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Review Article

The Role of Plant Mic robiomes in Sustainable Production of Horticultural Crops with a Focus on the Synthesis of Primary and Secondary Metabolites: A Review

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ABSTRACT

Plant microbiomes are recognized as key factors in improving the quantity and quality of horticultural crops. This systematic review, examining studies published in 2018 to date, analyzed the role of microbial communities residing in the rhizosphere, endosphere, and phyllosphere in regulating primary and secondary metabolites of horticultural crops. The findings indicate that beneficial microorganisms affect plant metabolism through multiple direct and indirect mechanisms. Direct mechanisms include improving the uptake of less mobile nutrients such as phosphorus and zinc, producing growth-promoting phytohormones, and fixing nitrogen. In contrast, indirect mechanisms include inducing systemic resistance in plants and suppressing pathogens through the production of antimicrobial metabolites and activation of defense signaling pathways such as jasmonic acid and salicylic acid. These effects not only lead to increased plant growth and yield but also significantly enhance the accumulation of valuable secondary metabolites related to quality, aroma, flavor, medicinal properties, and resistance to biotic and abiotic stresses. The practical application of this knowledge in the form of biofertilizers and designed microbial consortia has shown great potential to move towards sustainable horticultural production systems with reduced dependence on chemical inputs. However, there are several challenges, including the inherent complexity of host-microbiome interactions, variability of performance under different environmental and field conditions, and technical and regulatory barriers to commercialization of this technology. Overcoming these challenges requires the use of integrated approaches such as holomics, novel imaging technologies, and artificial intelligence-assisted modeling to gain a deeper understanding of this complex biological system. Finally, the application of microbiome-based strategies as a new and promising paradigm seems essential for ensuring food security and realizing sustainable agriculture in the current century.

Keywords: Biofertilizers, Plant microbiomes, Systemic resistance, Metabolite synthesis

INTRODUCTION

Horticultural crops, as major sources of human nutrition and health, are highly dependent on their production of primary and secondary metabolites. Primary metabolites, such as carbohydrates, proteins, and lipids, form the basis of growth and nutritional value. Secondary metabolites, such as alkaloids, phenols, and carotenoids, play vital roles in plant defense against stresses. Metabolites are concentrated in specific plant structures and determine the medicinal properties, appearance, flavor, and overall economic value of the products (Fig 1). Therefore, understanding and enhancing these metabolites can be highly beneficial for improving quality, enhancing stress resistance, extending shelf life, and promoting sustainable agriculture [1–3]. However, multiple factors in crop production affect metabolite synthesis, ultimately altering yield and product quality [4–6]. Metabolite production in horticultural crops is challenged by climate change, various stresses, soil limitations, chemical input risks, and emerging diseases [7–9]. Excessive chemical inputs also increase pathogen resistance, reduce product quality, pose risks to consumer health, and harm the environment [10–12]. Such challenges can reduce the quantity and quality of plant metabolites [13]. This situation makes the need to move towards sustainable agricultural methods more evident than ever. Therefore, finding alternative and sustainable solutions to reduce these inputs, increase plant resistance , and develop sustainable and low-input agricultural systems seems essential [14]. In this regard, the use of biological solutions has been considered as a new alternative in the production of horticultural products [15].

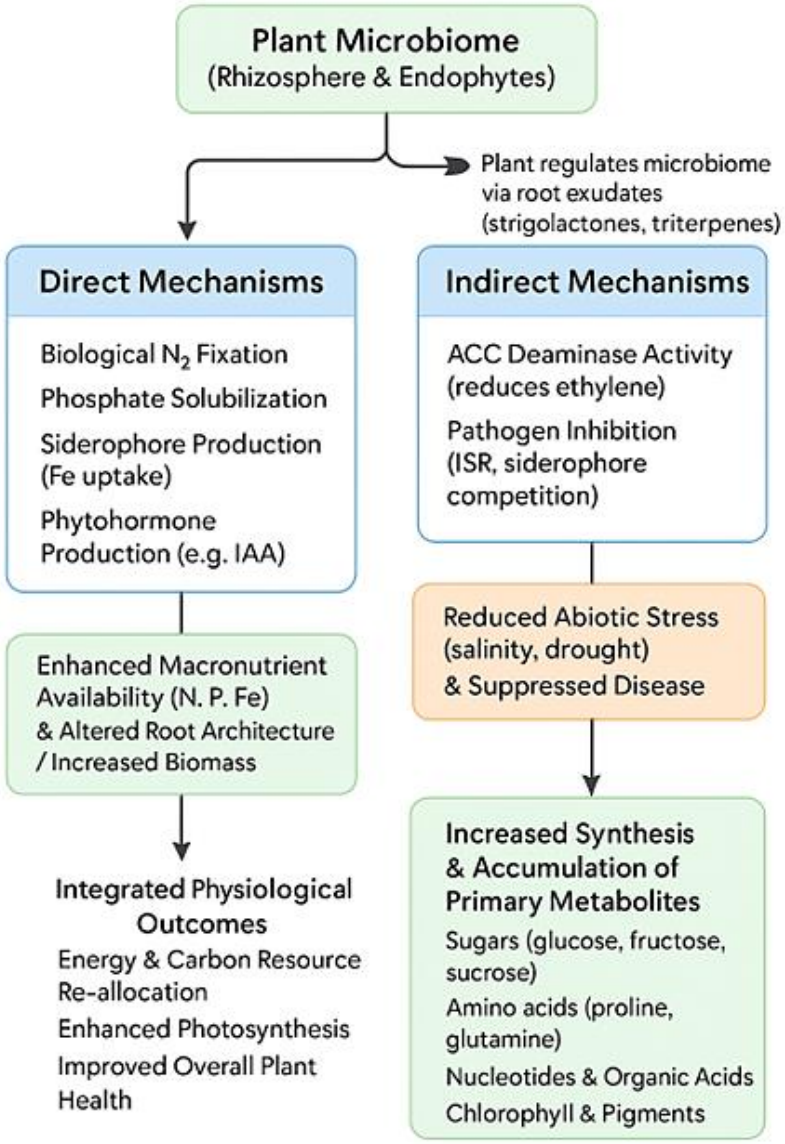

Fig. 1 Plant microbiome mechanisms and their benefits for growth enhancement and stress tolerance

Studies have shown that one of the factors affecting the accumulation of plant metabolites and maintaining their value is microbial interactions. These metabolites are signaling molecules in plant–microbiome interactions. They function bidirectionally: defending the plant and shaping the rhizosphere (the soil zone influenced by root secretions) microbiome [16, 17]. The plant microbiome is defined as an ecosystem composed of symbiotic microorganisms, including bacteria, fungi, archaea, protozoa, and viruses, that inhabit different ecological habitats of the plant, including the rhizosphere, endosphere (internal plant tissues) , and phyllosphere (leaves and aerial parts) , and have coevolved with their host. This microbial community plays a vital role in plant physiology and health [18, 19]. Rhizosphere microbiomes boost plant metabolism by enhancing nutrient uptake, regulating immunity, synthesizing compounds, and improving stress tolerance [20, 21]. This occurs by regulating metabolic pathways and secreting specialized metabolites, which tailor the microbiome to the plant's needs [22]. Specifically, rhizosphere microbiomes influence plant primary and secondary metabolites through the production of growth hormones, metabolic enzymes, and signaling compounds [23, 24]. Evidence suggests that these microorganisms are involved in both the production of primary metabolites and the biosynthesis and accumulation of valuable plant secondary metabolites [25]. Rhizosphere growth-promoting bacteria and arbuscular mycorrhizal fungi can enhance plant growth and metabolic quality by establishing symbiotic relationships through direct and indirect mechanisms [26, 27]. Direct mechanisms include facilitating the uptake of less mobile nutrients (e.g., zinc and phosphorus) via the expansion of the extraradical hyphal network in mycorrhizae, the production of phytohormones, and nitrogen fixation [15, 28]. Indirect mechanisms such as induction of systemic resistance and establishment of a plant primed state led to enhanced defense responses and accumulation of defense-related metabolites [29, 30]. Overall, the plant microbiome acts as a broad immune and regulatory system that has the ability to fundamentally influence plant nutrition, growth, immunity, and metabolites [31, 32]. Understanding the complex interactions between the microbiome and the plant could provide a new approach to sustainable agriculture and introduce these beneficial microorganisms as a promising strategy to enhance the quality of horticultural products [33, 34]. It should be noted that the successful establishment and reproduction of microorganisms within plant tissues (i.e., colonization) , and their sustained presence and activity, are necessary conditions for sustaining their beneficial effects on plant metabolism [35].

