomer, the target DNA molecule always contains the same nucleotide, thymidine that affects the binding efficiency. The last tandem repeat that binds a nucleotide at the 3′-end of the recognition site consists only of 20 amino acid residues and is known as a half-repeat.

TALENs can be applied to help protect plants from the effects of climate change. Genes that play a role in resistance to pests, diseases, or protection from harsh environmental conditions, such as drought and salinity, can be edited to enhance resilience. TALENS have also been used to improve the quality of products derived from crops. TALENs can be very easily and rapidly designed by researchers using a simple "protein-DNA code" that relates modular DNA-binding TALE repeat domains to individual bases in a target-binding site. Several studies have used TALENs to modify endogenous genes in rice (Chen et al. 2014; Miller et al. 2011; Takatsuka et al. 2022). Shan et al. (2018) have reported the use of TALENs in making knockout mutations in Arabidopsis. Another study by Li et al. (2012) introduced resistance to infection by *Xanthomonas* in rice by disrupting the target sites of naturally occurring TALEs that contribute to pathogenicity. They introduced a double-strand break into the wild-type pathogen TAL effector recognition site at the locus of the Os11N3 gene of rice using artificial TALENs against *Xanthomonas*. Soybean lines with low levels of polyunsaturated fats were developed using TALENs by introducing stacked mutations in two fatty acid desaturase 2 genes (FAD2-1A and FAD2-1B) which confer changes in fatty acid desaturase 3A (FAD3A) which increased the oleic acid levels of over 80% and decreased linoleic acid levels under 4% (Demorest et al. 2016). TALENs was also used to introduce mutations in all six sets of *mlo* genes that essentially resulted in plants with complete resistance to the wheat powdery mildew pathogen (Wang et al. 2014). Researchers have further used TALENS to further enhance the biofuel yield from sugarcanes. By targeting about 107 out of 109 copies of sugarcane's lignin biosynthetic gene caffeic acid O-methyltransferase (COMT) using TALENs, sugarcanes were mutated with 19.7% lignin reduction and up to 43.8% improved saccharification efficiency without affecting biomass production and maintaining good agronomic performance (Kannan et al. 2018).

14.3.5 Oligonucleotide-Directed Mutagenesis (ODM)

ODM, a recent and important tool, uses around 20–100 base long oligonucleotides. The sequence used is identical to the target sequence in the genome except that it contains a single base pair change (intended mutation to be inserted in the genome) toward achieving site-directed editing of the gene/sequence of interest (Abdurakhmonov 2016; Sauer et al. 2016b). Synthetic oligonucleotides with homology to a specific region of the target gene are transiently exposed to the plant cells followed by their binding to the targets and activating the cell's natural repair mechanisms. With the binding, the single mismatch is recognized in the template and then copies that mismatch or mutation into the target sequence through the repair process (Sauer et al. 2016a). The desired targeted single nucleotide or base editing in the plant genome is thus produced (Fig. 14.1d). Thus, the process confers a novel function or trait while the plant cell degrades the repair template oligonucleotide.

In crop plants, the ODM technology was successfully applied in tobacco (Beetham et al. 1999; Ruiter et al. 2004), maize (Zhu et al. 1999), *Arabidopsis* (Kochevenko and Willmitzer 2003), rice (Okuzaki and Toriyama 2004), oil seed rape (Gocal et al. 2015). Recently, the combination of ODN and CRISPR/Cas9 was used to develop an herbicide tolerance trait in *Linum usitatissimum* by precisely editing the EPSPS genes (Sauer et al. 2016a). In case of microbes, the system was used to edit *rpsL* and *rpoB* genes in *E. coli* and *rpsL* gene in *Pseudomonas syringae* (Swingle et al. 2010). In the cyanobacterium *Synechocystis* sp. PCC 6803, the ODM was used to specifically delete the *psbB* (the gene encoding for the chlorophyll alpha-binding protein CP47 in Photosystem II) to enable the reintroduction of modified psbB (Eaton-Rye and Vermaas 1991).

14.4 Conclusion

Millets are cultivated in semi-arid and arid environments where abiotic stresses including drought, salinity, extreme temperatures, and associated biotic stresses affect the crop's productivity. In the face of climate change, the rethinking of millets as alternative climate-smart crops holds enormous potential in ensuring the livelihood and nutritional security of the marginalized population living in extreme environments. Toward this, enhancing its productivity requires concerted efforts encompassing conventional and modern crop improvement programs as well as non-conventional techniques such as multi-omics approaches, rhizosphere engineering, etc. However, millet has long been neglected as an "orphan crop" and only recently garnered scientific attention due to the fact that its adaptations to challenging environments are better compared to other cereals. In this chapter, we discussed various emerging genome-editing techniques such as CRISPR/Cas9 system, ZFN, TALENS, base editing, prime editing, etc. which offer tremendous opportunities for increasing nutritional status, disease resistance, and crop yield in millets. Overall, these techniques can increase the crop's fitness in hostile environments by modulating several features of the plant such as germination rate, above- and belowground biomass, relative water content, photosynthesis ability, proline accumulation, and lipid peroxidation. Further studies utilizing a combination of genome-editing techniques and rhizosphere microbiome engineering will leverage the positive plant–microbe interactions toward improving millets productivity under environmental stress conditions.

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Chapter 15
Effect of Nano-Formulated Agrochemicals on Rhizospheric Communities in Millets

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Abstract Nanotechnology is an emerging technology that combines materials science and engineering fundamentals, altogether synthesizing materials at the nanoscale to solve various current problems. Agrochemicals are often used in modern intensive agriculture to protect crops from biotic stress, provide vital nutrients, and boost millet growth and yield. Although they are beneficial in the short term, their long-term and persistent applications damage soil fertility and negatively affect the rhizospheric microbiome. Nanotechnology in the form of nano-based agro-formulations is an innovative, environmentally acceptable, and practical solution for substitute synthetic fertilizers. Nanotechnological formulations and nanoparticles in the form of nanopesticides, nanoherbicides, nanogels, nanofertilizer, and nanofungicides are reported to have efficacy in continuous release of nutrients regulated distribution to plant nutrients very effectively. These

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_15

nanoformulations helps in fighting phytopathological diseases, promotes plant, and microbiome productivity. In addition, they are effective in alleviating biotic and abiotic stress in crop plants. However, despite their extraordinary effectiveness, they also have several drawbacks, such as a tedious manufacturing process, shaky delivery, and dosage-sensitive effectiveness. Learning and acknowledging the influence of nano-based agrochemicals on highly nutritious crops such as millets is important for further commercialization and wide utilization in agriculture. Hence, this chapter focuses on the usage of nanoformulations on millet crops, their effects on soil rhizospheric communities, and soil fertility.

Keywords Nanoformulations · Rhizosphere · Millets · Soil biota · Millet-grown soils

15.1 Introduction

The sustainable increase in food production and eradication of hunger and poverty are the main goals of agricultural sustainability and food safety in world countries. The inorganic synthetic fertilizers provide macronutrients and micronutrients for plant growth, but many agrochemical applications in agricultural fields have shown several disadvantages in the long term. Nutrient use efficiency (NUE) of soil reduces with continuous crop growth and application of various chemicals in the soil, leading to imbalanced nutrients and low nutrient uptake by plants. More than 90% of the applied chemicals are lost in the environment, while others are unable to reach the target locations required for efficient use (Nizzetto et al. 2016; Chen et al. 2022). For many years, fertilizers and insecticides have been crucial in ensuring that food production demands are met (Guo et al. 2018). However, excessive usage of these chemicals also impacts sustainable agriculture development. Nanotechnology has revolutionized its application in agriculture, medicine, and pharmaceuticals (Pudake et al. 2019; Raliya et al. 2017; Singh et al. 2021). Nano-based agrochemical formulations are introduced in the agroindustry to reduce the excessive utilization of chemical fertilizers and improve soil nutrition with minor alternations to soil properties and microbial communities (Arora et al. 2022; Rana et al. 2019). The engineered nanoparticles such as nanofertilizers, nanopesticides, nanoinsecticides, nanorodenticides, and other nanoformulations are utilized in agriculture for their broader scope, and distinct advantages such as controlled release of loaded substances, loss of nutrients, bioavailability, solubility, and desirable synthesis methods and are found as alternatives for conventional chemical fertilizers. A very limited number of highly tiny particles are used in the nanoformulation of agricultural chemicals, which act on the plant rhizosphere as active ingredients in fertilizers or other agricultural chemicals (Ul Haq and Ijaz 2019; Pudake et al. 2019). They have been extensively tested for their positive and negative impacts on soil health for the past two decades since they are considered as future enhancers of food production (Kumari and Singh 2020; An et al. 2022).

Millets are the most popular crops grown in arid and semi-arid regions of Asia and southern Europe. The agrochemicals such as fertilizers and pesticides have been reported to influence the yield, soil fertility, microbial communities, and enzymatic activities in agriculture. The rhizosphere communities of millets are less focused than rice, wheat, and maize, and these microbial populations participate in all biogeochemical cycles and enhance the nutrient availability to millets (Xu et al. 2018). The first part of this chapter focuses on the influence of agrochemicals and nanoformulations on soil microbiota (rhizosphere communities, soil properties, soil fauna). The latter focuses on existing nano-based agro-formulations and possible impacts on millet rhizosphere communities.

15.2 Current Trends in Nanoagrochemical Formulations

In recent years, nanotechnology interventions in agriculture have extended substantially, including nanoscale carriers, nanosensors, clay nanoparticles, nanopolymeric composites, nanoemulsions, and nanofood packaging materials. These novel technologies are also utilized to combat plant diseases, identify pathogens, boost plant resistance, and reduce post-harvest losses. Nano-based agrochemical formulations provide a wide range of benefits (more effectiveness and durability, better distribution and permeability, ability to disintegrate in the soil and environment, lack of toxicity, and photo generative nature), as well as a reduced amount of agro-inputs with convenient pesticide properties to protect crops against insect pests and diseases effectively (Fig. 15.1).

Nano carriers are utilized for slow and sustained delivery of fertilizers, fungicides, herbicides, pesticides, growth regulators, and other target active compounds (Balah and Pudake 2019; Vega-Vásquez et al. 2020). Nanopolymeric compounds from plants, animals, and microbe-based by-products create opportunities for exploring innovative and value-added nanomaterials and products. For example, clay-based nanomaterials can serve as versatile carriers of nutrients and pesticides with the advantage of extended release and could reduce 70–80% of pesticide use (Merino et al. 2020).

15.2.1 Nano Carriers for Agrochemical Formulations

Potential applications of nanotechnology for integrated pest management include the controlled administration of artificial intelligence (AIs) with increased activity at lessening drug concentrations. Nanocarriers significantly improve the chemical stability of agrochemicals while protecting them from harsh environmental factors (such as radiation and high temperatures) (Kumar et al. 2019). Nano encapsulation, also known as an encapsulation of active ingredients with nanoparticles, is a technique for the sustained but effective release of chemicals to a specific host

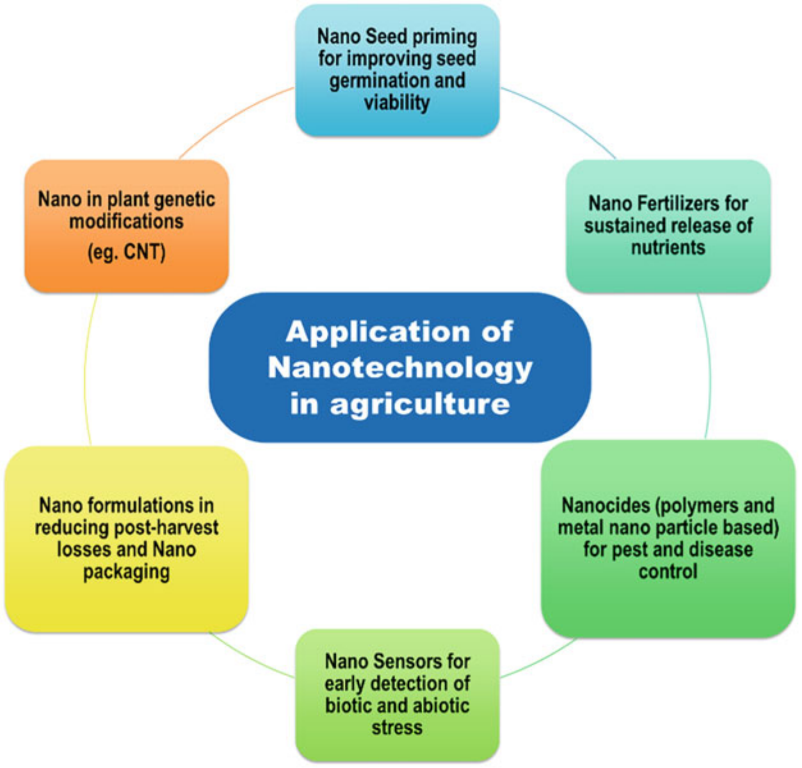

Fig. 15.1 Summary of application of nanotechnology in agriculture

plant. The biphasic dispersion of two or more immiscible liquids (water in oil or oil in water) to form droplets stabilized using any amphiphilic surfactant to create an emulsion droplet less than 100 nm is referred to as nanoemulsions. Nanoemulsions are transparent or translucent oil–water emulsions used in agriculture to promote physio-chemical stability, improve the properties of pesticides (bioavailability), and sustainable release of chemicals (Bhattacharyya et al. 2016).

