



MICROBIAL BIOTECHNOLOGY FOR FOOD, HEALTH, AND THE ENVIRONMENT

# PLANT-MICROBIAL INTERACTIONS AND SMART AGRICULTURAL BIOTECHNOLOGY

EDITED BY

Swati Tyagi, Robin Kumar,  
Baljeet Singh Saharan,  
and Ashok Kumar Nadda



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# Plant-Microbial Interactions and Smart Agricultural Biotechnology

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Swati Tyagi, Robin Kumar, Baljeet Singh Saharan,  
and Ashok Kumar Nadda



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# Preface

Farmers have great potential to adapt to and accept climate change. The global economy has changed and has been able to develop sustainable agriculture with high rates of growth. This helped us to solve the problem of food shortage. In this age, climate change has brought new and complicated challenges. To combat these challenges, it is necessary to understand the root causes, after which proper implementation of the solutions is required. On the other hand, the overuse of chemical fertilizers and pesticides has caused many environmental problems, including water acidification, greenhouse effect, and depletion of the ozone layer. These problems can be addressed using biopesticides and biofertilizers, which are beneficial, environmentally friendly, natural, and easy to use. Microbial biofertilizers control soil-borne diseases, maintain soil structure, and provide nutrients to plants. They play a vital role in sustainable agriculture. Arbuscular mycorrhizal fungi are important soil microbes that form symbiotic associations with most terrestrial plants and are mainly responsible for the uptake of phosphorus. Another group of microbes are biological nitrogen-fixing bacteria, which are universally potent bioinoculants used to promote plant growth. The use of *Rhizobium* in legume crops to support agricultural productivity is increasing day by day. Such types of bioinoculants are easily available to farmers. Moreover, the mass production of these biofertilizers is done in laboratories, and they can easily be stored as liquid or carrier-based bioformulations at room temperature. Phosphate-solubilizing bacteria are also extremely important, as they have been reported to increase P uptake by converting insoluble to soluble forms. *Azotobacter* and *Azospirillum* are two other very important bacteria. The response of these organisms is commonly seen in increasing yields. Apart from these microbes, cyanobacteria also contribute significantly to the nitrogen economy of sustainable agriculture. The tripartite relationship between legumes, rhizobia, and mycorrhiza is the most efficient combination to promote growth and achieve higher productivity in plants, trees, and vegetables. Cyanobacteria are excellent suppliers of nitrogen. Their importance for abundant crop production cannot be ignored and has been felt by farmers. They are easy to multiply and can now be obtained in bottles or packets throughout the year. In fact, the application of microbial bioinoculants is a very effective and natural method to increase and maintain the mineral economy of nature. Their use reduces the need for chemical fertilizers, making them the only effective alternative for self-sustaining farming.

Microbe–host or microbe–microbe interactions are important strategies for colonization and establishment in different agro-climatic conditions/regions. This communication/crosstalk covers all aspects of the microbial communities', e.g., signaling, chemotaxis, genetic exchange, metabolite conversion, intermediary metabolism, and physicochemical changes that lead to genotype selection. The survival rate of microbes in the environment depends on biodiversity because the high genetic variability increases the competitiveness and reduces the chances of intruders settling in the respective climatic conditions. Hence, these interactions are the result of

a coordination that results in acclimatization and adroitness and enables the filling of various niches by limiting biological and abiotic stresses or exchanging growth factors and signals. Several mechanisms might be involved in this exchange, such as secondary metabolites, biofilm formation, cellular transduction signaling, quorum sensing system, and siderophores. The final unit of interaction is the expression of each organism's genes in response to environmental stimuli responsible for the production of the biomolecules involved in such associations. Hence, in this book, we focus on the molecular mechanisms involved in such interactions in the molecular strategy used by various microbes, which influence the constitution and structure of microbes.

The challenges are varied and unpredictable, but with the experience of farmers and expertise of our scientists, it is possible to make agriculture smarter. There has been great demand for a book on *Plant-Microbial Interactions and Smart Agricultural Biotechnology*, wherein different issues concerning microbial interactions and smart agriculture have been described.