In this review, we seek to understand how the microbiome can be used to enhance the qualitative and quantitative value of horticultural crops by influencing metabolite synthesis, and to explore the strategies the plant microbiome uses to influence plant metabolism, leading to a favorable balance between growth (primary metabolites) and quality (secondary metabolites). This article provides a systematic review of the role of plant microbiomes in regulating primary and secondary metabolites of horticultural crops. To ensure the comprehensiveness and impartiality of this review, a systematic search strategy was implemented in reputable scientific databases (Scopus, Web of Science, PubMed, Google Scholar, CAB Abstracts, ScienceDirect) using targeted keywords (such as: plant microbiome, plant growth-promoting bacteria, microbial consortia, biofertilizers, metabolic pathway, primary and secondary metabolites). The main inclusion criterion for selecting articles was their publication in the period 2018–2024 in order to obtain recent findings in this field. After removing duplicate articles and those not relevant to the main topic, the remaining articles were subjected to content analysis. Studies were included only if they investigated the role of plant-associated microbiomes and reported quantitative or qualitative data on changes in primary or secondary metabolites. Finally, the findings were synthesized and presented in an integrated structure to meet the objectives of this review.

Practical Applications of Microorganisms and Microbial Consortia as Biofertilizers

Improving Crop Growth, Yield and Quality

To enhance readability and emphasize the most significant studies, we have consolidated the key findings of this section in Table 1. The table offers a structured summary of how microbial biofertilizers can improve the growth, yield, and quality of horticultural crops, detailing target crops, effective microorganisms, application methods, and observed outcomes. The practical applications outlined in the table are supported by specific molecular and physiological mechanisms, which are explored in the following sections to elucidate how these microbiomes regulate metabolite synthesis.

Increasing Tolerance to Environmental Stresses and Disease Control

Beneficial microorganisms enhance horticultural crop resilience and productivity through diverse, interconnected mechanisms. They directly improve plant health by increasing nutrient availability (e.g., solubilizing phosphorus, biofixing nitrogen) and producing growth-promoting hormones. Indirectly, they suppress pathogens through antimicrobial compounds and induce systemic resistance in plants by priming defense signaling pathways, such as those involving jasmonic, salicylic, and ethylene hormones. These actions lead to tangible agronomic benefits, including increased growth, yield, and stress tolerance (to drought, salinity, and disease). Critically, they also elevate crop quality by enhancing the synthesis of valuable secondary metabolites related to aroma, flavor, and medicinal properties. The practical application of this knowledge is realized through biofertilizers and designed microbial consortia, which restructure soil and plant-associated microbiomes. This approach represents a promising paradigm for sustainable horticulture, aiming to reduce dependence on chemical inputs while supporting food security. Relevant practical examples are summarized in Table 2.

Table 1 Practical applications of microorganisms and microbial consortia as biofertilizers for improving crop growth, yield, and quality.

Crop	Microorganism(s) Used	Application Method	Main Observed Effects	Reference (s)
Thyme	Arbuscular mycorrhizal fungi (AMF) and *Pseudomonas fluorescens*	Seed and Root inoculation	Positive effect on thyme growth such as root volume, plant height, dry and fresh weight; increase the percentage of essential oil.	[36]
Strawberry	*Glomus mosseae*, *Rhizophagus irregularis*, phosphate-solubilizing bacteria (*Pseudomonas*, *Bacillus*) combined with silicon	Mixing inoculum with growing medium	Improved growth, increased chlorophyll content, photosynthesis, and phosphorus uptake.	[37]
Peppermint	Bacterial consortium (*Azotobacter crococcum* and *Azospirillum*)	Seed inoculation	Enhanced drought tolerance, improved relative water content, proline, and water use efficiency; increased biosynthesis of phenolic, flavonoid, and monoterpene compounds.	[38]
Peppermint	*Bacillus subtilis* and *Pseudomonas fluorescens*	Soil inoculation	Increased defense hormones (jasmonic and salicylic acid), increased density of essential oil-producing glandular trichomes, enhanced production of menthol, menthone, and total antioxidant capacity.	[39]
Saffron	*Rhizophagus intraradices* (alone and mixed with *Funneliformis mosseae*)	Placement of fungal inoculum near roots in pots	Increased daughter corm size; enhanced product quality (safranal, picrocrocin, crocins).	[40]
Lavender	A mixture of six AMF species	Placement of inoculum near roots of young seedlings in organic medium	Increased total plant biomass, root biomass, leaf area, photosynthesis rate, chlorophyll a, b and carotenoid content, flowering stem length.	[41]
Vegetable crops	Plant growth-promoting bacteria (e.g., *Pseudomonas*, *Bacillus*, *Rhizobium*, *Azospirillum*) and fungi (e.g., *Trichoderma* spp.)	Soil/seed inoculation, Rhizosphere application	Improved root architecture, enhanced nutrient uptake efficiency (N, P, Fe), phytohormone production, tolerance to salinity/drought, suppression of soil-borne diseases, improved crop quality (e.g., elevated antioxidants in broccoli).	[42, 43]

Table 2 Applications of microorganisms and microbial consortia for increasing tolerance to environmental stresses and disease control.

Stress/ Disease	Crop	Microorganism(s) / Treatment	Application Method	Key Effects and Mechanisms	Reference
Drought Stress	Apple rootstock (M9-T337)	4-methylumbelliferone (4-MU) solution	Soil application	Increased populations of beneficial bacteria (*Pseudomonas*, *Bacillus*) and fungi (*Trichoderma*); improved water use efficiency, photosynthetic parameters, and drought tolerance.	[44]
Salinity Stress	Peppermint (*Mentha piperita*)	*Bacillus amyloliquefaciens* GB03 (via mVOCs)	Volatile exposure in split-chamber system	Increased fresh/dry weight, leaf number, total chlorophyll, and essential oil yield; modulated hormone response (increased SA, decreased ABA).	[45]
Pest Resistance (Thrips)	Chrysanthemum	Soil microbiomes from different plant species	Soil inoculation with microbial inoculum	Increased plant resistance linked to changes in defense compounds.	[46]
Disease Control (Fusarium Wilt)	Banana	Biofertilizer (mushroom waste & manure with *B. amyloliquefaciens*)	Application after soil fumigation	Controlled Fusarium wilt, increased soil pH and nutrient richness.	[47]
Disease Control (Fusarium Wilt)	Tomato	Rhizosphere microbiota from resistant plants	Microbiota transfer	Reduced *Ralstonia solanacearum* symptoms; enriched beneficial *Flavobacterium.*	[48]
Disease Control (Root Rot)	*Panax notoginseng*	*Rhodococcus sp.* and *Microbacterium oxydans* consortium	Addition to rhizosphere soil	Suppression of root rot fungus (*Ilyonectria sp.*); increased plant survival and biomass; root metabolite secretion stimulated beneficial bacteria.	[49]
Drought Stress	Tomato	*Streptomyces* spp.	Soil irrigation with bacterial suspension	Upregulated expression of *ERF1* and *WRKY70* genes; increased proline and sugar accumulation; enhanced drought resistance.	[50]
Disease Control (Downy & Gray Mold)	Grapevine	*Pseudomonas fluorescens* PTA-CT2	Applied to root zone	Induced systemic resistance against *Plasmopara viticola* and *Botrytis cinerea* via priming; enhanced SA, JA/ET pathways; improved photosynthetic efficiency.	[51]
Disease Control (Anthracnose)	Chili	*Bacillus* sp. BSp.3/aM	Seed biopriming with bacterial suspension	Reduced disease incidence; increased germination and seedling vigor; enhanced defense enzymes (POD, PPO, PAL, etc.) and phenolic compounds.	[52]
Disease Control (Bacterial Pathogen)	*Arabidopsis*, Tobacco	*Bacillus subtilis* GB03 & *B. amyloliquefaciens*	Volatile exposure or direct soil application	Increased biomass; induced systemic resistance against *Erwinia carotovora*.	[53]
Multiple Stresses	Various crops	*Azospirillum*, *Bacillus*, *Rhizobium*	Rhizosphere inoculation, foliar spray, co-inoculation	Enhanced salt tolerance, induced systemic resistance, improved iron uptake, pathogen inhibition via antibiotic production, promoted root growth and nodulation.	[54]