The chemical or physical confinement of gels in nanosize, which serves as nanocarriers for delivery of agrochemicals or drugs, is referred to as nanogels. These nanogels are swellable cross-linked polymer networks with a high ability to hold water without dissolving it into an aqueous medium. These polymers are either natural or synthetic and combination of both with properties such as amphiphilicity, softness, porosity, and degradability according to the variability in composition. The materials (i.e., nanoparticles) loaded into the nanogels protect the loaded material from degradation and elimination, and additionally, they actively participate in the delivery at the target site. The stimuli-responsive behaviour of nanogels with stability makes them suitable for the carrier of desired nanoparticles. Polymeric nanogels are a new hybrid to overcome poor colloidal stability, elimination, and

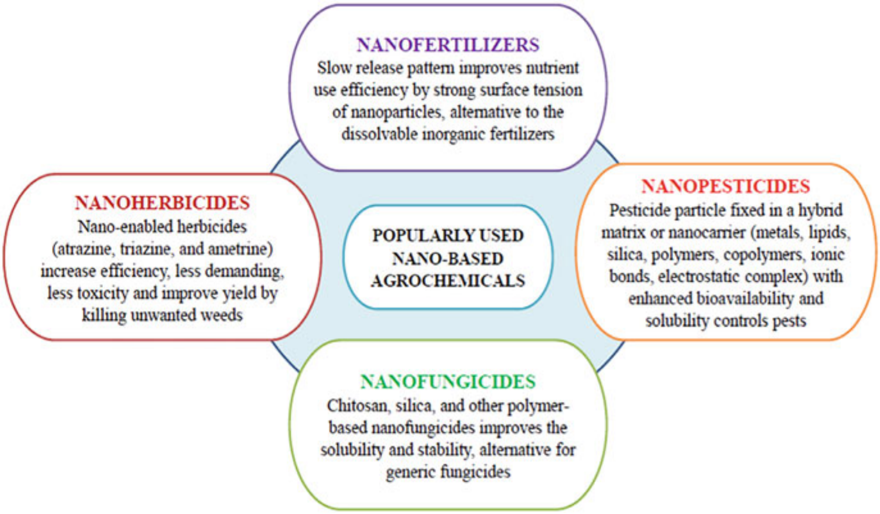

Fig. 15.2 Commercially used nano-based agrochemicals

low aqueous solubility. Aide variety of molecules can be incorporated to increase their utility (Soni et al. 2016). For instance, the core-shell type lignin nanogels formed using lignin methacrylate, emulsified and graft copolymerized along with acrylic acid, were stimuli-responsive (pH-responsive swelling). Therefore, lignin bio-based nanogels could be used as a nanocarrier for the controlled release of pesticides or fungicides (Yiamsawas et al. 2021). The different types of nanoagrochemical formulations are used in the manufacturing of nanofertilizer, nanopesticides, nanoherbicides, nanofungicides, etc. (Fig. 15.2).

15.2.2 Soil Rhizosphere Communities: Role, Interactions, and Importance in Crop Production

Nanotechnology provides a novel agrochemical tool that helps in high crop productivity, hazardous chemical reduction, plant-pathogen management, and crop protection through plant adaptation (Shang et al. 2019). Nanoparticles may have an impact on the soil microorganisms in the environment via (1) physical direct contact, (2) alteration of available nutrients and toxins, and (3) indirect effects due to their interaction with soil organic materials, therefore, leading to alterations in cell surface morphology and growth behaviour, disruption of biofilm, and core microorganisms of the millet rhizosphere including many organisms (Xie et al. 2017). Microbial communities in the rhizosphere are well known to be closely linked to plant roots (El-Ghamry et al. 2018). In addition, rhizosphere bacteria significantly impact nutrient uptake by increasing or lowering nutrient availability. Microbial communities in the rhizosphere are a subset of the soil microbial community but are frequently distinct from those in the bulk soil (Dotaniya and Meena 2015).

Furthermore, rhizosphere microorganisms are recognized to have a crucial role in plant health, and plant health positively influences rhizosphere microbes in millets (Babu et al. 2019; Tyagi et al. 2017). It has been found that the rhizosphere microbial communities associated with healthy and diseased plants differ, although rhizospheres associated with infected and dead plants have comparable microbial compositions (Liu et al. 2020). In millets, the rhizosphere is the most active microdomain for plant–microbe interactions, and it contains microorganisms vital for plant growth and health maintenance. Rhizosphere bacteria, for example, can provide nutrients to plants by speeding up nutrient cycling or nitrogen fixation and generating hormones or antibiotics that improve plant drought, salt, and disease tolerance.

The microbiota can either hurt or help the host plant. Beneficial microbes in the soil rhizosphere can aid plant adaptation to be natural and artificial environmental challenges and climate change (Zhou et al. 2009). Microorganisms in the rhizosphere have positive and negative interactions with plants and roots because soil habitats vary due to various circumstances, defining the microbiome for millets becomes critical (Elhady et al. 2018). Due to their quick sensitivity to environmental changes, microorganisms serve as early warning biological indices of soil and root health. Microbial diversity in the rhizosphere fluctuates as a result of such variables, as well as farming patterns and climate (Pudake et al. 2021; Rosen and Allan 2007). The microbial diversity in the rhizosphere of millet is unknown currently; thus, the millet microbiome is essential for future nutritional and food security.

Furthermore, almost all the minor millets can withstand harsh conditions especially finger millet plant indicates its potential involvement in reducing pathogenic strains and offering medicinal and nutritional benefits to the linked plants. In environmentally problematic settings, such as high salinity and locations with severe water shortages, it would be worthwhile to include beneficial species of this genus as bioinoculants to boost stress tolerance and produce finger millet (Kour et al. 2020). Root-driven soil aggregation and microbiological diversity are significant guides to long-term studies on climatic stress and early warning cues of aphids and other pathogen attacks. To measure the diversity of the ratio between the mass of root-adhering soil and the mass of root tissue, researchers looked at the relationships between soil adherence to roots, aggregation, microbiological activity, and root exudation (Amellal et al. 1998). Furthermore, the nutritional quality of the sediment and the vegetation mix of the patches may influence plant species' ability to shape their rhizosphere microbial community (Zhang et al. 2020). Microbes in the rhizosphere are essential for plant health and ecosystem functioning in terrestrial ecosystems. Yet, our knowledge of microbial co-occurrences and assembly processes in the millet rhizosphere is limited.

15.3 Influence of Agrochemicals on Soil Microbiota

The unsustainable agricultural practices in many developing countries resulted in direct and indirect ecosystem contamination and pollution (Dinesh et al. 2018). The new technologies have further influenced the adverse impact of unsustainable practices, leading to land, water, and atmospheric degradation. The various agrochemicals such as herbicides, pesticides, insecticides, molluscicides, nematicides, rodenticides, fungicides, and many other chemical or inorganic compounds have impacted all the living organisms especially soil microbiota (Meena et al. 2020; Dinesh et al. 2022). A large quantity of agrochemicals used in agriculture has affected soil microbiota directly, which are considered as biological indicators for soil fertility. The nutrient status of soil, microbial carbon biomass, and non-target biota are disturbed by the impact of various pesticides (Dinesh et al. 2018). They also affect the soil enzymes such as urease, dehydrogenase, nitrate reductase, oxidoreductase, and hydrolyses (Wang et al. 2006; Hussain et al. 2009).

15.3.1 Effects of Fungicides on Soil Microbiota

The harmful effects on microbial growth, functions, metabolism, and survival alter soil fertility (Venkatesh et al. 2022). Some of the negative impacts of fungicides on microbiota are (1) mancozeb and chlorothalonil could reduce the nitrification and denitrification process; (2) carbendazim is highly toxic to a potential biocontrol, *Trichoderma harzianum* (anti-fungal against Fusarium, Rhizoctonia, and Pythium); (3) copper-based fungicides affect the N-fixing bacterial population; (4) dimethomorph inhibits ammonification and nitrification in sandy soil; (5) fludioxonil is toxic to algae; (6) captan decreases nitrogenase activity and reduces viable counts of nitrifying and denitrifying bacteria, *Rhizobium cicero*; (7). Dinocap affects the ammonifying bacteria (Kyei-Boahen et al. 2001; Virág et al. 2007; Cycoń et al. 2010). In addition, many non-target fungicides cause inhibitory effects by reducing the beneficial microbiota in soil.

15.3.2 Impact of Herbicides on Soil Microbiota

Based upon the type of molecule present in the applied herbicide, the microbial population decreases within 7–30 days. Microbes' biosynthetic mechanisms or physiology are affected indirectly, affecting soil enzymes, protein biosynthesis, and plant growth regulators (ethylene, gibberellins, and indole acetic acid) (Kremer and Means 2009; Meena et al. 2020). The effects of herbicides on soil microbiota depend on concentration, degradability, adsorption, desorption, persistence, bioactivity, and toxic dosage level. Some microbes which can withstand a single application (Butachlor) are more sensitive to combination (Butachlor with cadmium) (Hussain et al. 2009). The inorganic fertilizers combined with heavy metals suppress soil microbial activities, and secondary metabolites produced by the transformation of herbicides are more severe than direct effects (Sinduja et al. 2022a, b). For instance, metabolites produced by 2,4-D affected a group of gram-negative bacteria, *Burkholderia cepacia.* Even after the N fertilization, prosulfuron in soil affects the nitrification and denitrification process. Atrazine and simazine (10 mg/L) were toxic for the growth and biological activities of Xanthobacter autotrophic. Acetochlor and its derivatives negatively impact bacterial communities such as *florescens, Mycobacterium phlei, and Bacillus subtilis*. The enzymatic activities such as dehydrogenase and invertase are affected by atrazine and metolachlor (Sáez et al. 2006).

15.3.3 Effects of Insecticides on Soil Microbiota

The growth and population of bacterial species such as Azotobacter, Pseudomonas, Bacillus, Penicillium, and many others are affected by long-term insecticides such as fenthion parathion and malathion. The soil polluted with 15 organochlorine pesticides exerted severe ecological risk, among which 76% of ecotoxicity was exhibited by endosulfan. The bioindicators, including 61,005 bacterial species and 33,397 fungal species were not found in organochlorine pesticide-affected soil, whereas they were present in uncontaminated soil (Egbe et al. 2021). In addition, the short-term application of thiamethoxam reduced the microbial diversity, bacterial abundance, and structure during short-term application (Actinobacteria) (Wu et al. 2021). Like other pesticides, insecticides inhibit the soil enzyme activities (urease, phosphatase, and nitrogenase), microbial biomass, and beneficial species population reduction.

15.4 Application of Nano-Formulated Agrochemicals for Various Crops

15.4.1 Enhancing Crop Quality and Growth Using Nano-Formulated Fertilizers

Nanofertilizers are a noteworthy recent development in decreasing the loss of applied fertilizers to the environment. By combining plant nutrients with nanomaterials, coating nutrient molecules with a thin layer of nanomaterials, and creating nano-sized emulsions, nanofertilizers are created that intelligently increase soil fertility and bioavailability (Sidorowicz et al. 2019). Additionally, studies of nutrients on slow/controlled release or control loss of nanofertilizers conducted in soil and water have confirmed that the long-term availability of all doped nutrients to the plant over the whole crop period of cultivation is essential for promoting germination, growth, flowering, and fruiting (Lateef et al. 2016). Different nanomaterials have been used to encapsulate macronutrients like carbon (C), nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), sulphur (S), and magnesium (Mg) to increase crop fertilizer uptake and reduce fertilizer outflow (Singh et al. 2021). For instance, urea-hydroxyapatite (HA) nanoformulations have the potential to increase release time and decrease nitrogen fertilizer consumption (Kottegoda et al. 2017). The application of nanodiammonium phosphate (n-DAP) in low input range has increased the yield of monocot and dicot plants such as wheat and tomato, respectively, with increase in the bioavailability of optimum phosphorous to the plants (Singh et al. 2021b).

Recent research has demonstrated that NPs, including CNTs, silicon dioxide (SiO_2) NPs, zinc oxide (ZnO) NPs, titanium dioxide (TiO_2) NPs, and even gold (Au) NPs, have favourable impacts on seed germination in agricultural plants, including tomato, wheat, rice, pearl millet, soybean, barley, and maize (Singh et al. 2016). Compared to untreated plants, the germination rate of pearl millet was dramatically improved by Au Nano formulations (Parveen et al. 2016). Numerous studies have demonstrated the benefits of using nanoparticles on germination, plant growth, and development (Table 15.1).

Synthesis proteins, carbohydrates, and hormones (such as auxins) are all regulated by micronutrients, which shield plants from infections and pests. Fe/SiO_2 nanoparticles have a great potential to enhance seed germination in barley and maize (Najafi Disfani et al. 2017). In addition to germination, nanomaterials such as ZnO, TiO_2, MWCNTs, FeO, $CuZnFe_4O_4$-oxide, and hydroxyl fullerenes are said to promote crop growth and development with quality improvement in a variety of crop species, including mustard, onion, spinach, tomato, potato, and wheat (Shalaby et al. 2016). For example, Zn nanoparticles were used to boost grain output in pearl millet (*Pennisetum americanum* L.) by roughly 38% (Moghaddasi et al. 2017). In several horticultural crop plants, including garden pea, cucumber, spinach, tomato, potato, eggplants, chilli, coriander, and onion, the usefulness of zinc-based nanofertilizers is documented (Ahmed et al. 2018b; Pullagurala et al. 2018) (Table 15.1).