This book will certainly provide useful information dealing with a diverse group of microbes, beneficial effects, and the bottlenecks in their implementation. Students, researchers, biotechnologists, microbiologists, botanists, soil biologists, industrialists involved in the mass production of biofertilizers, producers, environmentalists, and all other users should find this book extremely useful. The goal of this book is to provide essential information on the use of different microbes and their secondary metabolites for the treatment of various diseases affecting crops. This book also describes the potential of microbes (tiny factories) in improving crop yield, plant health, and the biomolecules' production efficiency for smart agriculture.

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# Editors

**Swati Tyagi** is currently working as a Project Scientist at the International Rice Research Institute – South Asia Regional Centre (ISARC), Varanasi, India. She holds more than 5 years' research experience in the area of plant-microbe interaction, genomics, transcriptomics, and next generation sequencing and analysis. Dr. Swati has published more than 10 research papers in journals of national and international repute. She graduated with a degree in microbiology from Kurukshetra University (Haryana) in 2012 and earned her PhD from the Division of Biotechnology, Jeonbuk National University, Republic of Korea. She was awarded Brain Korea 21 plus (BK21Plus) fellowship for her doctoral studies. She also worked as an International Researcher at Jeonbuk National University, Republic of Korea, and as a Post-Doctoral Research Fellow at the genomics division of the National Institute of Agriculture Science, Rural Development Administration, Republic of Korea. During her early career stages, she worked as a Research Assistant and Senior Research Fellow at the Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. Her keen research interest is in uncovering the secrets of plant genomes by next generation sequencing and computational analysis, understanding complex plant microbes' interactions, and characterizing and validating the effect of microbial volatile compounds on plants and pathogens. Her work has been published in various internationally reputed journals, namely *Scientific Reports*, *Journal of Biotechnology*, *Plants*, *Food and Crops*, *PeerJ*, *Mitochondria B*, *Critical Reviews in Biotechnologies*, and so on. Dr. Tyagi has also published more than 10 book chapters, and 3 books. She is also a member of the editorial board and reviewer committee of various journals of international repute such as MDPI, *Agronomy*, *IJM*, *Vaccine*, and so on. She has presented her research findings at more than 10 national and international conferences. She has attended more than 50 conferences, workshops, colloquia, seminars, and so on, both in India and abroad. Dr. Tyagi has delivered several invited lectures and has been involved in various social services such as teaching and providing free career counselling to rural area students.

**Robin Kumar** is presently working as Assistant Professor at Acharya Narendra Dev University of Agriculture and Technology Ayodhya, Uttar Pradesh, India. He did his undergraduate degree, M.Sc., and Ph.D. in Soil Science at Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India. He was IRRI research fellow during his M.Sc. studies and CIMMYT young fellowship for his Ph.D. studies. Dr. Kumar worked as a Research Associate at IIFSR, Meerut, India. Dr. Robin is handling projects on integrated farming management system and guiding several undergraduate and master students. His area of specialization is conservation agriculture, integrated farming, and organic farming. Dr. Kumar has published more than 70 research papers, book chapters, and review articles in journals of national and international repute. Dr. Kumar has attended several national and