Application Strategies: Consortia, Application Methods, and Combination with Other Inputs

The use of microbial inoculation in two tomato production systems (greenhouse and field) was investigated in two ways: foliar spraying at the transplanting stage and irrigation after transplanting to the main field. The microorganisms *Pseudomonas*, *Bacillus*, and *Penicillium* and a microbial consortium of various bacteria and fungi were used. The results showed that under controlled greenhouse conditions, all microbial treatments improved yield, fruit size, and crop distribution. Under field conditions (alkaline soil, phosphorus deficiency, salinity, and high temperature), the microbial consortium showed a clear advantage in improving phosphorus uptake, increasing biomass, and final yield. Furthermore, the microbial consortium increased bacterial diversity in the rhizosphere and altered microbial populations associated with salt and drought tolerance, such as *Sphingobacteriia* and *Flavobacteria* [55]. The application of biofertilizers in combination with other biological inputs such as humic acids and arbuscular mycorrhizal fungi was investigated in corn. Phosphorus-solubilizing bacteria *Pseudomonas spp*. and *Bacillus amyloliquefaciens* were applied as a suspension on the seeds and in the root zone of corn plants. Humic acids were added to the soil in solution, and mycorrhizal fungi were placed in the soil in granules. The results showed that the combined application of these agents, especially the combination of *Bacillus amyloliquefaciens* with humic acids and mycorrhizal fungi (B3HAM), had the greatest effect on increasing phosphorus uptake, improving plant growth, and changing the structure of the rhizosphere microbial community. These changes included an increase in the ratio of gram-positive to gram-negative bacteria, an increase in the ratio of mycorrhizal to saprophytic fungi, and stimulation of the indigenous soil microbial community [56]. On tomatoes, one method of biofertilizer application involved using an inoculum of *Bacillus sp*. JA strain and *Pseudomonas putida* strain JX14. They were applied in four stages: seed soaking, root irrigation on the seventh day of planting, root immersion of the seedling before transferring to the main soil, and plant irrigation two weeks after transfer. This application method resulted in the production of the hormone auxin, phosphate dissolution, siderophore production for iron absorption, and ACC deaminase enzyme production to reduce ethylene stress in the plant. These activities increased the absorption of nitrogen, phosphorus, and potassium, leading to increased tomato fruit yield and weight. Importantly, the results showed that the simultaneous application of these two bacteria was superior to the separate application of each [57]. According to a study on the ornamental plant *Limonium sinuatum*, the application of arbuscular mycorrhizal fungi in greenhouse cultivation was carried out as a direct inoculation of 25 g of fungal inoculum (containing spores, hyphae, and colonized roots) into the potting soil. The best results were obtained using the fungus *Glomus mosseae* in soil with a medium concentration of phosphorus (20 mg/kg), which led to an increase in biomass, flowering stem length, leaf area, and uptake of phosphorus, nitrogen, calcium, and zinc, while also delaying flowering [58]. In another study, the effect of the fungus *Acrophialophora jodhpurensis* on tomato was investigated by seed coating with a suspension of fungal spores using adhesives such as sugar, carboxymethyl cellulose, and molasses. This method led to successful colonization of tomato roots by the fungus. The results showed that the application of this fungus through seed coating not only induced resistance to fungal diseases such as root wilt (caused by *Rhizoctonia solani*) and early leaf spot (caused by *Alternaria alternata*), but also enhanced growth parameters. By producing auxin, siderophores, and phosphatases, treated plants showed increases in fresh and dry weight of the stem and root, as well as stem and root length, compared to control plants. Among the different adhesives, seed coating with sugar showed the highest efficiency in promoting growth and controlling disease [59, 60]. In an experiment on the ornamental plant *Peperomia pellucida*, direct inoculation of a bacterial suspension of *Enterobacter asburiae* and *Klebsiella variicola* was carried out into the soil around the roots. The application method consisted of preparing a bacterial suspension and injecting 5 ml of it to a depth of 3–5 cm. This treatment resulted in an increase in leaf and node number, root fresh weight, PAL (phenylalanine ammonia-lyase) enzyme activity, and total phenolic compound content. Changes in secondary metabolites included an increase in 2,4,5-trimethoxystyrene and a decrease in ishwarane. The decrease in ishwarane did not indicate a weakening of the plant, but rather a shift in the plant's metabolic strategy in response to bacterial symbiosis; the plant redirects resources from the production of one compound (ishwarane) to another (phenylpropanoids such as 2,4,5-trimethoxystyrene) [61]. Seed inoculation with mycorrhizal fungi in cassava and potato plants increased the yield and quality of these crops. These fungi reduce the need for chemical fertilizers by improving nutrient absorption. Other benefits include increased drought resistance, help in forming soil structure and reducing erosion, and increased yield stability against environmental changes. To achieve these benefits, farm management must shift toward more mycorrhiza-friendly practices, including reducing the use of phosphorus fertilizers, minimizing tillage operations, and avoiding the use of fungicides that are harmful to these beneficial symbionts.

Mechanisms of Action at the Molecular Level and Signaling

The volatile methyl jasmonate secreted by plant roots signals the formation of beneficial soil biofilms, alters the microbial composition of the rhizosphere, and forms biofilms that enhance plant growth. This phenomenon has been observed in various horticultural plants, suggesting a defense mechanism. These findings pave the way for the development of new strategies in agriculture that take advantage of the enhancement of the soil microbiome and the formation of beneficial biofilms [62]. A study showed that different cultivars of *Camellia sinensis* naturally regulate the community of leaf-resident fungi (the phyllosphere) by producing phenolic compounds and catechins. This microbial balance, which is established by secondary metabolites of the host plant, is a natural and inherent mechanism for shaping the microbiome. This finding emphasizes the importance of genotype or cultivar selection as a strategy for managing beneficial microbial communities [63]. Bacteria such as *Pseudomonas fulva*, *P. orientalis*, *Bacillus megaterium*, and *B. subtilis* act as both growth promoters and antagonists against the pathogens *Botrytis cinerea* and *Fusarium oxysporum*. They achieve this through siderophore production, phosphate solubilization, auxin synthesis, and hydrogen cyanide production. Endophytic fungi *Penicillium copticola* and *Paecilomyces lilacinus* have the ability to inhibit the plant pathogen *Trichothecium roseum*. Some endophytes such as *Pantoea vagans* and various *Pseudomonas* strains stimulate plant growth by producing IAA and GAs [64]. Based on a case study conducted on *Cymbidium aloifolium*, sterile seedlings were inoculated with fungal discs under in vitro conditions. The fungi *Aspergillus fumigatus*, *A. niger*, *A. oryzae*, and *Penicillium citrinum*—isolated from the same plant—directly altered the physiology of the host plant by producing the hormone auxin from the precursor tryptophan. This change was manifested in the form of cell elongation and division in the root, which led to the development of the root system, improving water and nutrient uptake, and increasing overall plant growth. This microbial consortium also

enhanced the production of antimicrobial and antioxidant metabolites and the potential to protect the plant against pathogens and stresses [65].