15.4.2 Nano-Formulated Chemicals for Crop Development and Protection

Insecticide, herbicide, fungicide, and bactericide nanoformulation or encapsulation using nanomaterials holds immense potential for reducing agrochemical doses, boosting crop productivity, and fostering sustainable development (Liu and Lal 2015). Various nano-formulations of agricultural chemicals are summarized in Table 15.2. Additionally, using fewer pesticides lowers the cost of growing crops. In these situations, the application of NPs is reported as an effective alternative to

Table 15.1 Application of nano-formulated fertilizers in crop growth and development for various crops

Nanomaterials	Crop species	Mode of application	Concentrations used	Responses	References
Field crops					
Multiwalled carbon nanotubes (MWCNTs)	*Allium sativum*, *Arachis hypogaea*, *Hordeum vulgare*, *Glycine max*, *Triticum aestivum*, *Zea mays*	Seed priming	100 g/mL	Enhanced germination, growth of seedlings, and water absorption potential of seeds	Lahiani et al. (2013) and Srivastava and Rao (2014)
ZnO	*Triticum aestivum*, *Cyamopsis tetragonoloba*, *Nicotiana tabacum*	Foliar spray	20 mg/L	Improved plant growth, biomass accumulation, and nutrient content	Du et al. (2019), Raliya and Tarafdar (2013), Tirani et al. (2019) and Wu et al. (2020)
	Sorghum bicolor	Foliar spray	–	Boosted sorghum production and grain nutritional quality	Dimkpa et al. (2017)
	Gossypium sp.	Soil application	–	Increased the biomass of the roots and above-ground tissues of the seedlings under salinity stress	Hussein and Abou-Baker (2018)
	Vigna radiate	Foliar spray	–		Dhoke et al. (2013)
FeS_2	*Brassica juncea*, *Cicer arietinum*, *Daucus carota*, *Spinacia oleracea*, *Sesamum indicum*	Seed priming	80–100 g/mL	Increased germination and crop yield	Das et al. (2016) and Srivastava et al. (2014)
	Arachis hypogaea, *Zea mays*	As fertilizers	15 mg/kg	Enhanced plants growth and biomass accumulation	Najafi Disfani et al. (2017)
TiO_2 NPs	*S. oleracea L*	Seeds soaked	–	Enhance seedling growth, biomass, and chlorophyll content	Singh and Kumar (2020)
	C. arietinum L	Spray on leaves	–	Reduce membrane damage during cold stress, increase the mineral uptake in plant	Faraji and Sepehri (2020)
	Oryza sativa	Foliar application	20 and 30 mg/L	Improved growth by stimulating antioxidant potential and inhibiting Cd translocation	Rizwan et al. (2019)

	Triticum aestivum L. c.v “Pishtaz”	Spraying by back-pack sprayer	0.01, 0.02, and 0.03%	Starch content increased, enhanced growth, yield, and gluten	Jaberzadeh et al. (2013)
	Triticum aestivum	Amended soil	500, 1000, and 2000 mg/kg	Improved metabolism, total chlorophyll, and carotenoids in the seedlings	Faraji and Sepehri (2020)
CuNPs	*Zea mays*	Plants priming	3.333, 4.444, and 5.556 mg/L	Higher leaf water content and plant biomass, increased metabolism contents, increased yield	Ali et al. (2020)
Ag NPs	*Vigna unguiculata*	Foliar spray	50–100 g/mL	Enhanced growth and biomass by stimulating root nodulation and soil bacterial diversity	Vanti et al. (2019)
	Vigna sinensis	Foliar spray	50 mg/L		
	Triticum aestivum	Mixed with pot soils	50 mg/L and 75 mg/L	Improved growth and tolerance to heat stress	Iqbal et al. (2019)
	Trigonella foenum-graecum	Petri dish exposure	0, 10, 20, 30, and 40 μg/mL	Improved fresh and dry mass, and increased germination rate	Hojjat and Kamyab (2017)
Nano chitosan	*Z. mays L*	Foliar spray	–	Promote seedling growth, overall plant height, and biomass	Deshpande et al. (2017) and Khati et al. (2017)
	T. durum L	Foliar spray	–	Enhance zinc content accumulation	Maluin and Hussein (2020)
	Triticum aestivum	–	–	Improved biochemical attributes	Abdel-Aziz et al. (2018)
	Hordeum vulgare L.	Foliar application	0, 30, 60, and 90 ppm	Relative water content, grain weight, protein, proline, CAT, and SOD levels were all raised	Behboudi et al. (2018)
MgONPs	*Nicotiana tabacum*	–	50, 150, and 250 mg/L	Improved SOD and POD activities in grown in matrix media	Cai et al. (2018)

(continued)

Table 15.1 (continued)

Nanomaterials	Crop species	Mode of application	Concentrations used	Responses	References
CeO_2 NPs	*Z. mays L*	Foliar spray	50 mg-Ce/L hydroponic	Promote photosynthesis and gas exchange, enhance percent germination and vigour index	Prakash and Deswal (2020)
	S. oleracea L				
Horticultural crops					
MWCNT	*Dodonaea viscosa L.*	Nano priming	10, 30, 50, 100, and 200 mg/L	Improved seed germination percentage	Yousefi et al. (2017)
ZnO	*Cucumis sativus*	–	–	Root tip deformation and growth inhibition	Zhao et al. (2020) and Liu et al. (2022)
	L. esculentum Mill.	–	–	Reduces fungal disease	Khan and Siddiqui (2018)
	Capsicum frutescens	–	–	Improves germination	Tantawy et al. (2015)
	Coriandrum sativum	–	–	Improves pigment contents	Pullagurala et al. (2018)
	Allium cepa	–	–	Inhibits root growth	Ahmed et al. (2018a)
	Coffea arabica	Foliar spray	10 mg/L	Enhanced growth and biomass accumulation	Rossi et al. (2019)
	Mangifera indica L.	Foliar spray	50, 100, 150 mg/L	Improved plant growth, nutrients uptake, and carbon assimilation	Elsheery et al. (2020)
FeS_2	*Cucumis sativus*, *Pisum sativum*	–	–	Increases biomass production and antioxidant enzyme activity	Giorgetti et al. (2019) and Moghaddasi et al. (2017)
SiO_2 NPs	*L. esculentum Mill.*	–	8 g/L (12 nm)	Significantly enhanced seed germination percent	Siddiqui et al. (2014)
	Solanum lycopersicum L.	Exposure in vitro	0.5, 1, 2, and 3 mM		Almutairi (2016)
	Fragaria ananassa	Exposure to nutrient sol	50 and 100 mg/L	Improved vegetative growth, increased metabolism rate	Avestan et al. (2019)
	Musa acuminate "Grand Nain"	In vitro	200, 400, and 600 mg/L	Enhanced shoot growth and improved photosynthesis	Mahmoud et al. (2020)

Nanochitosan	*Coffea canephora*	Foliar spray	–	Increase chlorophyll and photo-synthetic rate	Pereira et al. (2017)
	Capsicum annuum L	Seed treatment	–	Enhance the root biomass and fresh leaf biomass	Mirbolook et al. (2021)
	Psidium guajava	–	–	Reduces respiratory rate and anti-oxidant process and enhances firmness	Silva et al. (2018)
	Vitis vinifera	–	–	Enhances the activities of the enzymes such as chitinase, lyase, and glucanase	Shen and Yang (2017)
	Mangifera indica L.	–	–	Delays senescence process, loss of water, and firmness of fruits	Silva et al. (2017)

Table 15.2 Application of nano-formulated agrochemicals for crop protection on various crops

Nanomaterials	Disease	Host plant	Effects	References
AgNPs	Early blight/ *Alternaria solani*	*Solanum lycopersicum*	Reduction in fungal growth	Abdel-Hafez et al. (2016)
	Wilt/*Fusarium oxysporum*	*Solanum lycopersicum*	Antifungal effects	Madbouly et al. (2017)
	Different fungal diseases	*S. lycopersicum*	Inhibition in fungal growth reduces disease symptoms	Elshahawy et al. (2018)
	Bacterial spot/ *Xanthomonas perforans*	*Solanum lycopersicum*	Significantly decreased the pathogen activity	Ocsoy et al. (2013)
	Nematode/ *Meloidogyne* spp.	*Solanum lycopersicum*	99% of the nematodes died within 6 days	Cromwell et al. (2014)
	The spot blotch/ *Bipolaris sorokiniana*	*Triticum aestivum*	Biosynthesized AgNPs exhibited antifungal activity	Mishra et al. (2014)
	Phytophthora blight/ *Phytophthora capsici*	*Capsicum annuum*	Suppressed Phytophthora infection on plants and improved plant survival	Ali et al. (2020)
	Spot blotch/ *Bipolaris sorokiniana*	*Triticum aestivum*	Enhance lignification of vascular bundles	Ghorbani et al. (2021)
	Bacterial spot/ *X. perforans*	*Solanum lycopersicum*	Significantly decrease the activity of *X. perforans*	Tan et al. (2020)
	Spot blotch/ *B. sorokiniana*	*Triticum aestivum*	Inhibit conidial germination	Ali et al. (2020)
	Bacterial blight/ *Xanthomonas campestris pv. Campestris*	*Brassicaceae*	Enhance antioxidant enzyme activity	Noshad et al. (2020)
Chitosan NP	*Colletotrichum gloeosporioides*	*Capsicum annuum*	Inhibition growth of mycelia	Dawadi et al. (2021)
	Leaf spot/ *A. alternata*	*Oryza sativa*	Inhibit spore germination	Pereira et al. (2017)
	Rhizopus sp., *Colletotrichum capsici, Colletotrichum gloeosporioides, and Aspergillus niger*	*Capsicum annuum*	Delayed mycelia growth	Chookhongkha et al. (2012)
	Fusarium wilt/ *Fusarium andiyazi*	*Nicotiana tabacum*	Inhibition in mycelia growth	Chun and Chandrasekaran (2019)

(continued)

Table 15.2 (continued)

Nanomaterials	Disease	Host plant	Effects	References
	Downy mildew/*Sclerospora graminicola*	*Pennisetum glaucum*	Induced resistance to disease	Siddaiah et al. (2018)
	Early blight/*Rhizoctonia solani*	*S. lycopersicum*	Reduced pathogenic infection	Nadendla et al. (2018)
Cu-based NPs	Root fungal disease/*Fusarium oxysporum* f. sp. niveum	*Citrullus lanatus*	58% Decrease in disease progression	Borgatta et al. (2018)
	Clavibacter michiganensis	*Solanum lycopersicum*	Stimulates the levels of the enzymatic and non-enzymatic metabolites essential for the defence of tomato plants	Cumplido-Nájera et al. (2019)
	S. littoralis	*Arachis hypogea*	Enhanced the expression of exogenous genes encoding Bt toxin in cotton plant tissues	Ayoub et al. (2018)
	Bacterial blight/*Xanthomonas axonopodis pv. Punicae*	*Punica granatum*	Inhabited bacterial growth	Mondal and Mani (2012)
	Phytophthora infestans	*Solanum lycopersicum*	Active at low concentration of antifungal agent	Giannousi et al. (2013)
	Leaf blight/*Escherichia coli, Bacillus subtilis* and *F. oxysporum*	*Zea mays*	Decrease the number of conidia	Khan et al. (2020)
	Root fungal disease/*F. oxysporum*	*Citrullus lanatus*	Inhibit the fungus growth	
	Bacterial wilt/*Ralstonia solanacearum*	*Nicotiana tabacum*	Antibacterial potentials	
CeO_2	Wilt/*Fusarium oxysporum*	*Solanum lycopersicum*	Disease suppression by 35–57%	Adisa et al. (2018)
	Panama disease/*F. oxysporum*	*Musa paradisiaca Linn*	Enhance antioxidant enzyme activity	De Lamo and Takken (2020)
Ti-based NPs	Leaf spot/*X. perforans*	*Solanum lycopersicum*	High photocatalytic activity and antibacterial potential to reduce bacterial spot severity	Paret et al. (2013)

(continued)

Table 15.2 (continued)

Nanomaterials	Disease	Host plant	Effects	References
Mg-based NPs	*Ralstonia solanacearum*	*Solanum lycopersicum*	The mechanism included the upregulation of salicylic acid-inducible PR1, jasmonic-acid-inducible LoxA, ethylene inducible Osm, and systemic-resistance-related GluA to defense response	Imada et al. (2016)
	Vascular wilt disease/*Ralstonia solanacearum*	*Capsicum annuum*	Inhibit bacterial activity	Tailor et al. (2020)
	Bacterial leaf spot/*Xanthomonas alfalfae*	*Medicago sativa*	Significantly decrease the activity of *X. alfalfae*	Salem and Fouda (2021)

directly decrease pest and disease infection and activity, resulting in higher crop development and output (Servin et al. 2015). Polymeric NPs (like chitosan and solid lipids), inorganic nonmetallic NPs (like silica NPs and nanoclays), and metallic NPs (like Cu NPs and ZnO NPs) are examples of agrochemical nanocarriers (Shukla et al. 2020).

Furthermore, numerous studies have demonstrated that nano-formulated pesticides are more effective at eliminating pests without harming people or the environment. For instance, when applied to *Drosophila melanogaster*, spinosad- and permethrin-loaded chitosan NPs showed enhanced bioavailability even at lower dosages than free spinosad and free permethrin, and the nanocomposites caused less harm to people and the environment (Sharma et al. 2019). The nanoinsecticide particles shrink and become more concentrated after being encapsulated by NPs, giving them stability and a gradual release capability. Furthermore, pesticides can exert systemic effects to suppress chewing or sucking-type insects like aphids by increasing their ability to penetrate through plants and reach the cell sap (Li et al. 2007). For instance, several nanoinsecticides benefit from the poisonous properties of metallic NPs. Compared to bulk Al_2O_3 treatment, aluminium oxide (Al_2O_3) NPs showed excellent capability for eradicating *Sitophilus oryzae* on stored rice (Das et al. 2019). It has been established that using pheromones to manage insect populations is a promising and successful strategy (Dweck et al. 2015).

The foliar application of polysaccharide NPs loaded with metsulfuron-methyl to wheat-growing weeds dramatically reduced weed biomass compared to conventional pesticides. Additionally, the cytotoxicity of conventional and nanoherbicides was determined by culturing cells, and the results revealed that the herbicide-loaded NPs were less hazardous than the conventional herbicides (Kumar et al. 2017).

According to a different study, solid lipid NP-based nanoherbicides had improved release patterns and herbicidal action than conventional herbicides. *Raphanus raphanistrum* (a weed species) development was considerably slowed down when atrazine was encapsulated by solid lipid NPs as opposed to the untreated group.