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**Baljeet Singh Saharan's** area of research includes PGPR, bioremediation, biofertilizers, biosurfactants, bacteriocins, plant-microbial interactions, and molecular microbiology. He completed his M.Sc. and Ph.D. in Microbiology at CCS Haryana Agricultural University Hisar, India. He has worked as Assistant Professor in Microbiology in the Department of Microbiology, SBS, PGI, Dehradun, JCDV, Sirsa, and Kurukshetra University, India. He worked as Associate Professor at Kurukshetra University, India. He joined as Senior Scientist in the Department of Microbiology, CCS HAU, Hisar, in 2019. At present, he is working as Senior Scientist and is in charge of the Biofertilizer Production Centre, Department of Microbiology, CCS HAU, Hisar. He has guided 30 M.Sc. and 17 Ph.D. students as a supervisor. At present, one M.Sc. student and five Ph.D. students are working under his supervision. Dr. Saharan was given the C.V. Raman award (INDO-US) for research in the USA. He has been Visiting Research Scholar at Washington State University, Pullman, WA USA. Dr. Saharan has successfully completed three major research projects financed by the University Grant Commission (UGC), Department of Science & Technology (DST), and Haryana State Council for Science and Technology (HSCST). Dr. Saharan is a recipient of the DAAD (Indo-German) fellowship 2003–2004 for doing post-doctoral research in the Department of Bioremediation (now Department of Environmental Biotechnology), Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany. He has been given the Raman (Indo-US) fellowship for post-doctoral studies in the Department of Plant Pathology, USDA, Washington State University, Pullman, Washington, USA. He worked on plant growth-promoting rhizobacteria. Dr. Saharan has published more than 70 research papers in journals of national and international repute. He has published two books through international publishers including Springer and CRC Press. He has presented his research findings in more than 40 national/international conferences through funding provided by DST, DBT, HSCST, UGC, KUK, and so on. He was recently nominated to be a member of a national committee by the Director General (Indian Council of Agricultural Research) for the revalidation of results of ZBNF (SPNF – Model) for natural farming. He has attended more than 50 conferences, workshops, refresher courses, colloquia, seminars, and so on, in India and abroad.

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# 1 Plant-Microbe Interaction for Sustainable Agriculture

*Swati Tyagi, Robin Kumar, Baljeet Singh Saharan, and Ashok Kumar Nadda*

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## 1.1 INTRODUCTION

The world's population is growing exponentially, and, with this increasing population, the rate of urbanization/industrialization has also increased. This has increased the demand for food, which has accelerated the depletion of natural resources (Vejan et al. 2016). To meet this demand, an excessive and irrational use of agrochemicals, such as fertilizers, herbicides, and fungicides, has been adopted in the past, influencing agriculture practices and polluting soil, water, and ecosystems and causing an ecological imbalance. However, these practices cause more serious challenges rather than solving the demand; therefore, it is necessary to improve crop productivity organically or naturally in a sustainable manner in harmony with the ecosystem. These issues pushed agricultural scientists to look for alternative options and understand the underlying molecular mechanism of plant-microbe interactions that can provide fresh perspectives (Tyagi et al. 2021). In the natural environment, plants and microbes constantly interact with each other, and these interactions are highly diverse and can be broadly categorized as favourable, neutral, or harmful, depending on their effect on the plant health and development process (Tyagi, Lee et al. 2020).

In past decades, many studies have been conducted to understand the important molecular players involved in plant-microbe interactions, and a lot of information has been discovered; however, this information is not enough, and many queries still need to be addressed. Microorganisms interacting with plants exhibit enormous potential to improve plant health and development as a natural catalyst or to trigger a negative response in the form of disease or stress (van de Mortel et al. 2012). Queries such as how do these microbes interact and exhibit different responses, what are the signalling pathways involved, what makes the interaction harmful or beneficial, what are the immune factors in plant and microbes that affect the overall response in both the interacting partners must be answered to understand the overall interaction process in plants and microbes and will provide the landmark information that can be used to identify and mark the pathogens or beneficial microbes and their interaction effect on the crop plants.