Mechanisms of the Effect of Plant Microbiomes on Primary Metabolites

Direct Nutritional Enhancements: Improving Nutrient Availability and Primary Metabolism

Beneficial rhizosphere microorganisms increase primary metabolites in plants by improving nutrient availability and optimizing intraplant metabolism. In one study, microbial consortia symbiotic with corn roots were able to provide up to 82% of the plant's nitrogen needs by biofixing atmospheric nitrogen. This process provides nitrogen for the synthesis of amino acids and nucleotides, and by reducing the metabolic burden of synthesizing nitrogen-containing compounds, energy and carbon flow were directed to the synthesis of sugars and amino acids [66]. In *Arabidopsis*, mVOCs from *Pseudomonas* and *Burkholderia* enhanced carboxylate secretion from roots, improving iron uptake by inducing an iron-deficiency response. These changes led to the modulation of primary metabolism, including changes in the content of carbohydrates and organic acids, as well as the accumulation of secondary defensive metabolites, including phenols and glucosinolates [67]. In tomato and lettuce, *Bacillus* and *Pseudomonas* spp. increased phosphorus uptake via phosphate solubilization and siderophore production, promoting the synthesis of nucleotides and phospholipids. Arbuscular mycorrhizal fungi on basil plants improved the uptake of nitrogen and phosphorus by expanding the hyphal network and increasing the root absorption surface, leading to the synthesis of primary metabolites such as the amino acids glutamine and asparagine. Additionally, microbiomes containing *Azotobacter* and *Azospirillum* on pepper plants increased nitrogen availability through biological nitrogen fixation, resulting in the synthesis of primary metabolites including proteins, chlorophyll, and sugars [68]. Regarding micronutrient bioavailability mediated by microbiomes, it has been reported that *Pseudomonas* bacteria, by secreting siderophores such as pyoverdin, compete for iron with plant pathogens and suppress them by creating iron deficiency for the pathogen. This process leads to biological control of the pathogen and, considering that iron acts as a cofactor for many enzymes involved in primary metabolism, ultimately improves plant health [69]. Another study indicated that arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria in *Cyamopsis tetragonoloba* increased the activity of soil-active enzymes, including phosphatase, dehydrogenase, protease, and invertase, improving nutrient absorption and increasing the synthesis of the primary metabolite guar gum, which has a storage and protective role, as well as increasing grain yield [70]. In a study on *Populus euphratica*, phosphate-solubilizing bacteria facilitated the mineralization of organic phosphorus compounds by secreting the enzyme phosphatase. This process resulted in increased phosphorus concentration in the leaves and roots of the plant. Phosphorus, an essential element for the synthesis of ATP, DNA, RNA, and phospholipids, subsequently enabled the synthesis of primary metabolites and promoted plant growth [71]. Rhizobia in legume plants carried out nitrogen fixation by producing Nod factors and activating *nif/fix* genes. Increased nitrogen availability led to improved synthesis of vital primary metabolites such as amino acids and proteins in the host plant. Arbuscular mycorrhizal fungi in various plants increased the uptake of phosphorus and other nutrients by expanding their hyphal network. In return, the host plant provided the fungus with primary metabolites obtained from photosynthesis, such as carbohydrates and lipids. The *Gluconacetobacter diazotrophicus* Pa15 strain on rice plants caused the synthesis of the primary metabolites trehalose and alpha-tocopherol through the mechanism of increasing ABA production and chlorophyll content. The *Azospirillum* spp. Az19 strain on corn plants also caused the synthesis of the primary metabolite trehalose by increasing proline production [72]. The *Pseudomonas oryzihabitans* microbiome increased the primary metabolites glucose and fructose in the stems of *Salicornia europaea*. However, it was later reported that the *Brevibacterium casei* microbiome decreased the primary metabolite sucrose in the roots of *Salicornia europaea* [73].

Modulation of Plant Growth and Architecture: The Role of Microbial Phytohormones

Endophytic and rhizosphere bacteria *Pantoea agglomerans*, *P. dispersa*, *P. ananatis*, *P. eucalypti*, and *P. cypripedii* isolated from maize and wheat significantly increased shoot and root length, as well as the fresh and dry weight of shoots and roots, in maize, wheat, and rice plants by producing growth-promoting compounds such as IAA as a primary metabolite. This increase in biomass is the result of stimulating and improving growth-related metabolic processes in the host plant [74]. *Pseudomonas* sp. CM11 induced lateral root formation in *Arabidopsis* by activating the PLETHORA (PLT3/5/7) transcription factor-dependent pathway. This change in root structure led to an increase in root and shoot biomass, including primary structural metabolites such as cellulose, and ultimately an increase in seed number and weight [75]. The results of several experiments showed that *Bacillus* and *Pseudomonas* strains on different plants synthesized primary metabolites related to root growth and development through the mechanism of auxin hormone production. The microorganism *Bacillus velezensis* on pepper plants increased primary metabolites of soluble sugars, amino acids, and organic acids through the mechanism of auxin and gibberellin hormone production. It also protected the plant against gray mold disease by producing antifungal metabolites such as surfactant lipopeptides and inducing systemic resistance in the plant. These changes in primary metabolites were attributed to the overall improvement in plant health and physiological status under the influence of this bacterium [76].

Induced Systemic Tolerance: Microbiome-Mediated Amelioration of Abiotic Stresses

The consortium of bacteria *Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp. containing ACC deaminase enzyme increased the synthesis of primary metabolites, including chlorophyll, carotenoid, proline, and total soluble sugars, in *Phaseolus vulgaris* under salt stress. This occurred through the mechanism of reducing ethylene levels and strengthening the antioxidant system by increasing the activity of SOD, CAT, and POD enzymes [77]. Additionally, several bacterial species have demonstrated this ACC deaminase activity, including *Pseudomonas migulae* on tomato, *Variovorax paradoxus* on melon, *Achromobacter piechaudii* on pepper and tomato, *Pseudomonas fluorescens* on wheat, *Rhizobium* spp. on chickpea, *Pseudomonas putida* on canola, and *Azospirillum brasilense* on rice [78]. By decomposing the ethylene precursor (ACC) in the rhizosphere, these bacteria reduce ethylene levels in the plant, thereby mitigating the negative effects of stress. Consequently, a plant under less stress can direct its energy resources (e.g., ATP), carbon skeletons (e.g., sugars), proline, and chlorophyll activity towards growth and primary metabolism instead of expending them on stress responses [78]. The bacterium *Pseudomonas simiae* strain AU inoculated into *Glycine max* L. Merrill has been shown to regulate the

expression of drought response genes (*DREB2A*, *EREB*, *PIP*, *TIP*, *GOLS*, *P5CS*), reduce ethylene production, and increase abscisic acid and salicylic acid production, leading to the synthesis of primary metabolites (proline, soluble sugars, chlorophyll) [79]. Under stress conditions, plants attract arbuscular mycorrhizal fungi to the rhizosphere through the secretion of compounds such as strigolactones. This symbiosis enhances stress tolerance by improving water and mineral uptake, thereby maintaining the synthesis of primary metabolites [80]. Under water stress conditions, the application of arbuscular mycorrhizal fungi and a combined *Azotobacter* + *Pseudomonas* (AzPs) treatment can independently enhance seed germination and improve the biochemical properties of lavender [81].

Indirect Effects by Pathogen Inhibition and Root Microbiome Selection

The hemp root microbiome increases the synthesis of specialized metabolites, including cannabinoids, terpenes, and flavonoids, by activating the plant's defense system and the jasmonic acid signaling pathway. In contrast, *Arabidopsis* selectively shapes its root bacterial community by promoting the growth of beneficial bacteria and inhibiting undesirable ones through the production of a complex network of triterpenes, such as thalianin and arabidin. This bidirectional interaction between the plant and the microbiome creates a dynamic symbiotic relationship in which the plant manages its microbiome through specialized metabolites, and the microbiome in turn directly influences the quantity and quality of these metabolites produced in the plant [81, 82]. Colonization of tomato roots by the bacterium *Pseudomonas chlororaphis* leads to induced systemic resistance (ISR) in the plant. This process is accompanied by hormonal signaling networks, the most important of which is the reduction of ABA hormone in the root and the activation of SA and JA signaling pathways. In this case, when exposed to drought stress, the plant maintains a higher photosynthetic activity and metabolic capacity. This leads to the efficient accumulation of protective metabolites, including soluble sugars such as glucose, fructose, and sucrose, and amino acids including proline [83].