Additionally, *Zea mays* were unaffected by the tested nanoherbicide concentration (de Oliveira et al. 2015). In weeds treated to SiO_2 nanoparticles, Sharifi-Rad et al. (2018) found that germination, root and shoot lengths, fresh and dried weights, and photosynthetic pigments with total protein all fell dramatically. Similarly, Kumar et al. (2017) demonstrated that pectin (polysaccharide) nanoparticles loaded with the herbicide (metsulfuron methyl) are more cytotoxic to *Chenopodium album* plants both in the laboratory and in field conditions, and only a very low amount of AI is needed in comparison to the commercial herbicide. Commercial herbicides only control or eradicate the weeds' above-ground sections, leaving their underground components like rhizomes or tubers unaffected. As a result, weeds begin to grow again; however, nanoherbicides stop this from happening (Dwivedi et al. 2016).

15.5 Impact of Nanoformulations on Plant Growth (Root and Plant Endophytes)

The interaction and uptake of nanoparticles are influenced by several factors such as the nature of nanoparticles, plant physiology, the interaction of nanomaterials with the environment, etc. The mechanism of entry of nanoparticles, their mode of application, and their penetration through plant cells are detailed in Fig. 15.3. The common factors influencing nanoparticle uptake and transport in plants are (a) Nature of nanoparticle, its surface charge, and chemical properties; (b) Method of application of nanoparticle; (c) Nanoparticles interact with microorganism in soil, which might impede their absorption into the plant system via several tissues such as epidermis, endodermis, casparian strips, and cuticle till vascular bundles; (d) Mechanisms involved in nanoparticle internalization within the plant tissues, such as mediation of carrier proteins, pore formation, endocytosis, etc.

The entry of nanoparticles occurs via root system through cells using symplastic pathway or around the cells to reach xylem via apoplastic pathway (Lv et al. 2019). The nanoparticle uptake involves diffusion, gated channel confirmation, symporter, and antiporter active transport. Diffusion involves the movement of ion molecules from a region of higher concentration to a region of lower concentration. There are two types of gated ion channels, one, the voltage-gated ion channels, and two, ligand-gated ion channels. These are the class of transmembrane proteins. In the case of voltage-gated ion channels, it forms the ion channel activated near the channel by changes in the electrical membrane potential, which alters the conformation of the channel proteins, regulating their opening and closing. While in the case of ligand-gated ion channels, they provide a means of converting the

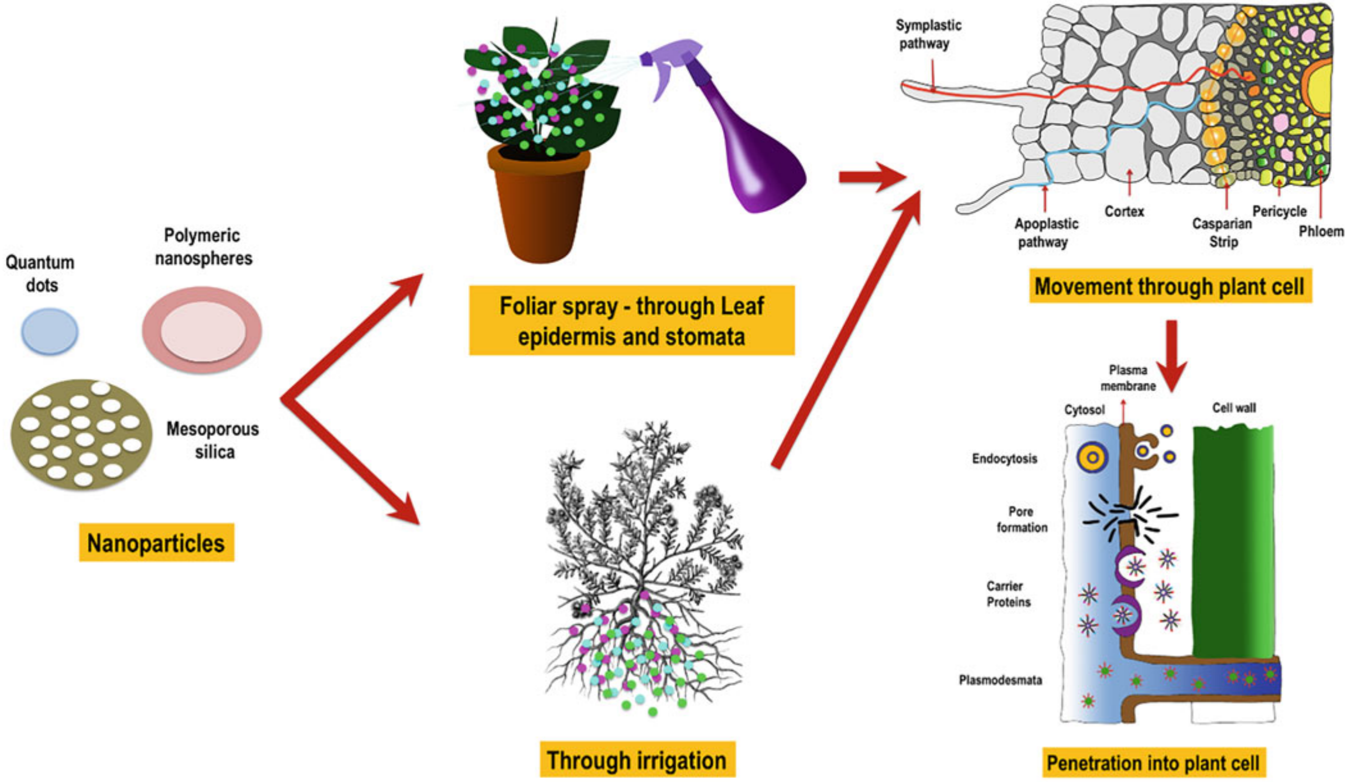

Fig. 15.3 Interaction of nanoparticles in soil–plant interface

biochemical signals into ion flux and electrical events near the membrane (Banerjee et al. 2019). In plants, the intracellular and extracellular signalling ligands are represented by cyclic nucleotides and glutamate, respectively.

A symporter and the antiporters are integral membrane proteins and a type of co-transporter and exchanger, respectively. The symporter in the plasma membrane involves in exchange of two dissimilar molecules in the same direction through the cell membrane, whereas the antiporter exchange two or more different molecules or ions across a phospholipid membrane termed "secondary active transport" in opposite directions, one into the cell and one out of the cell (Lv et al. 2019).

15.6 Impact of Nano-Formulated Agrochemicals on Soil Health

15.6.1 Soil Properties

Soil structure and functions are vital systems for ecosystem sustainability. The primary sink for all the chemicals applied for crop growth and production is soil and sediments. Clay composition, pH, temperature, minerals, and organic matter play an adverse role in agrochemical absorption and activities in soil. The interaction between nanoformulations and soil decides the leaching, runoff, bioavailability, distribution, uptake, and action. Or else, chemical residues remain in the soil for years inhibiting microbial and other enzymatic activities in soil due to long-term persistence (Parada et al. 2019). For instance, alachlor and metolachlor were reported to persist in soil water and were detected after 39 and 18 months, respectively (Vryzas 2018). A slow-release nanofertilizer with chitosan nanoparticles incorporated with potassium improved the soil's physical properties such as high-water conductivity, porosity, and enhanced friability, supporting *Zea mays*' root growth. Its dry density reduction induced the higher uptake of nutrients by plants and doubled the root biomass. Better carbon-cycling activities, higher microbial activities, and soil conditioning by stabilizing the soil aggregates and cohesive bonding of soil particles prevented soil degradation. As a result of slow-release nanofertilizer with chitosan nanoparticles, the increased crop productivity was observed with slow release of nutrients from the nanofertilizers (Kubavat et al. 2020).

Although nano-based agrochemicals tend to have many advantages on soil properties, it also exhibits adverse effects. For example, nanofertilizer was observed to decrease the soil pH level and increase exchangeable potassium but has no significant impact on total nitrogen and available phosphorous (Pide et al. 2022). On the other hand, nanocarriers used to deliver fungicides, insecticides, and fertilizers anchor the plant roots to soil organic matter and structure to reduce the runoff of applied chemicals (Bratovcic et al. 2021). Therefore, a sustainable and slow-release pattern of nanoformulations can be achieved based on the soil structure and properties.

15.6.2 Rhizospheric Interaction and Microbial Communities

Agrochemical formulated nanoparticles may have a noticeable impact on the rhizospheric communities of soil. It has been discovered that the uptake of synthetic agrochemical nanoparticles into roots and root nodules eliminates N_2 fixation potentials and impairs the growth of numerous crops (Priester et al. 2012). Investigations have shown that carbon-based and chemical NPs can produce stress, generating excess ROS that can affect many plants' proteins, lipids, carbohydrates, and DNA (Zhai 2020). Carbon nanotubes, one of the carbon-based ENPs, have been reported to induce ROS accumulation, enhancing lipid peroxidation in cell culture (Liu et al. 2010) and seedlings' root tips. Agrochemical formulations' effects on rhizobium in crops were also studied by Fan and Smith (2021), who discovered that nanoagrochemicals cause morphological alterations in bacterial cells. Chlorophyll *a*, the major photosynthetic pigment in plants, is more sensitive to photodegradation than other pigments and could be a more useful indicator of agrochemical NPs toxicity compared with growth characteristics of the crops and rhizospheric communities.

Furthermore, the growth of root nodules and the consequent delay in initiating nitrogen fixation were observed as signs that the relationship between these microbial organisms was disrupted. According to reports, modifying bacterial communities was dose-dependent, with some taxa increasing as a percentage of the community while more taxa decreased, leading to lower diversity. The soil bacteria may be in contact with NPs when applied directly to land containing mobile NPs. Some of these microorganisms are also effective at adsorbing and accumulating nanomaterials of one kind or another, which starts the mobilization of nanomaterials via food chains and can change communities of different populations (Holden et al. 2013). The well-known agricultural microbicide NPs has a detrimental effect on plant growth and destroys the soil microorganisms supporting it (Yadav et al. 2018). Nano formulations were found to drastically inhibit the activity of various soil enzymes, including soil protease, catalase, and peroxidase, in addition to microorganisms (Du et al. 2014). Additionally, it was shown that inorganic nanoparticles harmed soil microbial communities. These microbes' toxicity is further significantly enhanced by the presence of light (Adams et al. 2020).

The unique toxicity can be attributable to the nanoparticles and their microbial interactions, which correlate the physicochemical features of agrochemical formulations with their biological response species. However, the material's surface coating, which environmental factors can considerably change, can reduce or increase microbial toxicity (Suresh et al. 2013). NPs can be ingested by microorganisms, according to studies on ecologically significant bacterial species such as *E. coli*, *Bacillus subtilis*, *Pseudomonas putida*, and others. Most microorganisms had learned how to efflux, detoxify, and accumulate the metal ions, and the bioremediating plants also took major role in bioaccumulation and detoxification (Sinduja et al. 2022a, b). They also created efficient molecular mechanisms and operated specific metabolic pathways (Sinduja et al. 2022c). Additionally, bacteria can volatilize certain ions to remove their acute toxicity (De Souza et al. 1999). More research is required in agrochemical formulations for millets regarding beneficial soil microbes like N_2 fixing, phosphate solubilizers, and AM fungi to establish the uptake mechanisms and outcomes in soil and microbe interactions, even though microbes have evolved resistance and avoidance mechanisms.

15.6.3 Soil Fauna

Nanoformulations, including nano zero-valent iron (nZVI), have been utilized for years to remediate polluted soils and aquifer (Karthika et al. 2019). However, little research has examined its secondary environmental consequences and ecotoxicity on soil organisms (El-Temsah and Joner 2012a). Hence, the translocation of NMs into microorganisms, soil fauna, edible food tissues, and their influence on food quality and safety are critical concerns in nanotoxicology research (Zhao et al. 2014). Soil fauna may also change rhizosphere soil's physical and biochemical environment (Bray et al. 2019). Hence, soil fauna is regarded as an ecosystem engineer (Cunha et al. 2016) and plays a significant role in soil processes, including soil formation, waste decomposition, and nitrogen mineralization (Bardgett and Chan 1999). Large numbers of microorganisms (e.g., bacteria and fungus) and soil fauna (e.g., earthworms) assemble in the rhizosphere around plant roots, producing a distinct biotic and abiotic environment via root secretion, microbial growth, and earthworm activity in soils (Berendsen et al. 2012). Earthworms are ubiquitous in many types of soil and makeup 60–80% of the total soil biomass, making them excellent for soil toxicity studies (Bouché 1992). Earthworms are delicate, simple to handle, and toxicity test data is accessible. *E. fetida* is an easy-to-cultivate test species (OECD 1986). However, earthworm research on the transport and availability of NMs in soils is sparse. X-ray tomography demonstrated that earthworm burrowing governed nanoparticle movement and fate in soil (Baccaro et al. 2019). Numerous studies show that earthworms promote the abundance and activity of soil microbes (e.g., PGPR, plant symbiotic microbes) in the rhizosphere by burrowing and secreting mucus (Braga et al. 2016). This indirectly mediates the geochemical behaviour of NMs driven by microbes in the rhizosphere.

NMs interact with soil components (minerals and organic matter) before entering plants; hence their bioavailability may be affected by soil physicochemical parameters, especially pH and ionic strength of soil pore water (Tourinho et al. 2012). Soil texture and organic matter impact earthworm behaviour and nanoparticle mobility and availability. Clay and organic matter may alter the toxicity of nanoparticles in soil. For nZVI, clay content affects plant toxicity (El-Temsah and Joner 2012b).

Earthworms help move pore water, air, and solutes to horizontal and vertical soil layers, which increases metal mobility and availability (Leveque et al. 2014). Thus, earthworm bioturbation may facilitate the transport of NMs from bulk soils to the rhizosphere. Rhizosphere microorganisms drive the geochemical behaviour of NMs in the rhizosphere. Earthworms secrete mucus from their body surface, a combination of water, electrolyte, indoleacetic acid, and amino acid (Puga-Freitas and Blouin 2015), which may infiltrate the rhizosphere and interact with NMs. Wang et al. (2020) have shown that earthworm mucus impacts metal oxide NMs' suspension, hydrodynamic size, and solubility. These active groups of amino acids in mucus (e.g., carboxyl and amino) may speed the dissolution and reduction of NMs, particularly CuO and CeO_2 NMs, and increase their bioavailability (Huang et al. 2017).