In this context, beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPR) or plant growth-promoting fungi (PGPF) are considered potential elements to improve plant growth and development and, hence, can serve as natural and sustainable alternatives to fertilizers and pesticides (van Loon et al. 1998). In past decades, several PGPFs and PGPRs were identified, characterized, and found to improve plant growth and nutritional quality, soil health, and fertility in a sustained manner. For example, some members of the bacterial genera *Bacillus*, *Trichoderma*, and *Fusarium* prevent plant diseases by subduing plant pathogens, thereby serving as biocontrol agents (Tyagi et al. 2020). Application of PGPR and mycorrhizal fungi enhance plant growth under various stress conditions. Both fungal and bacterial endophytes are found to be active stress relievers of the host plant (Voisard et al. 1989). Studies with many microbial inoculants have demonstrated their beneficial role in plant growth through effective root colonization and induction of plant growth support mechanisms. Overall, the exploitation of beneficial microorganisms and their useful interactions with plants offer promising and eco-friendly strategies in the development of organic agriculture globally (Eckardt 2002). However, microbes can cause a serious threat to agricultural production by causing severe diseases and are considered to be phytopathogens (El-Tarabily et al. 2000). Plant-pathogen studies must be undertaken to identify new pathogens, how they affect plant health, and how the already reported pathogens are developing resistance and evolving. All this information about plant-microbe interaction, whether it is beneficial or harmful, lies in the genetic material, and, thus, the genes involved in these interactions must be identified, characterized, and annotated. There is an urgent need to link the plant studies with “OMICS” to address these questions and provide some useful gene quantitative trait loci (QTLs) controlling specific traits to the breeders that can be used in breeding programs and thus improve the crop varieties in terms of productivity or quality.

## 1.2 BENEFICIAL PLANT-MICROBE INTERACTIONS

With the start of the green revolution, the use of chemical fertilizers, insecticides, and pesticides increased dramatically to improve agricultural yield and productivity.



However, despite several studies reporting their negative impact on the ecosystems, soil health, and humans, they are still in use (Tyagi et al. 2021). To overcome these potential hazards, the use of microorganisms for crop improvement has been proposed and, over the years, this proposal has been widely accepted by innovative farmers and agriculturists. Several plant growth-promoting microorganisms (PGPM) – including PGPRs, PGPFs, arbuscular mycorrhizae (AMF), and endophytes (bacteria/fungi) – have been reported to influence plant growth positively (Tyagi et al. 2021). These PGPMs either directly secrete plant growth-promoting compounds in the form of volatile organic compounds (VOCs), plant hormones, siderophores, etc. to boost the plant health or use indirect mechanisms such as the release of lytic enzymes, antibiotics, and other defence molecules which antagonise the pathogen growth and promote the plant growth as well as develop resistance against the plant pathogens (Asari et al. 2016). VOCs are compounds with a high vapour pressure that makes them highly diffusible in soil and the plant canopy, thus, making them an ideal molecule for crosstalk between plants and microbes (Asari et al. 2016). VOCs generated by plants and microorganisms can act as signalling molecules activating a series of molecular events that ultimately regulate a wide range of physiological processes of plants and microorganisms (Tyagi et al. 2019). VOCs released by plants determine the type of microbiota that can live in the phytosphere and prime the plant defensive system to upcoming stresses (Tyagi et al. 2018; Tyagi et al. 2019; Tyagi, Lee et al. 2020). Microbes also emit VOCs in response to environmental conditions and can stimulate plant growth and induce resistance/tolerance to biotic/abiotic anxious factors (Tahir et al. 2017; Tahir et al. 2017). Thus, biogenic VOCs represent a rich and complex chemical vocabulary that can help to uncover the hidden secrets that can be used for modern sustainable agriculture. On the other hand, metal-resistant siderophore-producing bacteria help a plant to thrive under heavy metal stress by alleviating the metal toxicity. Heavy metals, being noxious in nature, enter the food chain and result in the toxicity of plants and animals (Chen et al. 2016). With the expansion of industries, the pollution of these toxic metals is increasing at a very fast pace. The removal of these heavy metals by natural means is the need of the hour. The toxication of soil by these metals can be removed through phytoremediation, mycoremediation, and microbial remediation (Chen et al. 2016). One such way of microbial remediation includes the application of siderophores that are synthesized naturally by microbes and are helpful in forming a complex with heavy metal. Siderophores have been used mostly for clinical studies but have the potential to play a critical role in cleaning the environment (Rajkumar et al. 2010). Siderophores provide heavy-metal detoxification and cleaning of the environment by natural means. Some microbes improve the growth of the plants and serve as biofertilizers (Rajkumar et al. 2010). These microbes form spores and provide a competitive environment for other microbes. Many PGPR/F(s) secrete lytic enzymes such as chitinase, cellulase that can degrade the insoluble organic polymers into soluble compounds that can be utilized by the plants (Tyagi, Lee et al. 2020). Additionally, microbes induce systemic resistance in plants and provide protection against biotic and abiotic stress. Several reports have been published that show the potential and mechanism of action of how microbes secrete different molecules that