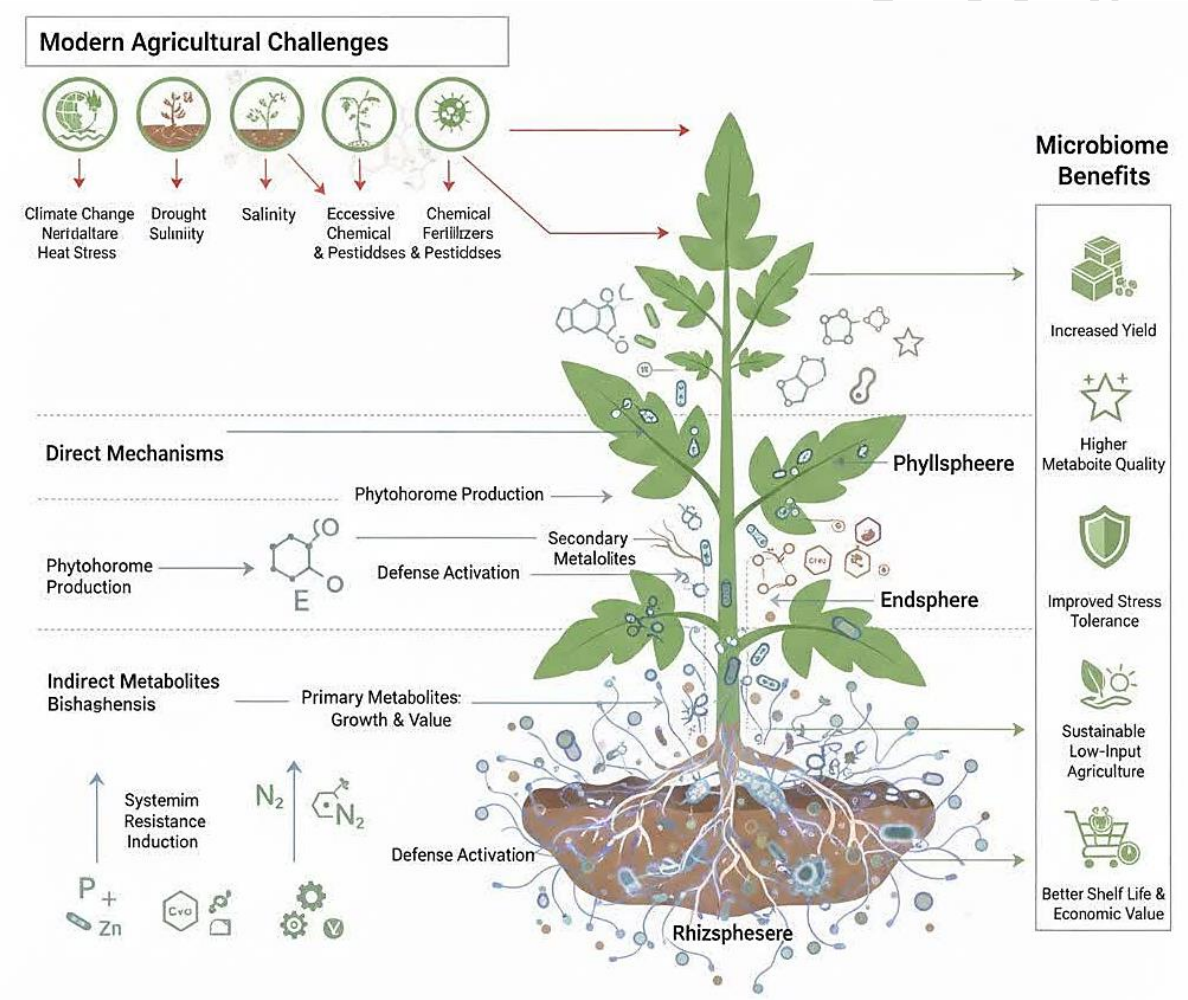

Fig. 2 Modern agricultural challenges and the plant microbiome's role in sustainable agriculture

Mechanisms of Plant Microbiome Effects on Secondary Metabolites

Basic Mechanisms and Systemic Induction in Plant-Microbiome Interactions

The bacteria *Brevibacterium casei* and *Pseudomonas oryzihabitans* increased the synthesis of secondary metabolites of phenolic compounds, including caffeic acid, chlorogenic acid, and rutin, in the plant *Salicornia europaea* by increasing the activity of the enzyme phenylalanine ammonia lyase (PAL) [73]. An important mechanism by which microbiomes can alter the synthesis of secondary metabolites is systemic induction. Colonization of tomato roots by *Bacillus subtilis* strain 3610 resulted in the systemic excretion of the secondary metabolite acylsucrose (S4:17) from the roots. This phenomenon, known as systemic root-induced excretion of secondary metabolites (SIREM), is mediated by the rhizosphere microbiome, and these metabolites play a defensive role for the plant [85]. Rhizobacterium *Pseudomonas simiae* WCS417 induced the expression of coumarin biosynthesis genes, including *F6'H1* and *BGLU42*,

by activating the transcription factor MYB72 in *Arabidopsis* roots. This activation led to the accumulation and secretion of the secondary metabolite scopoletin in the root. Scopoletin had antimicrobial properties against soil-borne fungal pathogens such as *Fusarium oxysporum* and *Verticillium dahliae* and also functioned in shaping the root microbiome by selecting for beneficial bacteria and eliminating pathogens. In addition, scopoletin helped the plant absorb iron by chelating and reducing trivalent iron under iron deficiency conditions [86]. *Bacillus amyloliquefaciens* FZB42 induced systemic resistance and increased expression of defense genes related to antioxidant and phenylpropanoid pathways in tomato plants through the production of the secondary metabolite fungicin [87]. *Bacillus cereus* strain C1L induced systemic resistance in tobacco (*Nicotiana tabacum*) by producing the volatile compound dimethyl disulfide (DMDS) as an elicitor, activating the jasmonic acid and ethylene signaling pathways, and increasing the activity of defense enzymes related to secondary metabolites, including phenylalanine ammonia lyase (PAL), peroxidase (PO), and polyphenol oxidase (PPO) [88]. *Bacillus proteolyticus* OSUB18 activated the plant's defense mode by producing the volatile compound acetoin. Following infection with the bacterial pathogen *Pseudomonas syringae*, the hormone salicylic acid (SA) and the expression of its related genes (such as *PR1*) were increased. Similarly, after infection with the fungal pathogen *Botrytis cinerea*, the hormone jasmonic acid (JA) and its related genes (such as *PDF1.2*) were increased. In both cases, the plant's physical and chemical defenses, including the accumulation of callose (a sugar polymer in the cell wall) and the production of reactive oxygen species (ROS), were more potent. The expression of key genes regulating systemic defense (*MYC2*) and ROS production (*RBOHD*) was also increased. In addition to stimulating indirect defense, this bacterium also directly inhibited the growth of pathogens and enhanced plant growth by producing substances such as siderophores and ammonia [89]. *Pseudomonas* and *Bacillus* bacteria, by secreting the lipopeptide elicitor surfactin, increased the synthesis of phenolic compounds and phytoalexins in bean, tomato, and tobacco plants through the ethylene and jasmonic acid signaling pathways. The fungus *Trichoderma virens*, by producing the siderophore ferricrocin, increased the synthesis of secondary defense metabolites in corn by inducing systemic resistance. The bacterium *Azospirillum brasilense* induced the accumulation of phenolic compounds and callose in wheat by secreting lipopolysaccharide (LPS). *Aspergillus niger* cellulase enzyme, used as a fungal elicitor in licorice cell suspension culture, led to an increase in phenolic, flavonoid, and tannin content, as well as antioxidant activity [90]. The bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* increased the synthesis of phytoalexins and phenolic compounds in *Arabidopsis* and tomato by producing the DAPG (2,4-diacetylphloroglucinol) elicitor and activating the ethylene-jasmonate signaling pathway. This process represents an effective biocontrol strategy in which microorganisms enhance systemic resistance by modulating plant secondary metabolism [26]. The bacterium *Streptomyces* AgN23 in *Arabidopsis* modulates the plant sphingolipid signaling pathway by inhibiting the IPCS enzyme, inducing the production of camalexin. This interaction leads to the activation of the plant defense system, including the production of the secondary phytoalexin camalexin and the activation of defense genes. These metabolic changes increase the plant's resistance to pathogens and indirectly provide conditions for better establishment of the bacteria themselves. By producing secondary defense compounds in response to microbial signals, the plant prepares the rhizosphere environment for the presence of beneficial microbial populations and prevents the growth of harmful microorganisms. This indicates that the production of secondary compounds in plants is not just a passive defense response but is part of a complex molecular interaction with the microbiome that ultimately leads to the formation of a beneficial microbiome and increased plant resistance [91]. Beneficial rhizosphere bacteria, including *Pseudomonas* and *Bacillus*, activated jasmonic acid and ethylene signaling pathways in tomato and tobacco plants through the production of volatile compounds such as 2,3-butanediol and increased the synthesis of secondary defense metabolites including phytoalexins (capsidiol), phenolic compounds, and lignin, which made the plants resistant to various pathogens [92]. Application of humic acids to rice plants changed the structure of the root bacterial community by enriching the genera *Chitinophaga*, *Mucilaginibacter*, *Pseudomonas*, and GpI of *Acidobacteria*. These bacteria have the ability to suppress pathogens through the production of hydrolytic enzymes and siderophores. Humic acids also increased the activity of the enzyme phenylalanine ammonia lyase (PAL), which is a key enzyme in the biosynthesis of phenolic secondary metabolites (phenylpropanoids). These findings indicate that humic acids, by enriching the microbial community around the plant root, activate the plant defense system through the synthesis of secondary metabolites, thereby strengthening plant defense against pathogens [93].