The rhizosphere regulates earthworm geochemical behaviour and NM bioavailability in two phases. Physical consequences of earthworm bioturbation on NMs migration from bulk soils to rhizosphere; direct and indirect influences of earthworm mucus on NMs geochemical behaviours in the rhizosphere (Wang et al. 2020). Rhizosphere mechanisms might change NM geochemical processes such as aggregation, anti-aggregation, redox reaction, and transformation, regulating the translocation of NMs into plants and the NMs-mediated physiological and biochemical response of edible crop tissue (Wang et al. 2020). Soil organic matter and root exudates may adsorb on NMs after exposure, changing their hydrophobicity, charge, and metamorphosis (Layet et al. 2017).

A study conducted in Nanjing, China, reveals that TiO_2 and ZnO nanoparticles (NPs) have a detrimental effect on earthworms (Hu et al. 2010). Artificial soil systems containing distilled water, 0.1, 0.5, 1.0, or 5.0 g/kg of NPs were created and earthworms were exposed for 7 days. After an acute toxicity test, Zn and Ti were studied in earthworms, antioxidant enzyme activities, DNA damage, cellulase activity, and gut mitochondria damage. TiO_2 and ZnO NPs may cause substantial harm to earthworms at concentrations over 1.0 g/kg. Ti and Zn, particularly Zn, bioaccumulated, and the most significant dosage in soil (5.0 g/kg) harmed mitochondria. In addition, 5.0 g/kg of ZnO NPs reduced cellulase activity. TiO_2 and ZnO NPs are toxic to *E. fetida* at values over 1.0 g/kg in soil, although ZnO is more toxic (Hu et al. 2010).

Another study in Norway studied nZVI coated with carboxymethyl cellulose on *Eisenia fetida* and *Lumbricus rubellus* utilizing Organisation for Economic Co-operation and Development (OECD) procedures using sandy loam and artificial OECD soil. Earthworms were subjected to 0–2000 mg nZVI per kg soil and added fresh or aged in non-saturated soil for 30 days. nZVI concentrations in 500 mg/kg soil negatively influenced earthworm, weight fluctuations, and death. Furthermore, 100 mg/kg nZVI also influenced reproduction. nZVI's toxicity was decreased after some time, with significant variations between old and non-aged soils (El-Temsah and Joner 2012b).

No meta-analysis studies addressed NMs-mediated changes in the rhizosphere soil environment (Wang et al. 2020). Furthermore, the control of soil fauna and earthworms on the chemical behaviour of NMs in the rhizosphere is still in its infancy; hence, more advanced studies for better integration of earthworms into the geochemical behaviour of NMs driven by rhizosphere processes are needed in the future (Wang et al. 2020).

15.7 Existing and Possible Effects of Nano-Formulated Agrochemicals on Millets

Nanomaterials are used as nanofertilizers, nanopesticides, and nanosensors to boost agricultural output. Engineered nanomaterials (ENMs) affect fertilizer nutrient availability in soil and plant uptake. These materials can control crop diseases by directly acting on pathogens and generating reactive oxygen species (ROS). ENMs may potentially reduce disease by boosting crop nutrition and plant defence. In addition, ENMs may supplement traditional fertilizers and insecticides, lowering agricultural and environmental impact. Usually, millets utilize significantly less quantity of fertilizers and other agrochemicals. In India, it is mainly grown in the rain-fed areas during the summer season; habitually, the field and agronomic operations are much less than other food crops (Faiz et al. 2022). There are 10 millets that are eaten globally (Srivastava and Bisht 2021), and further we discuss about the effects of nano-formulated agrochemicals on millets.

15.7.1 Effect of Nanoformulation on Disease Suppression on Millets

Chitosan nanomaterials (CNM) delayed blast disease signs in infected finger millet from 15 to 25 days and decreased the severity by 64% on day 50. Increases in peroxidase activity and ROS generation led to disease suppression (Sathiyabama and Manikandan 2016). This shows that CNMs may boost plant growth during disease attacks via generating ROS. In other plant-pathogen, downy mildew disease induced by *Sclerospora graminicola* was significantly decreased (82%) in millet seeds exposed to CNMs (250 mg/kg soil), although less effective than the commercial fungicide (metalaxyl was 92% effective). 143 Ammonia lyase, catalase, peroxidase, polyphenol oxidase, phenylalanine, and superoxide dismutase increased in treated plants, suppressing disease severity (Siddaiah et al. 2018). Like finger millet, a Cu–chitosan nanocomposite boosted maize's defenses against *Curvularia lunata* (Sathiyabama and Charles 2015) and *Fusarium verticillioides* (Adisa et al. 2019). Some studies reported that nanocomposite improved agricultural yields (Choudhary et al. 2017). Combining seed treatment with foliar nanocomposite was more effective than foliar treatment alone against *P. grisea* in finger millet. The seed treatment was proposed to safeguard the plant by boosting defense enzymes (Adisa et al. 2019).

Pre-soaked seeds in chitosan nanoformulation at the rate of 0.1% w/v in Finger millet suppresses the growth and incidence of *Pyricularia grisea* and blast disease by 64% at day 50 after inoculation (Sathiyabama and Manikandan 2016). Nanoformulation of selenium with *Trichoderma asperellum* at the rate of 150–250 mg/L on pearl millet in the form of Foliar spray suppresses the downy mildew disease *Sclerospora graminicola*. In addition, it improves early plant growth

(Nandini et al. 2017). Pre-soaked seed treatment and foliar treatment at the rate of 0.1% w/v concentration of copper(II) oxide (CuO) on Finger millet has a positive effect on controlling the disease in *Pyricularia grisea* and blast disease incidence by 75%. As CuO can also act as a copper micronutrient, it increased the number of leaves by 22% when used as a foliar spray and by 33% as combined exposure of seed soaking and foliar spray (Guan et al. 2020). It also increased the defense enzymes in the finger millet (Sathiyabama and Manikandan 2018).

15.7.2 Effect of Nanoformulations as Nutrition and Fertilizer on Millets

Application of Zn nanofertilizers on 6-week-old finger millet plants improved shoot length (15.1%), root length (4.2%), root area (24.2%), chlorophyll content (24.4%), total soluble leaf protein (38.7%), dry plant biomass (12.5%), and enzyme activities of acid phosphatase (76.9%), alkaline phosphatase (61.7%), phytase (322.2%), and dehydrogenase (21%). In addition, zinc nanofertilizer increased crop output by 37.7% (Tarafdar et al. 2014).

Plant height, thousand-grain weight, and grain yield quantitative characteristics of foxtail millet did not change statistically between ZnO NP-treated and untreated plants; however, ZnO NP-treated plant grains had considerably greater oil and total nitrogen contents and reduced crop water stress index (CWSI) (Kolenčík et al. 2019). The slow-releasing nano-fertilizer increases plant physiological qualities and grain nutritional parameters, which benefits nanomaterial-based companies. In addition, the photocatalytic impact of ZnO NPs on leaf surfaces speeds up photosynthesis (Kolenčík et al. 2019; Morab et al. 2021).

In a study conducted in India, nitrogen and zinc nanofertilizers were employed with organic farming on wheat, pearl millet, mustard, and sesame. Organic farming using nitrogen and zinc nanofertilizers increased wheat, sesame, pearl millet, and mustard yields by 5.35, 24.24, 4.2, and 8.4%, respectively (Kumar et al. 2022). Increased yield correlated with wheat tillers, pearl millet ear head length, sesame capsule number per plant, and mustard siliquae number per plant. Organic manure, bio-fertilizer, and nanofertilizers produced greater yields and better plant development than standard chemical fertilizers alone. The findings imply that nanotechnology and organic farming may reduce the need for chemical fertilizer while increasing crop yield.

15.7.3 Nanoformulations as Pesticides on Millets

Nanotechnology is a potential crop protection technique, especially in lowering the quantity of active ingredients (AI) used in the field. Despite this technology's safety, efficiency, sustainability, and field performance, a big regulatory concern persists. Despite the promises and problems of developing, scaling up, and applying nanoformulations in the field, the worldwide market indicates a consistent expansion of this technology and a push toward innovation by employing sustainable components and new application models (Ferreira et al. 2022). Nanopesticides have the potential to be safer and more effective than their traditional pesticides. However, nanopesticides affect insect physiology and metabolism in plants during their interactions with them, especially regarding their method of action, which is little known. The current primary concern of scientists is to study the interaction of nanopesticides with plants.

Inorganic, porous nanoparticles may help deploy nanotechnology in the form of nanopesticides. Inorganic porous nanoparticles may encapsulate and transport insecticides and fertilizers. Porous nanocarriers may boost pesticide solubility and mobility and manage their release. Silica, iron oxide, zinc oxide, copper oxide, clays, and hydroxyapatites are common inorganic nanomaterials researched for crop development and protection (Bueno and Ghoshal 2022). This approach may give shelf-stable formulations, improved field performance with a lower AI dosage, and fewer tank combination difficulties. Nanoformulations applied by drones are a developing technology in precision agriculture that may expand their usage (Ferreira et al. 2022).

Copper-based nanoparticles offer minimal manufacturing costs and powerful antibacterial activities at acceptable doses, making them excellent for agricultural applications. Copper-based nanomaterials may be made by classic chemical, physical, and biogenic processes. Copper aids plant development, metabolism, and defence, and it is used in fungicides to fight plant diseases. In addition, copper-based nanoparticles have created new ways to protect and defend crops, with better outcomes and lesser toxicity than conventional copper (Gomes et al. 2022).

Nanosystem comprising chlorpyrifos (CPF), polydopamine (PA), attapulgite, and calcium alginate (CA) was used to create pH-responsive-controlled release chlorpyrifos. The mechanism protects CPF molecules from UV radiation. PA-CA hydrogel showed good biosafety on *Escherichia coli* and foxtail millet. It is a promising strategy to increase pesticide consumption efficiency and longevity, which has an extensive application opportunity in the future (Xiang et al. 2018). In one study, silver-doped MoO_3 and h-MoO_3 nanoparticle were produced by hydrothermal method; and *Staphylococcus aureus*, *Bacillus cereus*, *Citrobacter koseri*, and *Pseudomonas aeruginosa* were used to investigate the antibacterial activity of the nanoparticles found in the antimicrobial properties (Raj et al. 2021).

15.8 Advantages and Disadvantages

There is enormous pressure on the agriculture sector to find a solution for the continuous and bulky usage of chemicals in agriculture productivity to feed the growing population. This is because the nutrients applied to the soil are leached or lost than absorbed by plants. On the other hand, the soil rhizosphere communities of millets and other crops require fertilizers to retain their metabolisms and growth while increasing crop productivity. The development of nanoformulations with crucial properties is gaining attention worldwide; therefore, both sides of the coin need to be addressed for sustainable usage (Fig. 15.4).

15.9 Research Gaps and Future Perspective

In agriculture, more than 232 nano-enabled products are manufactured by 75 companies in 26 countries worldwide, most of which are in the fertilizer and pesticides category (Rajput et al. 2021). The optimal NPK fertilizers ratio of 4:2:1 is ideal for crop productivity, while the current ratio is maintained at 10:2.7:1 in India, with more heavily subsidized urea (Sharma et al. 2021). A clear understanding of the interaction between nanoparticles and rhizosphere reactions is necessary to assess the impact of nano-formulated agrochemicals on crop production through improved disease resistance, enhanced nutrient use efficiency, and better crop yield, mainly in millets. The interaction of nano-formulated agrochemicals in millets crops should have a better understanding of regarding the important aspects such as (1) mechanism behind the improvement of growth, resistance, and yield of millets, (2) impact on the rhizosphere environment, (3) adverse effects on millets if any. However, extensive research in millet crops regarding the impact of nano-formulated agrochemicals is needed. It is also essential to consider the factors such as environmental safety, cost efficiency, sustainability, and availability to farmers for large-scale applications.

As a result, fresh ways are required to safeguard the food plants with minimum or no adverse environmental consequences. Developing several nano-formulated agrochemicals has demonstrated higher agro-chemical activity, controlled release capacity, and targeted delivery qualities. They can also restore the oxidative stress symptoms induced by numerous stresses in roots and plants and stimulate plant adaptation to unfavourable conditions, making them an important agricultural tool. Although nanotechnology finds a vast relevance in agriculture, it is still in a very nascent stage, especially on nanobiomaterials for agriculture use. The nanoproducts developed so far have not yet penetrated the market and have not reached the common farmer. Concerted efforts are needed to promote the application of nanotechnology with multi-stakeholder participation, including the government, private sector industries, educational institutions, and other relevant stakeholders engaged in agriculture. Formulating suitable policies and educating and encouraging the farmers for wide-scale use will sustainably assist in food and nutritional security, contribute to a safeguarding environment, and combat climate change.