induce resistance against fungal/bacterial pathogens as well as improve the plant growth and development process (Tyagi et al. 2021). Also, the secondary metabolites released by microbes are taken by plants to resist the abiotic stresses such as drought, salt, etc. These interactions among plants and different classes of microbes are much more important to understand so that they can be utilized as alternatives for chemical based agri-products and to ensure the plant health under unfavourable conditions.

### 1.3 HARMFUL PLANT-MICROBE INTERACTIONS

In plant-microbe interaction studies, it is reported that fungi are more likely to cause more yield losses than other phytopathogens because of their highly evolving nature. Usually, the fungal phytopathogens are host specific, but survival on alternative hosts has also been reported for many of them, which helps the invading pathogen to breach the plant's immune system and develop resistance (Tyagi, Lee et al. 2020). Other phytopathogens, such as bacteria, viruses, and nematodes, also negatively impact plant health and decrease productivity. These pathogens interact with the plant host, breach the plant's immune system, and develop disease in the host, which, later in the course of infection, kills the plant or reduces productivity. Pathogens secrete effector proteins that interact with plant proteins and initiate the infection process. Also, pathogens secrete toxins that shut down the expression of defence-related genes, making them vulnerable to infection. Though previous studies provide informative insights regarding these interactions, much still remains unknown and needs to be addressed (Tyagi et al. 2021).

### 1.4 MICROBIAL IMMUNITY AGAINST HOST PLANTS

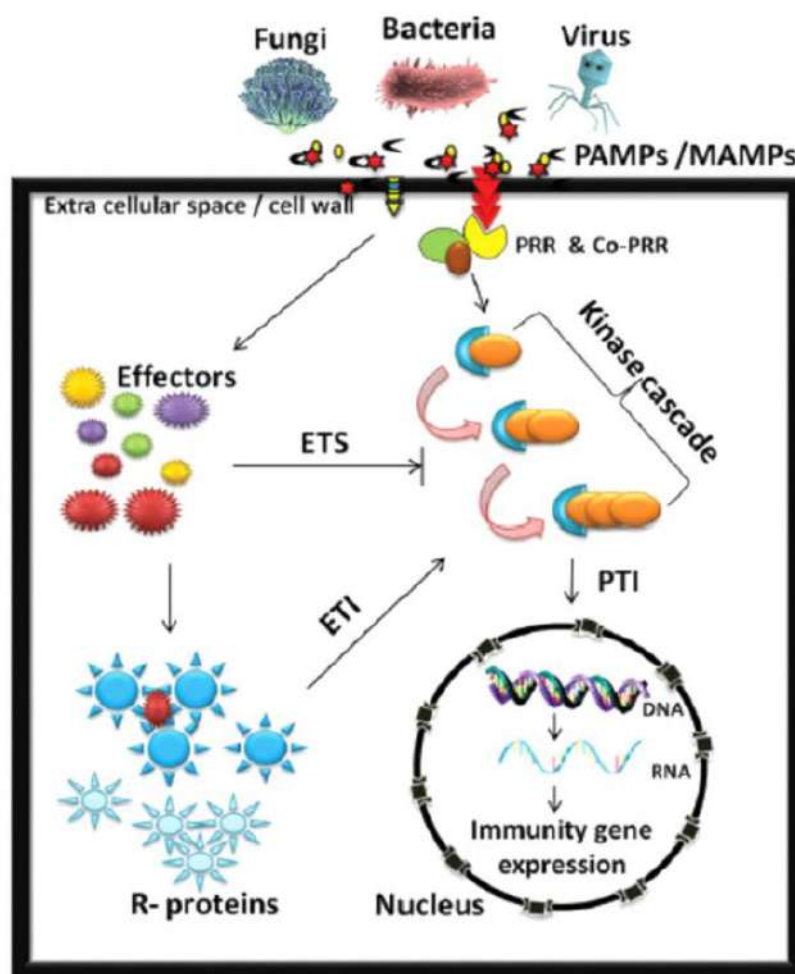
To initiate infection in the host plant, microbes must fight against the plant's defence system. Microbes use different virulence-related biomolecules that interact with the host. In the case of bacteria, type II, III, and IV secretion systems release virulence-related biomolecules that interact with the host to initiate and progress the infection (Tyagi et al. 2021). Similarly, fungi secrete enzymes that degrade the host cell wall/membrane-related molecules and allow the pathogens to enter into the host. On the other hand, viruses' particles enter the host through mechanical and chemical injury caused by external factors or by biological vectors (Tyagi et al. 2021). Once the viral particle enters the host, it can replicate itself and induce the infection response. It is very important to understand the mechanism of how pathogens enter the host and what molecules are involved in infection progression as well as how they initiate the infection. Understanding this molecular crosstalk between plant and microbes will help us to develop resistant or less susceptible plant/crop varieties (Tyagi et al. 2021).