Direct Modulation of Biosynthetic Pathways and Accumulation of Secondary Metabolites

Studies have shown that plant resident microbiomes, especially fungal endophytes, can play a crucial role in the synthesis and accumulation of medicinal secondary metabolites. For example, the endophytic fungus *Fusarium redolens* strain DKR2 in *Coleus forskohlii* has been shown to regulate the biosynthetic pathway of the terpenoid secondary metabolite forskolin in the roots of this plant, conferring a protective and defensive role to the plant [94]. Similarly, in an experiment in *Papaver somniferum*, an endophytic bacterial consortium consisting of *Acinetobacter* sp. (SM1B) and *Marmoricola* sp. (SM3B) increased endogenous morphine content by 2250% and thebaine content by 1067% compared to control plants by simultaneously inducing the expression of key genes of the benzylisoquinoline alkaloid biosynthesis pathway (TYDC, NCS, 6OMT, CNMT, NMCH, SalSyn, SalR, SalAT, and COR) [95]. The fungus *Trichoderma harzianum* and its secondary metabolite harzianic acid enhanced the accumulation of steroid glycoalkaloids in tomato plants by activating defense pathways dependent on the hormones ethylene, jasmonic acid, and salicylic acid, and by altering biosynthetic pathways. This led to an increase in secondary metabolites, including tomatine, dehydrotomatine, and tomatidine, which enhanced the overall resistance of the plant [96]. Plant growth-promoting rhizobacteria, including strains *Bacillus subtilis* and *Paenibacillus alvei* (T22), induced systemic resistance and metabolic priming in *Triticum aestivum* L. by reducing the levels of TCA cycle intermediates (such as malic acid and aconitic acid) and increasing aromatic precursors (such as phenylalanine). This caused a shift in carbon flow from primary to secondary metabolism, ultimately leading to the accumulation of defensive secondary metabolites including phenolic compounds, flavonoids, hydroxycinnamic acids (HCAs), and their amides (HCAAs) in response to infection with the pathogen *Puccinia striiformis* f. sp. *tritici* [97]. A bacterial consortium consisting of *Brevibacterium casei* EB3 and *Pseudomonas oryzihabitans* RL18 on *Salicornia europaea* induced the synthesis of secondary metabolites, including apigenin, quercetin, formononetin, caffeic acid, and caffeoylquinic acid, through a mechanism of metabolic reprogramming and enhancement of defense pathways. Under controlled conditions, this reprogramming was accompanied by an increase in growth-promoting metabolites, such as unsaturated fatty acids, sucrose, citric acid,

and acetic acid. Under field conditions, this mechanism led to a stronger accumulation of stress-resistant metabolites, including glucose, fructose, hydroxybenzoic acid, quercetin, and apigenin [98]. Due to their antioxidant and anti-inflammatory properties, phenolic and flavonoid compounds such as quercetin and apigenin play a role in both plant stress resistance and the nutritional value of horticultural crops [99, 100].

Practical Applications and Implications: From Increasing Resistance to Improving Nutritional and Medicinal Value

The application of rhizosphere growth-promoting bacteria, including a *Bacillus*-like bacterium and a *Pseudomonas*-like bacterium (identified by the codes SEB1 and SEB2), led to improved antioxidant status and increased production of secondary metabolites such as phenolic compounds in peppermint plants [101]. Arbuscular mycorrhizal fungi in tomato, lettuce, and spinach plants increase the production of polyphenols, flavonoids, and anthocyanins, enhancing the plant's innate immunity and producing a healthier, more resistant, and more nutritionally valuable crop. This effect is often due to the strengthening of the plant's immune and metabolic systems. By establishing a symbiotic relationship, the fungus makes the plant stronger, and the stronger plant naturally synthesizes more secondary metabolites, which often have a defensive and antioxidant role [102]. Applying suspensions of four plant growth-promoting rhizobacteria strains—*Pseudomonas fluorescens* N04, *P. koreensis* N19, *Paenibacillus alvei* T19, and *Lysinibacillus sphaericus* T22—directly to tomato plant roots resulted in the production and accumulation of defensive secondary metabolites, including hydroxycinnamic acid derivatives (such as caffeoylquinic acid and feruloylquinic acid), flavonoids (including rutin and eriodictyol glycoside), glycoalkaloids (such as alpha-tomatine and dehydrotomatine), and aromatic amino acids (phenylalanine, tyrosine, and tryptophan) [103]. *Bacillus amyloliquefaciens*, by activating PR genes and increasing the activities of phenylalanine ammonia lyase, beta-1,3-glucanase, and chitinase, led to the accumulation of defensive metabolites including phenols, flavonoids, and lignin, which conferred resistance to leaf curl virus in tomato plants [104]. The microbiome consisting of *Glomus* spp. fungi on *Ocimum basilicum* increased the synthesis of the secondary metabolites rosmarinic acid, caffeic acid, and essential oils. *Rhizophagus intraradices* on *Ocimum basilicum* regulated the expression of genes encoding key enzymes in the rosmarinic acid biosynthetic pathway. The microbial consortium consisting of *Rhizophagus irregularis* and *Gigaspora margarita* on *Echinacea purpurea* increased the concentration of caffeic acid derivatives, alkamides, and terpenes. *Rhizophagus irregularis* on *Stevia rebaudiana* increased the content of steviol glycosides. A comparison of *Rhizoglomus clarus* and *Rhizophagus irregularis* on *Inula ensifolia* showed that *Rhizoglomus clarus* was more effective in increasing thymol derivatives. *Gigaspora rosea* on *Ocimum basilicum* also increased the production of camphor and alpha-terpineol, while *Gigaspora margarita* reduced the total essential oil content, especially eucalyptol, linalool, and eugenol [105]. The rhizosphere core microbiome of the medicinal plant *Bletilla striata*, including the genera *Paraburkholderia*, *Methylibium*, *Bradyrhizobium*, *Chitinophaga*, and *Mycobacterium*, increased the accumulation of militarine (a secondary metabolite) and polysaccharides (BSP) in the plant through indirect mechanisms. These included improved nutrient uptake (such as nitrogen fixation by *Bradyrhizobium*), synthesis of microbial metabolic pathways (GAE, *bgIX*, and *TPS* genes related to militarine biosynthesis), and modulation of microbial community composition by soil organic carbon, which collectively enhanced the medicinal value of the plant [106]. Numerous studies have reported similar enhancements across a wide range of medicinal plants. For example, inoculation of *Glycyrrhiza uralensis* with *Glomus mosseae* resulted in increased accumulation of glycyrrhizin. In *Hypericum perforatum*, *Rhizophagus intraradices* increased hypericin and pseudohypericin. In *Dioscorea* species, *Glomus etunicatum* increased polyphenols, flavonoids, and anthocyanins. In *Camptotheca acuminata*, a consortium of *Glomus diaphanum*, *Acaulospora mellea*, and *Sclerocystis sinuosa* increased camptothecin. In *Cucumis sativus* seedlings, *Funneliformis mosseae* increased flavonoids, lignin, DPPH activity, and phenolic compounds. This pattern of enhanced secondary metabolite accumulation has been documented in numerous other species [107].