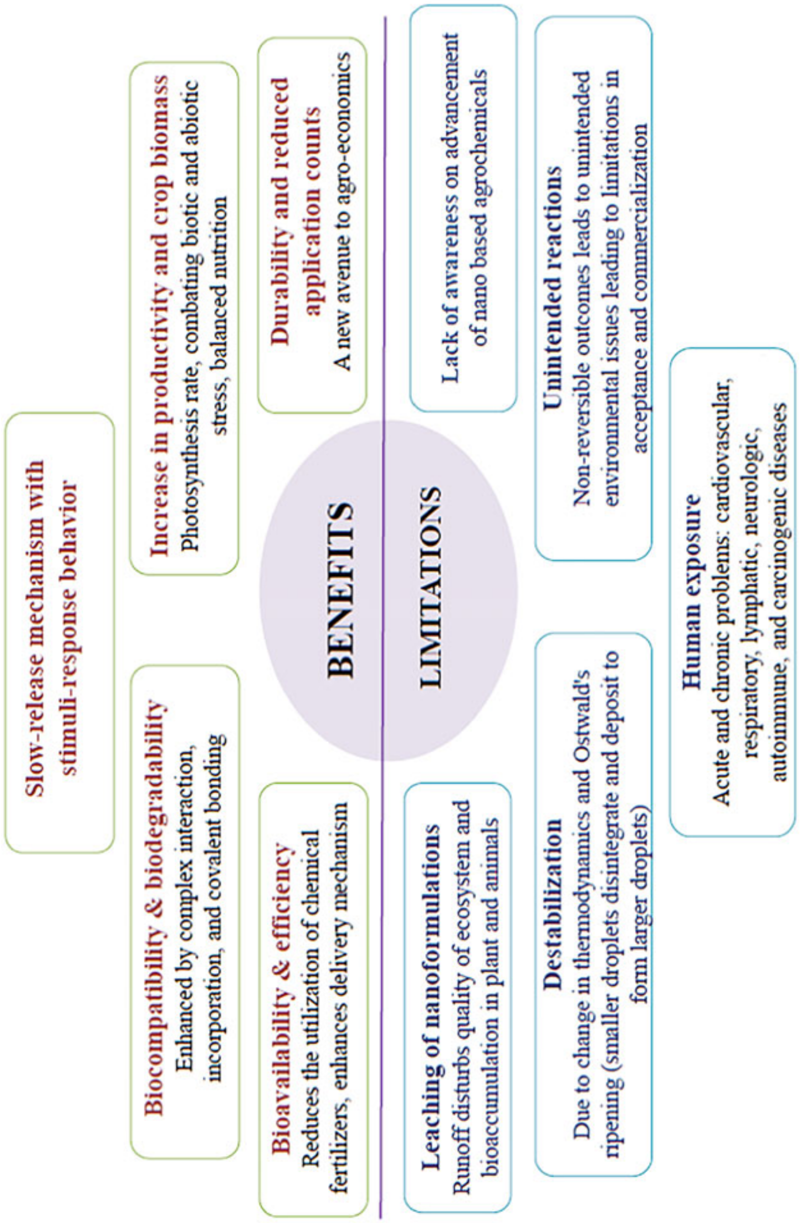

Fig. 15.4 Benefits and limitations of nano-based agroformulations

15.10 Conclusion

The chapter discussed the state of nanoformulations in rhizospheric communities focusing on the current status and possible effects on rhizosphere communities of millets. This groundwork on millets will help formulate further research on the influence of nanoformulations of microbial communities in millet farming and how it may pose challenges to humans through the food chain. The alteration in soil microbiota such as microbial biomass, the microbial population including rhizosphere communities, enzyme activities, and function are positively explored during nanoagrochemical application. But the uncertainty of nanomaterials in agriculture must be explored more, and a life cycle assessment of each nanoproduct should be discovered before commercialization. The possible effects of millet rhizosphere communities may differ based on the dosage and concentration of nanoformulations and synthesis method. Hence, exploration of toxicity on the environment should be a research priority, especially in millets, since research findings on millet rhizosphere communities are meagre comparatively. Biosynthesis of nanoagrochemicals should be encouraged to improve yield and productivity sustainably.

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Chapter 16
Potential Application of Nanotechnology in Biofertilizer Formulation for Millets

Kritika Rana and Ramesh Namdeo Pudake

Abstract With a rising population, there is an ever-growing demand for food grains and agriculture products. As a result, food security becomes of utmost importance to ensure a continuous supply of food. Chemical fertilizer and existing agriculture practices have so far helped us to keep up with the food demands. However, the over-dependence of agriculture on chemical fertilizers causes damage to both the environment and human health. Therefore, there is growing need to look for alternatives to chemical fertilizers without compromising the agricultural output. Recent advancements in nanotechnology provide a hope that nanoparticles can be used to protect crops from various pathogens, as well as used in biofertilizers to achieve higher yields. With the new advancement in the nanotechnology field, we are witnessing a rise in the studies on nanobiofertilizers which are not only efficient but help in high yield of crops. Recent studies mentioned in this chapter shed light on how nanobiofertilizers can provide a promising alternative to traditional chemical fertilizers.

Keywords Biofertilizer · Nanomaterials · Nanotechnology · Nanoformulation · Encapsulation · PGPR

16.1 Introduction

According to the World Population Prospect 2022, the global population is projected to reach 8 billion on 15 November 2022, and India is projected to surpass China as the world's most populous country in 2023 (United Nations 2022). To feed this growing population, 2–3 billion tons of grains (50% increase in annual production) is required (Mahapatra et al. 2022). The changing climatic conditions and indiscriminate use of chemicals has taken a heavy toll on agriculture. There is a critical need to improve the resiliency of agriculture against abiotic and biotic stresses in an

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R. N. Pudake et al. (eds.), *Millet Rhizosphere*, Rhizosphere Biology,
https://doi.org/10.1007/978-981-99-2166-9_16

ecofriendly and sustainable way to combat against dwindling agricultural production and rising population level. The need to produce food products extremely, quickly and in large quantities has led to a departure from traditional agricultural methods in order to obtain higher crop yields.

The use of chemical fertilizers has made it possible for the country to keep up with the food demands but at the cost of damaging the soil and environment to a great extent. These high yields are made possible using fertilizers that require great quantities of energy, mainly from fossil fuels. In addition, the disproportionate use of chemical fertilizers is one of the main causes of soil degradation (Sujanya and Chandra 2011). These chemicals affect the soil in terms of depletion of water holding capacity, soil fertility, increased salinity, and disparity in soil nutrients (Savci 2012). Due to several drawbacks of chemical fertilizers, efforts are being made to look for eco-friendly alternatives, it is thus necessary to increase the application of more innocuous fertilizers to replace chemical fertilizers or at least reduce their excessive use (Mahanty et al. 2017).

Organic farming is one such strategy which can ensure food safety without compromising the biodiversity of the soil (Bhardwaj et al. 2014). Organic farming employs natural microflora of the soil which is comprised of bacteria and fungi including arbuscular mycorrhiza fungi (AMF) called plant growth promoting rhizobacteria (PGPR). Biofertilizers are those substances that contain living microorganisms, and they colonize the rhizosphere of the plant and increase the supply or availability of primary nutrient and/or growth stimulus to the target crop (Ritika and Utpal 2014). Biofertilizers or microbial inoculants contain specific microorganisms which colonize the rhizosphere or plant and promote plant growth by increasing the supply of nutrient to the plants (Bhardwaj et al. 2014). Biofertilizers release various polysaccharides, metabolites, and various plant growth-promoting metabolites which help improve the soil quality and have an inhibitory effect on the proliferation of different plant pathogens, thereby improving plant growth (Mahanty et al. 2017). Additionally, biofertilizers make the soil environment rich in micro and macro nutrients via nitrogen fixation, phosphate and potassium solubilization, and production of antibiotics (Daniel et al. 2022). There is evidence that microbes inhabiting the plants are potential biofertilizers and can be used for sustainable crop production. The importance of the rhizospheric microorganism in crop production especially in millets is also highlighted in all the chapters of this book.

Biofertilizers, however, also have some drawbacks such as poor shelf-life, performance under fluctuating environmental conditions (temperature, radiation, pH sensitive), not suitable for long term, susceptible to desiccation, and most importantly the high requirement of dose for large coverage area. The emerging field of plant nanobiotechnology is helping to make agriculture profitable and sustainable at the same time (Ashraf et al. 2021; Pudake et al. 2019). Nanotechnology is a potential technology to revolutionize agriculture with powerful tools such as nanopesticides, nanosensors for detecting early symptoms of biotic/abiotic stresses in plants, NP-based plant growth stimulators, nanocarriers for targeted delivery of agrochemicals, improving soil quality, and more. To overcome the above drawbacks of biofertilizers, nanoparticle-based formulations can be used (Mahapatra et al.

2022). The term nanobiofertilizers signifies the formulation containing organic fertilizer, i.e., biofertilizer with nanomaterials. Nanomaterials can help in providing slow release of nutrients in controlled manner and can enhance the activity of biofertilizers thereby ultimately promoting the crop yield and productivity (Mahapatra et al. 2022). This chapter reviews the role of nanoparticles and nanoformulations of biofertilizers and their impact on various crop plants. It also summarizes the studies done in other crops that can be useful for millets cultivation.

16.2 Role of Nanoparticles in Biofertilizer

Globally, there are studies with the aim to elucidate how nanoparticles affect the PGPR's performance. In the below section, we tried to review the results with all the different nanoparticles used along with the biofertilizer/bioinoculants.

16.2.1 TiO_2 Nanoparticles

Titania (TiO_2) nanoparticles (TNs) have become important for various applications of nanomaterials in several fields of life science and other industries. The various sizes of TN that are biocompatible and are environmentally friendly are being synthesized by various methods (Timmusk et al. 2018). In one study, biocompatible titania nanoparticles were synthesized by acidic hydrolysis of titanium ethoxide, $Ti(OC_2H_5)_4$. They were evaluated along with *B. thuringiensis* and *P. polymyxa* for their effectiveness for improved wheat growth under the drought, salt, and pathogen stress. The changes in wheat biomass and TN-treated second inoculant colonization have a positive correlation. They also reported that TNs helped in stable attachment of PGPR on the wheat roots (Timmusk et al. 2018). In another study, the similar role the TNs play in helping adherence of PGPR to root surface by interacting in the form of inner-sphere complexes with the phosphate function in the membrane. The TNs helped in increased biomass of bacteria and ultimately, they resulted in growth promotion of Brassica under the stress condition (Palmqvist et al. 2015).

Shafiq and team studied the effect *Bacillus pumilus*, and ZnO and TiO_2 NPs on cadmium stress in maize plants. It was observed that under Cd stress conditions, *B. pumilus* and ZnO and TiO_2 NPs can successfully enhance maize's biomass, antioxidants, proline, nutritional content, and phytohormones. Combining plant growth promoting bacteria (PGPB) with NPs resulted in the maximum growth and improved defensive mechanisms against Cd stress (Shafiq et al. 2022). These all studies have indicated that TNs can be effectively used for PGPR formulation in many crops including millets.

16.2.2 Zinc Oxide Nanoparticles

Zn is an essential nutrient and plays key roles in many biological functions, and its deficiency might be a significant limiting factor in crop production. ZnO being a nanostructure exhibits gigantic area of applications and potential to boost the yield, development of food crops and their use as food additive (Czyżowska and Barbasz 2022; Rana et al. 2019; Sun et al. 2020). Recently, it has been found to alleviate drought-induced alterations in various crops such as rice, tomato, sorghum, cucumber (Dimkpa et al. 2019; El-Zohri et al. 2021; Ghani et al. 2022; Semida et al. 2021; Waqas Mazhar et al. 2022).

Azmat et al. (2022) investigated the effect of PGPR and green synthesized ZnO NPs against heat and drought stress in wheat. Treated plants showed improvement in biomass, photosynthetic pigments, nutrients, soluble sugar, and indole acetic acid content. When used together, ZnO NPs and *Pseudomonas* sp. protected plants by producing higher proline, antioxidant enzymes, and abscisic acid. The findings suggested that the synergistic effect of PGPR and ZnO-NPs can rescue plants from both single and combined heat and drought stress (Azmat et al. 2022).

Another study examined the potential of PGPR and NPs alone and combined for their drought-ameliorating characteristics. It was observed that *Azospirillum brasilense* alone (T_2) and ZnO NPs (T_3) improved wheat plant water relations, chlorophyll, proline, phenolics and grain quality, and yield. The best results were obtained when ZnO NPs and PGPR were used in combination. The combined treatment showed an enhancement in the antioxidant levels as compared to the control (Muhammad et al. 2022).

Similarly, ZnO NPs, and PGPRs have shown a positive effect under salinity stress by reducing genetic impairment in tomato seedlings. The study used different levels of ZnO NPs (0, 20, and 40 mg/L) and PGPBs (no bacteria, *Bacillus subtilis*, *Lactobacillus casei*, *Bacillus pumilus*). Salinity stress caused an increase in polymorphism rate assessed by Randomly Amplified Polymorphic DNA (RAPD), while PGPR and ZnO NPs reduced these adverse effects. A significant increase in the genomic template stability (GTS) was increased by the PGPBs (*Lactobacillus casei*) and 40 mg/L ZnO-NPs (Hosseinpour et al. 2020).

Heavy metal (HM) contamination in soil and plants is a serious concern, and in recent times, the problem has intensified considerably. Heavy metals are a potential threat to human health, thus there is a dire need to reduce the effects of heavy metals on plants and humans. Zinc application has been found to reduce HM-induced toxicity in plants. Use of zinc reduces HM uptake and translocation in plants. It also improves plant–water relationship, nutrient uptake, antioxidant activity, and gene expression. Additionally, it improves photosynthesis by enhancing photosystem activity, enzyme activity and more. Therefore, zinc can improve the performance of plant functions under HM stress. Several studies have been conducted which outline the effect of zinc oxide nanoparticles combined with PGPR on plants under stressful conditions.

Arsenic contamination is a serious threat to agriculture. Therefore, several studies have focused on mitigating the effects of arsenic contamination. Khan et al. investigated the potential of ZnO-NPs and PGPR mixed biofertilizer (BF) on maize plants under induced arsenic stress of 50 ppm and 100 ppm. The treated plants showed enhanced fresh and dry biomass, relative water content, protein content, soluble sugars, and enzymatic antioxidant defense mechanisms. A marked damage reduction was seen in cells treated with ZnO NPs and BF. Thus, the use of BF along with ZnO-NPs could be a very effective bio source for improving maize plant growth under As stress (Khan et al. 2022). Another study addressed this issue by checking the efficacy of synergistic effect of arsenic-tolerant PRPR (*Providencia vermicola*), ZnO NPs (10 ppm), and oxalic acid (100 ppm) in *Luffa acutangula* grown in arsenic-rich soil (150 ppm). *Providencia vermicola* exhibited features such as solubilization of phosphate, potassium, and siderophores production. Results revealed that there was a significant enhancement in photosynthetic pigments, relative water content, total sugars, proteins, and indole acetic acid along with As amelioration in *L. acutangular*. An improvement in nutrient levels and antioxidants was also seen. There was a significant reduction in arsenic bioaccumulation in *L. acutangula* shoots and roots by 40 and 58%, respectively, as compared with control (Tanveer et al. 2022).