### 1.5 PLANT IMMUNITY AGAINST INVADING PATHOGENS

In nature, plants, as a defending host, and microbes, as infecting pathogen, are constantly racing to initiate/mitigate infection. Several microbes, including bacteria, fungi, and viruses, cause a number of diseases in plants, and plants have to deal

with these invading pathogens to survive (Tyagi et al. 2020). The success rate of any infection is dependent on the susceptibility of the host and the environmental conditions favouring the establishment of infection. To fight invading pathogens, plants have two layers of defence: 1) constitutive and 2) induced. The constitutive defence system includes the physical and chemical barriers that are uniformly present in all plant species and act as the first line of defence (Tyagi et al. 2021). On the other hand, the induced defence system is activated when the pathogen attacks the host and either tries to breach or has breached the first line of defence (Figure 1.1).

Over time, different research groups have introduced different models of plant-microbe interaction to explain plant immunity and response toward the invading pathogens. The most accepted model among them is the zig-zag model (Tyagi et al. 2021). The plant defence system is activated by cell-to-cell communication followed by a complex series of events between the host plant and pathogen or its component. Depending upon the type (bacteria, fungi, virus, etc.) and nature (biotrophic, necrotrophic, etc.) of invading pathogen, the immune system employs different biomolecules (Tyagi et al. 2018). Generally, plants' cell walls or cell membranes have



**FIGURE 1.1** Plant immune system: The figure illustrates the interaction between plant microbes and the underlying mechanism involved in plant defence.

a different set of receptors often called protein recognition receptors (PRR) or wall-associated kinases (WAKs). On pathogen encounter, this set of receptors identify the pathogen or microbe associated molecular patterns (PAMP/MAMP) and recruit the PAMP/MAMP-triggered immune (PTI) system to fight the invading pathogen (Tyagi et al. 2018). To breach this defence, pathogens induce effector-triggered susceptibility (ETS), which causes some pathogens to secrete effector proteins that quash the PTI by employing susceptibility (S) proteins that enable the disease to advance. In this scenario, the plant's immune system initiates another line of defence by employing resistance (R) genes. R genes recognize the effectors or avirulence genes from pathogens and activate effector-triggered immunity (ETI) (Tyagi et al. 2021). These two induced defence systems (PTI and ETI) then alter the expression of different sets of genes, such as mitogen-activated protein kinase (MAPKs), plant hormones, transcription factors, and other pathogenesis-related genes that further induce hypersensitive response, reactive oxygen species (ROS) generation, cell-wall modification, stomata closure, or secretion of anti-microbial proteins and compounds, e.g., chitinases, protease inhibitors, defensins, and phytoalexins, etc. (Tyagi et al. 2018).