Challenges Facing the Use of Plant Microbiomes

Lack of Accurate Understanding of Plant-Microbiome Interactions

A major challenge in applying microbiome science to horticulture is the still-limited understanding of complex microbe-host interactions [108]. Our understanding of these relationships remains limited and requires more in-depth research to characterize the interactions between microorganisms, plants, and environmental factors [109–111]. The insufficient understanding of the metabolic and signaling networks between plants and microbiomes has made it difficult to design effective microbial consortia and introduce efficient formulations [112]. This lack of knowledge of the precise molecular mechanisms that govern host-microbiome interactions is a serious obstacle to exploiting the full potential of these microorganisms [113]. Furthermore, the effectiveness of a microbe or consortium is context-dependent, varying with host genotype, soil type, and environmental conditions. Competition with the native soil microbiome, nutritional conditions, pH, and moisture are known to be factors affecting the variability of microbial performance [114]. The colonization and persistence of introduced strains in new host rhizospheres are often unpredictable [115]. This context-dependency limits the generalizability of field results and hinders the development of universal solutions [3].

Studies have identified specific mechanisms, such as the role of beneficial rhizobacteria *Pseudomonas* and *Bacillus* in activating jasmonic acid and ethylene signaling pathways through volatile compounds like 2,3-butanediol, leading to increased synthesis of secondary defensive metabolites [92]. However, the integrated signaling networks, receptor-level interactions, and context-dependent regulation of these pathways remain poorly understood.

Implementation, Economic and Legal Challenges in the Exploitation of Microorganisms

Producing standardized, reliable microbial formulations is also challenging. The stability and colonization ability of introduced microbes often vary under field conditions, unlike in controlled lab environments, limiting the reliability of results across different settings [116–118]. From an applied perspective, the reproducibility of results in real field conditions is limited due to the variability of soil, climate, and native microflora [119–121]. Determining the optimal dose of inoculum is of great importance in the application of microorganisms. The results of various studies have shown that, in general, high doses do not necessarily provide the best performance, and more balanced doses are usually more effective in terms of growth, nutrition, and disease control [122]. Another challenge is the difficulty of in vitro

cultivation and mass production of many endophytes due to the inability to recreate the real conditions of the host plant [69]. The effectiveness of endophytes is highly dependent on internal and external factors, including the physiological state of the plant, species, geographical location, and even the sampling season [75]. More than 80% of endophytes cannot be identified with conventional culture media [123], and endophytes isolated from one species are not always compatible with other species [124]. The lack of knowledge of many endophyte–host–pathogen interactions has hindered the development of effective formulations [125, 126]. High production costs, difficulty in registering and commercializing microbial products, and biosafety issues are other obstacles to the development of this technology [127–129]. Legal challenges associated with engineered microorganisms require more rigorous regulatory frameworks to verify their biosafety [130, 131].

A study on the endophytic fungus *Acrophialophora jodhpurensis* demonstrated that seed coating with specific adhesives (e.g., sugar, carboxymethyl cellulose) could enhance root colonization and improve growth and disease resistance in tomato [59]. However, scaling up this formulation while maintaining spore viability, ensuring adhesion uniformity, and achieving cost-effectiveness under diverse storage and field conditions remains a major technical barrier. Moreover, regulatory approval for such a novel fungal agent requires extensive biosafety and efficacy data, which lengthens the time-to-market and increases development costs.

Computational and Analytical Limitations in Plant Microbiome Studies

A deeper understanding of plant-microbiome relationships requires integrating multi-omics data (e.g., metagenomics, metabolomics), which capture molecular information at different levels [132]. Integrating multi-omics data (e.g., metabolome, transcriptome, microbiome) remains challenging. To analyze these data, computational tools such as CCLasso and SparCC have been developed for networking and modeling microbial correlations, and the mmvec tool for predicting interactions between microorganisms and metabolites. However, specialized and integrated software for the application of these tools in plants is still in its early stages of development [133]. The lack of reference databases for the precise differentiation of metabolite origin (plant or microbial) is a serious challenge [134]. This lack has further increased the need to utilize technologies such as mass spectrometric imaging (MSI), which shows the spatial distribution of molecules, and high-performance liquid chromatography-mass spectrometry (LC-MS), which separates and identifies compounds in mixtures [135, 136]. Phylogenetic tools based on gene markers such as 16S rRNA (a gene region for determining the genus and species of bacteria) and ITS (a gene region for identifying fungal species), although efficient in identifying and quantifying the diversity of microorganisms, do not provide information about their metabolic or functional roles [137]. Also, the lack of distinction between statistical correlations and true biological interactions [138] and the lack of detailed environmental data [139] make the interpretation of results difficult. Most studies to date have used simplified systems. Although holo-omics approaches have transformative potential for deciphering complex microbial interactions, their adoption is hindered by high costs, a lack of specialized software, and the inherent difficulty of interpreting large, multi-layered datasets [111]. The development of computational tools, machine learning, and explainable artificial intelligence is essential for interpreting these networks [140, 141]. Understanding the dynamics of host–microbiome interactions requires time-consuming and controlled experiments, but conducting these studies is challenging due to the cost and difficulty of repeated sampling. It is also difficult to interpret omics data and correlate them with physiological traits of plants whose genome sequences are not fully determined and known [111]. The development of statistical models to integrate host and microbiome data [142] and bioinformatics tools specific to holomics (an integrated study of an organism at the molecular level along with all its associated microbes) analysis [143] are future priorities. Pilot studies with low-cost designs and limited sampling can pave the way for more comprehensive research. A deeper understanding of plant-microbiome interactions through holomics can facilitate the management of environmental changes and improve horticultural productivity [113]. Integrating holo-omic data with non-omic analyses also broadens ecological and evolutionary perspectives. The development of novel microscopic and molecular technologies can enable more detailed analysis of the plant–endophyte system. For example, single-cell sequencing allows for the investigation of the function of each microbe separately. Advanced fluorescence microscopy reveals the spatial location of endophytes in plant tissue with high resolution. In situ hybridization (FISH) techniques enable the simultaneous identification and localization of microbes. Molecular sensors track metabolites and exchange signals in real time. Raman microscopy offers non-destructive analysis of chemical compounds at the site of interaction. Together, these technologies have deepened our understanding of the molecular mechanisms governing this symbiosis [113, 123]. Overcoming these obstacles requires collaboration between plant biologists, microbiologists, and data specialists.

A study on *Papaver somniferum* found that a bacterial consortium significantly increased the accumulation of morphine, thebaine, and alkaloid biosynthesis genes [95]. Determining whether the observed metabolic changes result from plant metabolic reprogramming or direct microbial activity remains challenging due to the lack of comprehensive metabolite origin databases. Interpreting such multi-omics data requires advanced computational tools and reference databases, which are currently underdeveloped for plant-microbe systems. Moreover, modeling the intricate regulatory network coordinating gene expression and metabolic flux in response to microbial cues remains beyond the reach of current analytical frameworks.