Shafiq et al. (2022) demonstrated the efficiency of cadmium-tolerant PGPR (*Bacillus pumilus*), ZnO NPs, and TiO_2 NPs in maize grown in cadmium-rich conditions. *B. pumilus*, ZnO NPs (20 ppm), and TiO_2 NPs (10 ppm) showed a synergistic effect on plant growth promotion in pot experiments. All the treatments, alone and in combination, resulted in improved stress metabolism and defense responses. The combined treatment increased the levels of minerals and antioxidants and reduced the lipid peroxidation and cadmium bioaccumulation in shoots and roots by 40–60%. These findings suggest that *B. pumilus*, ZnO, and TiO_2 NPs, when used separately or in combination can be used as a viable strategy to reduce cadmium stress in maize (Shafiq et al. 2022). Another study focused on alleviating induced cadmium stress using ZnO NPs (20 mg/kg) and *Bacillus fortis* IAGS 223 in *Cucumis melo* plants. The plants were treated with *B. fortis* IAGS 223 and ZnO-NPs (20 mg/kg), either alone or in combination. It was seen that the activity of antioxidant enzymes was considerably modulated with the combined application of ZnO-NPs and *B. fortis* IAGS-223. Plants inoculated with *B. fortis* IAGS-223 supplemented with ZnO-NPs exhibited reduced amount of cadmium, protein-bound thiols, and non-protein-bound thiols under Cd stress (Shah et al. 2021).

A recent study used *Staphylococcus aureus* strain K1 with different amounts of ZnO NPs (0, 50, 100 mg/L) to remediate chromium stress in wheat. Wheat was grown in pot experiment under varying stress levels (0, 50, 100, and 200 mg/kg) of chromium. The treatment of *S. aureus* and ZnO NPs solely or in combination improved the wheat defense system nutrient uptake and increased chlorophyll and carotenoid contents, and also antioxidant enzymes. The combined treatment also substantially reduced chromium accumulation in different parts of the plants. It was thus concluded that the combination of *S. aureus* and ZnO NPs detoxify the effects of chromium on plants and boost its defense system (Ahmad et al. 2022). All these studies were of laboratory scale, and no study has evaluated the impact of these additives on microbial diversity and soil functions. These findings and structural morphology of ZnO/MgO nanoparticles draw attention to the need to consider their usage in biofertilizer formulation technology.

Recently, it has been reported that when *Piriformospora indica* DSM 11827 fungus was embedded with ZnO-nanorods, it formed a synergistic association and resulted in better growth of *Brassica oleracea* var. *botrytis* (Broccoli) (Singhal et al. 2017). They found that ZnO-nanorods at 500 ppm resulted in the increased biomass of *P. indica*. But they have not studied the mechanism behind the synergetic effect of nanoparticles on *P. indica.* Also, the impact on microbial diversity was not evaluated. In one collaborative study from Washington University in St. Louis, USA, and Central Arid Zone Research Institute, India, had reported that when biosynthesized ZnO NPs were added in soil, it increased the mobilization of native Phosphorus in Mung Bean (Raliya et al. 2016). However, this study has not evaluated the impact of NPs on native microbes in soil.

16.2.3 SiO_2 Nanoparticles

Silica nanoparticles can also be used as nano-fertilizers and nano-pesticides. Silica nanoparticles reduce biotic and abiotic stresses by enhancing the expression of antioxidant enzymes, osmoprotectants, proteins and secondary metabolites, such as phenolic compounds. Silica nanoparticles also induce non-enzymatic defense responses by increasing the synthesis of ascorbic acid, proline, glutathione, and phytohormones (Mathur and Srivastava 2022). Here, we review the use of silica nanoparticles in agriculture, with focus on synthesis, toxicity, mechanisms, uptake in plants, use as fertilizers and pesticides, and alleviation of biotic and abiotic stresses, such as pests, drought, and toxic metals.

One study investigated the effect of silica nanoparticles (SiO_2-NPs) on antioxidant enzymatic activity, phosphate solubilization capacity, and gibberellin production of *Bacillus cereus*-Amazcala (*B.c*-A). Furthermore, the effect of SiO_2-NPs and B.c-A was investigated on chili pepper plants under greenhouse condition. The result showed that SiO_2-NPs (100 ppm) enhanced the role of B.c-A by increasing its phosphate solubilization capacity. It was concluded that SiO_2-NPs acted as a eustressor by inducing defense-related responses. The co-application of SiO_2-NPs and B.c-A increased the seed germination percentage, plant height, number of leaves, and number and yield of fruits (Ferrusquía-Jiménez et al. 2022).

Akhtar et al. (2021) reported that drought-tolerant PGPR can confer drought tolerance in plants, and this effect can be enhanced by the application of silicon oxide nanoparticles (SiO_2 NPs). Drought-tolerant strains (*Bacillus* sp. *Azospirillum lipoferum* and *Azospirillum brasilense*) were isolated from the rhizospheric soil of arid regions. It was seen that the combination of all three strains gave the best results. Finally, in pot experiments, SiO_2 NPs and PGPR exhibited a synergistic role and improved the growth and yield of wheat. Moreover, the combined treatment led to improved biomass, relative water content, increased nutrient uptake, and chlorophyll-*a*, *b* content of wheat. An up-regulation of antioxidant enzymes was also observed. In conclusion, the combined application of SiO_2 NPs and PGPR strains induced drought tolerance in wheat which improved the growth and yield of wheat under drought stress (Akhtar et al. 2021).

Another study studied the impact of nanosilicon dioxide (10 mg/L) and PGPR (PC1-MK106029 and PC4-MK106024) on the growth of *Zea mays* in pot experiment. The combined treatment of both the NPs and PGPR enhanced plant height and number of leaves after 30 days of sowing. An enhancement of total chlorophyll, carotenoids, flavonoids, soluble protein, phenol was observed as compared to maize leaves. Treated plants also showed a substantial increase in catalase and peroxidase activity. Physicochemical and biochemical properties of soil also improved after treatment with PGPR and nanoparticles.

In one study, silica and PGPR were used to manage water-deficit stress in wheat. The combined effect of different amounts and sources of silicon (control, 100 mg nano-Si/kg, 200 mg nano-Si/kg, and 200 mg Si/kg from potassium silicate source) and PGPB (*Pseudomonas* sp. 19, *Bacillus* sp. 76, and consortium of 19 and 76 strains) were evaluated for canola and wheat under water-deficit stress. It was observed that Si application significantly enhanced water-deficit stress by increasing shoot and root dry weight in canola and wheat. Meanwhile, Si and PGPB application increased NPK and Si concentration in canola and wheat shoots. Treatment with 100 mg nano-Si/kg and *Pseudomonas* sp. 19 showed the best results in canola. Whereas treatment with 200 mg nano-Si/kg and *Pseudomonas* sp. 19 showed the best results (Valizadeh-rad et al. 2022). Similarly, the application of SiNP and PGPR together resulted in improvement in yield-related attributes in maize under the water and salt stress. Along with this nutrient uptake (N, P, and K) was improved, indicating that the combination can be a sustainable strategy for achieving drought and salt stress tolerance in maize (Hafez et al. 2021). Along with this, combination of PGPR and nanosilicon dioxide can be used as an alternative to pesticides, fertilizers, and it is an effective method to enhance plant growth and soil fertility by increasing enzyme activities of microbes (Kukreti et al. 2020).

16.2.4 Silver Nanoparticles

There are number of studies on utilization of silver nanoparticles (AgNP) with biological system, making them most abundantly studies metal nanoparticle. Generally, it shows a strong inhibitory and antimicrobial effect. They have also been found to be antioxidant and anti-inflammatory. Along with these, AgNPs will find novel applications in future application (García-Ovando et al. 2022). In one study, Kumar et al. (2020) investigated the impact of PGPR and silver nanoparticles on *Zea mays*. *Bacillus cereus* LPR2, AgNPs, and *Bacillus cereus* LPR2 + AgNPs were coated on maize seeds. The results were that seeds coated with *B. cereus* LPR2 showed maximum seed germination and shoot and root length followed by seeds treated with AgNPs. But *B. cereus* LPR2 + AgNPs did not show significant change compared to seeds treated with LPR2 and AgNPs separately. This suggests that nanoparticles can be beneficial, detrimental, or neutral for plants. The study also concluded that *B. cereus* LPR2 and AgNPs can be used as bioinoculant and growth stimulators, respectively, for maize (Kumar et al. 2020).

16.2.5 Gold Nanoparticles

A group investigated the effect of GNPs on selected PGPR, viz., *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Paenibacillus elgii*. It was found in in vitro examinations that GNPs resulted in accelerated growth of 63% in *P. elgii*, 57% in *P. fluorescens*, and 33% in *B. subtilis*. However, no acceleration or reduction on growth was observed in case of *P. putida* due to GNPs (Shukla et al. 2015).

Rhizosphere is a unique environment where chemical conversation between plants and microorganisms take place. The phytohormone, indole-3-acetic acid (IAA) produced by microbes has a significant role in interaction between plants and bacteria. Hence, any substance which has a modulating effect on IAA production can have an impact on plant–microbe interaction. Several studies by Panichikkal et al. have focused on analyzing the effect of nanoparticles on rhizospheric IAA.

One such study by Panichikkal et al. (2019) used rhizospheric *Pseudomonas monteilii* to investigate the concentration-dependent effect of biogenic Au NPs on the indole-3-acetic acid production. It was observed that in the presence of 50 μg/mL AuNPs, IAA production by *P. monteilii* was enhanced. Moreover, *Vigna unguiculata* seedlings grown in presence of 50 μg/mL of AuNP showed an increased growth. The results thus showed the ability of AuNPs to augment the IAA-producing potential of *P. monteilii* (Panichikkal et al. 2019).

Another study by Panichikkal et al. was done to evaluate the effect of chitosan nanoparticles (CNPs) and gold nanoparticles (AuNPs) on the IAA production by rhizospheric *Pseudomonas aeruginosa*. It was found that 5 mg/mL CNPs and 100 μg/mL AuNPs enhanced IAA production by *P. aeruginosa*. Additionally, CNPs and AuNPs-supplemented *P. aeruginosa* caused enhancement of shoot length and fresh weight of *V. unguiculata* plants (Panichikkal and Krishnankutty 2022).

16.2.6 CeO_2 Nanoparticles

The rare earth metal oxide—cerium oxide (CeO_2)—has been extensively used in agriculture due to its benefits. Generally, it has been reported that CeO_2 NPs helps in modulation of physiological activities in plant under stress, resulting into plant protection in stress tolerance (Hassanpouraghdam et al. 2022). Xie et al. (2022) studied the effect of CeO_2 NPs on soil microbial community, soil enzyme activity, and cucumber seedling growth. CeO_2 treatment induced oxidative stress in the roots but led to a significant increase in the biomass of the roots. However, the vitamin C content and soluble sugar content decreased and mineral nutrient contents were altered. Results showed that cerium was significantly accumulated in the cucumber tissue after CeO_2 NPs exposure. The study indicated that any environmental factors that alter the rhizosphere chemistry may affect the behavior and biological effects of NPs in soil-plant system (Xie et al. 2022).

16.2.7 Copper (II) Oxide (CuO) Nanoparticle

Copper being a plant micronutrient plays an important role in plant growth. Along with this, copper also shows the antimicrobial effects and so the amount of its nanoparticles in the agricultural system determines its positive or adverse effects. Haider et al. (2022) studied the effect of CuONPs combined with *Bacillus subtilis* on corn (*Zea mays*) and wheat (*Triticum aestivum*). *Bacillus subtilis* was used as a potential PGPR due to its prototrophic nature. The growth parameter (root length, shoot length, and plant biomass) of corn and wheat was recorded after 5 days. The results revealed that CuO NPs combined with *B. subtilis* improved root length, shoot length, and plant biomass of corn and wheat (Haider et al. 2022). Another study by Hosseinpour et al. demonstrated the use of CuO NPs (0, 50, and 100 mg/L) combined with PGPBs (*Bacillus subtilis*, *Lactobacillus casei*, *Bacillus pumilis)* in wheat plants exposed to salt stress (250 mM NaCl). An increase in the genomic template stability values with the application of PGPBs with CuO-NPs treatments was observed, especially *Lactobacillus casei* with 100 mg/L of CuO-Nps. Moreover, all treatments resulted in DNA hypo-methylation. In conclusion, PGPBs with CuO-NPs showed a strong anti-genotoxic effect against salt stress in wheat (Hosseinpour et al. 2022).

16.2.8 Molybdenum Nanoparticles

Abiotic constraints such as heavy metals have hampered the PGPR activity under field conditions. This calls for developing strategies to improve the efficacy of biological agents. Recently, metal nanoparticles have been reported as a tool for remediation of metal-contaminated environment (Baby et al. 2022). Ahmed and colleagues explored the use of molybdenum nanoparticles produced via biogenesis using *Bacillus* sp. strain ZH16 for mitigating arsenic stress in wheat. It was observed that application of biogenic MoNPs and bacterial strain ZH16 substantially improved the nutrient content and ionic balance of wheat under normal and spiked arsenic stress. The *Bacillus* sp. strain ZH16 has the potential to promote plant growth and mobilize nutrients; however, this ability is restrained under arsenic stress (Ahmed et al. 2022).