## 1.6 USE OF OMICS TECHNIQUES FOR PLANT-MICROBE INTERACTION STUDIES

The OMICS technologies that collect the information in the form of deoxyribonucleotide (DNA), ribonucleotide (RNA), proteins, or metabolites can shed light on the cellular structure and function of an organism. Out of these, the information that is stored in the form of DNA sequences is not much affected by the environmental condition; however, RNA, proteins, and metabolites are strongly influenced by external factors (Crandall et al. 2020; Sharma et al. 2020). The study of DNA sequences can be performed by genomics or next generation sequencing (NGS) techniques. While the study of RNA can be achieved by transcriptomics, proteins by proteomics, and metabolites by metabolomics. These technologies, either alone or in combination with others, can provide the desired information. The advancement of NGS made it possible to uncover the secrets lying within the genome of any organism (Crandall et al. 2020; Sharma et al. 2020). It became easy to sequence the genome of an organism, understand the mechanism of infection, detect the genetic variations, modify the gene/genome of an organism, and provide different prospects for developing resistance against plant pathogens or improving plant growth and development by introducing a specific gene or trait. It is now possible to study the genotype–phenotype relationship with the highest resolution through linkage mapping. Different approaches such as traits associated mapping, QTL mapping, genome-wide associate studies (GWAS), and haplotype analysis have been used to study these associations to improve plant traits and productivity (Crandall et al. 2020; Sharma et al. 2020). On the other hand, transcriptomic studies characterize and quantify the RNAs present in each sample (plant, organ, tissue) in a particular condition and provide the connection between the genotype and phenotype (Crandall et al. 2020; Sharma et al. 2020). RNA sequencing,

isoform sequencing, microarray analysis, etc. are different approaches that have been used to capture the transcriptomic information (Crandall et al. 2020; Sharma et al. 2020). In addition to this, proteomic studies are based on the full proteins present in a particular sample in a specific condition. The proteomic studies use two-dimension gel electrophoresis (2DE), liquid chromatography (LC), mass spectroscopy (MS), and coupled approaches such as LC/MS etc. Similarly, metabolomics is the study of by-products/metabolites as distinct molecules involved in a plant in a specific condition (Crandall et al. 2020; Sharma et al. 2020). The techniques employed for metabolomics also include LC/MS, nuclear magnetic resonance spectroscopy (NMR), capillary electrophoresis (CE), and time-of-flight (TOF)-MS devices. On the other hand, advances in molecular biology techniques made it easier to modify the genome of a host either by introducing some genes that can breakdown the toxins, abolish the activity of cell-wall degrading enzymes, excrete antimicrobial compounds, or delete the genes that are susceptible to pathogen attack (Crandall et al. 2020; Sharma et al. 2020). A number of gene editing approaches, such as RNA interference, zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), CRISPR : Cas9, etc., have been discovered that exhibit potential in the field of genome editing in agriculture and other fields of science (Tyagi et al. 2020). Though these molecular and OMICs techniques seem sophisticated and have proven to be very informative, and a wealth of receptors, genes, genomes, proteins, and metabolic products have been accumulated that can be used to improve the plant-microbe relationship for sustainable agriculture.

## 1.7 FUTURE PERSPECTIVES

Plant-microbe interaction studies can provide promising solutions for sustainable agriculture. These studies are very important to develop biofertilizers, biopesticides, and bioremediation processes. In the past, several studies were performed that have been very useful, but they are not enough. It is very important to find new genes and targets and understand their mechanisms of action during beneficial or harmful interaction with plants. Therefore, it is necessary to couple the OMICs and biotechnological approaches to understand the genetics of and in-depth knowledge of plant growth, development, disease, traits, and stress (abiotic/biotic) management.

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## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No conflict of interest.

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