16.2.9 Iron Nanoparticles

A recent study was conducted to understand the effect of nanoparticles and biofertilizers on grain yield of wheat under different irrigation levels. The study involved nanoparticles (nano zinc oxide, nano iron oxide, and nano zinc-iron oxide), biofertilizers (seed inoculation by *Azotobacter, Azosperilium*, and *Pseudomonas*), and three irrigation levels (normal irrigation, moderate and severe water limitation). Biofertilizers and nanooxides increased the proline, soluble sugars, and enzyme activities. Under severe water limitation, the combined application of *Azotobacter* and nano Zn-Fe oxide increased the grain yield by 88% comparatively. The study concluded that the combined application of biofertilizer and nano Zn-Fe oxide improved wheat yield under drought stress (Seyed Sharifi et al. 2020).

16.2.10 Chitosan Nanoparticles

A wide range of studies have demonstrated that use of PGPR with nanocompounds improve plant growth and soil health in pot experiments. One such work involved study of response of two PGPR (PS2-KX650178 and PS10-KX650179) combined with nanozeolite and nanochitosan on Fenugreek (*Trigonella foenum-graecum*). The experiments included study of physiological and biochemical parameters of soil and plant in pot experiment for 45 days. A marked increase in plant height (1.5–2 times), leaf number, fresh weight was observed with nanocompounds and PGPR treatment as compared to control. The combination treatment also showed the highest levels of total chlorophyll level, sugar, soluble leaf protein, and catalase activity. The study demonstrated that in pot experiments, nanocompounds (nanozeolite and nanochitosan) in combination with PGPR improved plant growth and soil health (Kumari et al. 2020).

Another study explored the effects of PGPR along with nanochitosan on maize including growth, productivity, and mechanisms involved in defense response under field conditions. Nanochitosan combined with PGPR enhanced various parameters by 1.5–2-folds such as seed germination, plant height, root length, fresh and dry weight of shoot and root, chlorophyll, carotenoids, total sugar, and protein content after 60 days of the field experiment. The treatment also enhanced the level of phenolic compounds, catalase, peroxidase, and superoxide dismutase. Moreover, a significant increase in cob length, cob weight/plot, grain yield/plot was observed. Thus, the combination treatment improved health and yield of maize plants (Chaudhary et al. 2021d).

Agri et al. (2022) also studied the effect of nanochitosan along with bioinoculant on maize. The treated plants considerably enhanced plant height, leaf number, photosynthetic pigments, sugar, protein, phenol, and flavonoid content as compared to control. Like other studies, a higher antioxidant enzyme activity was observed. Additionally, soil health was improved due to enhanced total bacterial count, phosphate, and potassium solubilizers, soil enzymes (such as dehydrogenase, fluorescein diacetate, and alkaline phosphatase). The treated samples had an abundance of bacterial genera such as *Proteobacteria, Actinobacteria, Chloroflexi,* and *Firmicutes*. The application of nanochitosan with bioinoculants supported PGPB which are involved in nutrient assimilation and growth stimulating activities (Agri et al. 2022).

Recently, CNPs were used to analyze their effect on plant beneficial properties of rhizospheric *Pseudomonas monteilii*, which are known for their multiple benefits to plant function. The impact of CNPs on IAA production of *P. monteilii* was analyzed and it was found that 1 mg/mL CNPs enhanced IAA production. In plant studies conducted on *Vigna unguiculata* showed an enhanced shoot length, leaf number, and fresh weight when treated with the culture filtrate collected from *P. monteilii* cultivated with 1 mg/mL CNPs. The results highlighted the beneficial effect of the CNPs on functioning of rhizobacteria (Panichikkal et al. 2022b).

Endophytic bacteria have several plant beneficial features which can enhance agricultural productivity by supporting plant growth, yield, and disease resistance. In one study, the biofilm formation and antifungal properties of *Paenibacillus* sp. CCB36, which was isolated from the rhizome of *Curcuma caesia* Roxb., was evaluated in the presence of zinc oxide and chitosan nanoparticles. The group studied the effect of zinc oxide nanoparticles and chitosan nanoparticles (CNP) on the biofilm formation of *Paenibacillus* sp. CCB36 using tissue culture plate assay. ZnO NPs reduced biofilm formation, whereas CNPs modulated the same, which is why CNPs were used for further experiments. The biocontrol properties of *Paenibacillus* sp. CCB36 against *Rhizoctonia solani* was found to get enhanced with the use of CNPs. The results indicated that the application of nanoparticles led to an improvement in colonization and functioning of endophytic bacteria (Panichikkal et al. 2022a).

16.2.11 Nano Zeolite

The use of soil mineral modifiers such as natural zeolite compounds to improve the physical and chemical properties of the soil and reduce the destructive effects of chemical fertilizers on agricultural fields is one of the modern technologies that have recently been considered. Recently, Chaudhary et al. observed the combined effect of two PGPRs (*Bacillus* sp.) and nanozeolites on maize plants under field conditions. It was seen that combination of nanozeolite and PGPR significantly promoted plant height, root length, fresh and dry weight of shoot and root, chlorophyll, carotenoids, total sugar, protein, and phenol contents. The plants also showed an enhanced level of catalase, peroxidase, superoxide dismutase, phenols, alcohols, and acid-esters which signifies stress management. Moreover, maize productivity increased by 29.80% due to the combined treatment of *Bacillus* sp. and nanozeolite. These results demonstrate that the application of bio-inoculants with nanozeolite showed a positive response on the health and productivity of maize plants (Chaudhary et al. 2021b).

In one study, the effect of zeolite and biofertilizers on yield components, yield, and nutrient uptake of corn cultivars (cv. 6010 and ns71) was analyzed. Application of zeolite and biofertilizer in six combinations was done. The results indicated that application of zeolite + *Bacillus* sp. + AMF led to an increase in yield components and seed yield of corn. Maximum seed yield was obtained with combined application of zeolite and biofertilizers, and the yield decreased with separate application. The application of zeolite + *Bacillus* sp. + AMF also resulted in the highest amount of seed phosphorus in the ns71 cultivar in the second year of experiment. Therefore, it was concluded that the combined application of zeolite and biofertilizers is recommended to increase the quantitative and qualitative yield of corn (Ebrahimi Chamani et al. 2021).

16.2.12 Nanogypsum

It is well known that gypsum is used to improve the chemical and physical properties of soil. It is very important agrochemical for achieving sustainability in agriculture. Some studies have reported that the application of gypsum in growth medium had enhanced the PGPR growth and specified for the increase in shelf life of bioinoculants (Chaudhary et al. 2021a). In one study, the effect of nanogypsum and *Pseudomonas taiwanensis* strain BCRC 17751 was examined on maize plants and soil health. When applied together, nanogypsum (13 nm size) and bacterial inoculum led to better soil physicochemical properties and agronomical parameters of maize plants. The combination led to a significant increase in total bacterial counts, NPK solubilizing bacterial population, and soil enzyme activities. Moreover, an increase in the population of beneficial bacteria like *Proteobacteria*, *Bacteriodetes*, *Planctomycetes*, *Acidobacteria,* and *Nitrospirae* in treated soil. It was concluded that the combined application of nanogypsum and *P. taiwanensis* helped in improving the structure and function of soil which affects the plant health without causing any toxic effect. However, like most of the studies, in-situ studies are required to get maximum benefits to the farmers and environment (Chaudhary et al. 2021c).

16.3 PGPR Encapsulation

As discussed earlier, we know that as compared to synthetic fertilizers, biofertilizer has low efficacy in field conditions and so needs new formulation techniques to increase its efficacy. In recent years, various polymers and biopolymers are being used for making this formulation by encapsulation or entrapment of beneficial microbes or bio-agents. In this section, we have summarized the studies that have used various polymers and other compounds for efficient delivery of PGPR.

Due to its advantages such as ionic crosslinking, pH responsiveness, excellent biocompatibility, biodegradability and low price, alginate has become one of the most important natural polysaccharides extensively used in constructing desired delivery systems for food bioactive ingredients (Li et al. 2021). *Pseudomonas* sp. DN18 was entrapped in an alginate bead and supplemented with salicylic acid (SA) and zinc oxide nanoparticles (ZnO NPs). This encapsulation of PGPR with ZnO NPs resulted in indole-3-acetic acid (IAA) production and antifungal activity against *Sclerotium rolfsii*. *Pseudomonas* sp. has been proven to produce IAA, an auxin critical for plant growth and development. The modified formulation was used in *Oryza sativa* seedlings. The roots and leaf of *O. sativa* seedlings were inoculated with *S. rolfsii* and were analyzed after 2 weeks. Plants treated with Pseudomonas encapsulated in alginate bead and supplemented with SA and ZNO NPs showed superior plant growth promoting and biocontrol properties as compared to free living *Pseudomonas* sp. DN18 (Panichikkal et al. 2021).

Enterobacter sp. and *Pseudomonas* sp. bioinoculants have shown over 30% growth in wheat grain production (Suleman et al. 2018). Roslan et al. (2020) recently demonstrated that *Enterobacter hormaechei* 40a has both a P- and K-solubilization ability which is beneficial in the promotion of the early vegetative growth of okra seedlings. Strain 40a was used in free cell and encapsulated form (alginate beads containing sugar-protein hydrolysate and molasses) and combined with different phosphate and potassium dosage. The study showed that half-dose PK-fertilizer + encapsulated strain 40a (3HB) showed the highest soil available phosphate (SAP) and potassium (SAK), along with highest P and K uptakes for all plant organs. This was followed by full-dose PK-fertilizer (3F), half-dose PK-fertilizer + free-cell strain 40a (3HI) and half-dose PK fertilizer (3H). Moreover, 3HB and 3F treatments resulted in increased abundance of *Acidobacteriales*, *Burkholderia caballeronia paraburkholderia*, *Gemmataceae*, and *Sphingomonas*. The effect one-time 3HB treatment on okra growth and yield was comparable to the biweekly inoculation of free-cell strain 40a (Roslan et al. 2020).

Moradipour and colleagues have demonstrated that the nanocapsules of *Pseudomonas fluorescens* VUPF5 and *Bacillus subtilis* VRU1 significantly enhanced root length and its proliferation in Pistachio micropropagation. Highest root length (6.26 cm) and shoot (3.34 cm) was observed with nanoformulation of VUPF5. It was observed that nanoencapsulation of bacterial metabolites was effective in enhancing proliferation of shoots and rooting during the tissue culture (Moradipour et al. 2019).

Chitosan has been used as a potential polymer for probiotic bacteria encapsulation. The properties of chitosan such as biocompatibility, non-toxicity, easy dissolution, high adsorption, wide-availability, biodegradation, and cost-effectiveness make it a good candidate for use in agriculture. Chitosan overcomes the problems of bacterial encapsulation which include degradability, survival, and long-term performance. However, more precision is required to improve encapsulation of PGPB in chitosan for its application in agriculture (Riseh et al. 2022).

A recent study reported the development of nanoclay-based delivery system for the encapsulation of PGPR. Five strains of *Pseudomonas* spp. were selected and encapsulated in laponite gel beads and used to treat *V. unguiculata* seedlings. The treatment showed a significant improvement in leaf length, leaf number, and shoot length as compared to the control plants. Moreover, enhanced photosynthetic potential was seen in the treated seedlings. Additionally, viability studies confirmed that the condition of rhizobacteria was stable within the laponite bead up to 60 days after entrapment (Snigdha et al. 2021a). Same group of researchers have also used poly (ethylene oxide) and laponite clay nanocomposite and significant growth improvement in *V. unguiculata* was observed (Snigdha et al. 2021b). Recent reviews have also highlighted the various formulation types of microbial encapsulation, along with their advantages to have better agricultural production (Rojas-Sánchez et al. 2022; Szopa et al. 2022). These studies and promising results have indicated that nanoencapulation of PGPR or nanoformulation of compounds produced by them can be an effective method for millet production. So further studies are required to be done in near future with millet associated PGPRs.

16.4 Conclusion and Prospects

The indiscriminate use of chemical fertilizers in agriculture has resulted in adverse effects on soil by altering its physicochemical properties, affecting plant nutrition quality, and leading to several other ecological consequences. Hence, there is a need for developing eco-friendly and sustainable techniques and practices to increase the agricultural output with minimum ill-effects. Nanobiotechnology can provide a viable option to implement sustainable agriculture practices. Nanoparticles loaded with biofertilizers have a great potential to revolutionize the agricultural sector. Organic manures and bioinoculants can be integrated with nanoparticles to ensure slow and sustained release of nutrients to plants which would ensure effective and efficient nutrients and better soil health. Other benefits of nanobiofertilizers include enhanced stability of functional ingredients, small dosage requirement, improving crop yield, enhancing stress tolerance in plants, and limited nutrient loss by degradation. Many biopolymers have also been identified for the encapsulation of PGPR, and new polymers are being researched. However, most biopolymers and synthetic polymers used for encapsulation of bacteria and cells have not been reviewed in a concise manner. The novel tool such as encapsulated PGPR was found to be effective in storing and delivering these microbes to crop plants and hence may have future application in crop production under challenging climatic conditions. Further this technique can be effectively applied for the encapsulation and delivery of various PGPR other biostimulating compounds to millets for enhancing their growth and production. More importantly, risks and negative aspects of nanotechnology applications in agriculture should not be overlooked and assessed properly. Considering both positive and negative aspects of nanobiofertilizers, there is a dire need to improve the research and compensate for the risk associated with the use of nanoparticles and biofertilizers. Several points need to be analyzed and considered in this regard. Laboratory-based and pot experiments alone cannot provide a complete picture about the real-life application of nanobiofertilizers in agriculture fields. Hence, experiments need to be done on the field to get an insight into the environmental impact of nanobiofertilizers. Moreover, a permissible safety limit of nanoparticle dosage needs to be set by government agencies after careful safety assessment. Finally, there needs to be a thorough understanding of the biomagnification transfer effects of nanobiofertilizers application in plant systems. In conclusion, we can say that this chapter reviewed the use of nanomaterials along with biofertilizers for the better knowledge of the readers.

Acknowledgements Authors are grateful to Science and Engineering Research Board, Department of Science and Technology, Govt. of India, New Delhi, for providing grant under the Teachers Associateship for Research Excellence (TARE) (TAR/2020/000166).

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