New Technologies in Studying Rhizosphere Interactions

Recent research is exploring how to manipulate the composition and function of the plant microbiome using Syn Coms (synthetic microbial communities), which are simplified versions of the natural plant microbiome. A key goal is to modulate plant secondary metabolite pathways. In fact, by creating a specific microbial community in the laboratory and adding it to the plant, the plant can be induced to produce more beneficial secondary compounds or to become more resistant to environmental stress conditions [121, 144]. The analysis of root-microorganism interactions in living tissue, under natural or near-natural conditions, has been made possible by advanced technologies. Microfluidic systems allow for the live and precise observation of the dynamics of interactions by creating a controlled environment on a micrometer scale (rhizosphere-on-a-chip). Biosensors, which combine a biological element and a transducer, are used to detect and measure signaling molecules and specific metabolic activities during these interactions in real time. The RMI-Chip, as a specialized microfluidic platform, allows for the simultaneous cultivation of roots and microbes in separate chambers for non-destructive observation and precise sampling. TRIS, a transparent alternative to soil, allows for direct 3D imaging of root structure and microbial

distribution by simulating the physical and chemical properties of the rhizosphere environment [145]. The plant microbiome has been proposed as a complex and selectable trait in modern breeding programs. Since the composition and function of the plant-associated microbial community is partly heritable, has a direct impact on plant health and performance, and can be measured using metagenomics (the study of the genes of an entire microbial community without the need for cultivation), it can be considered a new criterion for selecting superior varieties. This approach enables selection for plants that are superior not only genetically, but also due to their ability to establish symbiosis with beneficial microbial populations [146]. EcoFab is an automated platform for the design, construction, and analysis of artificial microbial communities. It serves as an engineered system for studying microbial ecosystems under fully controlled laboratory conditions. These systems eliminate human error and the inherent variability of manual methods through complete automation of experimental processes (e.g., cultivation, transfer, and dilution) and precise control of environmental parameters (e.g., temperature and nutrition). As a result, researchers can create a large number of completely identical samples (repeatable) and maintain constant conditions over time (controllable). The main advantage of this approach is to increase the reliability and validity of scientific data, accelerate the discovery of fundamental principles governing microbial interactions, and enable reliable engineering of microbial communities for specific applications such as agriculture [147, 148]. The use of stable isotopes to determine the origin of metabolites [149] and the application of genome-wide resolved metagenomics [150] are considered novel solutions. Furthermore, the implementation of FAIR principles in omics data—which ensures data is discoverable, accessible, interoperable, and reusable—guarantees the integrity, transparency, and long-term reusability of this valuable data, thereby paving the way for progress [151]. The application of artificial intelligence tools to analyze complex datasets allows for the identification of interaction patterns between genetic and environmental variables in the plant holobiont (i.e., a host plant and all its microbial symbionts functioning as an integrated ecological unit) [140]. Third-generation sequencing technologies, such as MinION and PacBio, increase genomic accuracy and continuity by generating long-read DNA sequences, thereby improving genomic transparency through better coverage of repetitive regions and reduction of sequencing gaps. These technologies, along with advanced computational methods, provide a more comprehensive understanding of the functional dynamics of holobionts [152].

As previously stated, microfluidic platforms such as rhizosphere-on-a-chip enable researchers to simulate soil conditions at a micro-scale while maintaining precise control over environmental parameters. Enhanced by biosensors for detecting signaling molecules and transparent soil substitutes (e.g., TRIS) for three-dimensional root imaging, these systems provide spatiotemporal resolution of microbial colonization, root secretions, and metabolic exchanges [145]. Although such tools are highly valuable for advancing from descriptive studies toward a mechanistic understanding of the rhizosphere, their widespread adoption remains limited due to technical complexity and operational costs.

Knowledge Gaps

Although the role of antimicrobial compounds in plant-microbe interactions is known, the mechanisms of their synthesis and the biochemical basis of these interactions remain unknown. Induction of systemic resistance by beneficial microbes has been observed, but the molecular details of these interactions, especially how microbes coordinate for successful colonization and activation of plant immunity, are not fully understood. There is also a discrepancy between the efficacy of these microbes under greenhouse conditions and their variable outcomes in the field, largely attributable to their inability to survive and establish in the rhizosphere. Although plants actively absorb and excrete microbes, the mechanisms controlling the entry and exit of beneficial or pathogenic microbes are unknown. It is also unclear whether plants selectively utilize microbes as a nutrient source [153]. Although the influence of environmental factors on microbiome composition is known, how environmental changes affect the dynamics and function of microbial communities under field conditions requires further investigation. Despite the introduction of beneficial microorganisms, there is a large gap between laboratory studies and validation of their practical application in the field. Technological advances have been effective in determining the composition and diversity of the microbiome, but our understanding of the physiological and molecular functions of these microbes and their precise role in influencing host genotype, phenotype, and productivity remains incomplete. To achieve valid results in microbiome studies, it is necessary to standardize the different stages of research (experimental design, sample preparation, data analysis) due to the significant variability observed across studies [154]. While the role of root exudates in recruiting rhizosphere microbes is known, their composition and quantity are quantitative traits controlled by multiple genes. This complexity obscures the specific contribution of rhizobia to disease resistance and the interplay between plant genes, root exudates, and resistance-associated microbes. Technologies such as QTL mapping and genome-wide association studies (GWAS) have enabled the identification of genes associated with these quantitative traits. To address these gaps, the Sterile Microcosm Culture System can be used to assess the contribution of rhizobia to disease resistance and, by employing QTL/gene analyses, metabolomics, and microbiome sequencing, understand the relationship between plant genes, root exudates, and resistance-associated microbes [155]. Root exudates play a role in shaping the rhizosphere microbiome, but the molecular mechanisms and factors influencing the composition and function of these microbial communities are still poorly understood. The development and application of synthesized microbial communities in agriculture requires further research, as the mechanisms of colonization, performance stability, and competitiveness with native microorganisms are not fully understood. Another knowledge gap is that soil management practices do not produce the same results at different locations and times, and environmental factors affect their effectiveness. Understanding the mechanisms of root colonization and microbial function is essential for rhizosphere engineering. The use of omics technologies and multidisciplinary approaches to decipher complex plant-microbe interactions and optimize microbial communities for stress resistance is a promising direction [156]. The research highlights the complexity of the molecular dialogue between plants and fungi and emphasizes the need for future studies to identify the precise function of secretome proteins and how these signals are integrated into plant signaling networks to regulate growth and defense [8]. Understanding the synergistic or antagonistic effects of environmental factors that simultaneously affect the plant is an important knowledge gap. Since under natural conditions, the accumulation of secondary metabolites is induced by several factors simultaneously, the question arises: what are the combined effects of environmental factors on biosynthetic pathways and secondary metabolite accumulation? Another issue is the lack of understanding of the molecular and regulatory

mechanisms that drive physiological responses. Although changes in gene expression have been noted, the comprehensive signaling networks and transcriptional regulators that integrate these responses are unknown. This raises the question: what are the precise molecular mechanisms and regulatory networks that mediate the response of secondary metabolites to environmental stimuli? Also, the response of secondary metabolites to an environmental factor can vary between different species, cultivars of the same species, and even tissues of the same plant. This diversity represents a knowledge gap about the genetic and epigenetic basis of these differences. Understanding these foundations for predicting plant responses and optimizing cultivation should be addressed in future studies. A final point is that transferring this knowledge from laboratory conditions to large-scale agricultural systems will require future research on the sustainability and efficiency of these strategies in real-world complex environments [157]. A study introduced the systemic release of root-derived secondary metabolites (SIREM), in which colonization of tomato roots by *Bacillus subtilis* 3610 triggers the systemic release of a specific acyl sugar (S4:17) into the rhizosphere [85]. While this response is clearly associated with plant defense, the signaling pathways that coordinate metabolite synthesis in the shoots, transport to the roots, and release into the soil remain largely unknown. Furthermore, it is unclear how SIREM interacts with the native soil microbiome, whether it is conserved across crop species, and how environmental stressors may modulate its efficacy.

CONCLUSION

This systematic review demonstrates that plant microbiomes residing in the rhizosphere, endosphere, and phyllosphere play an important role in improving the quantity and quality of primary and secondary metabolites in horticultural crops. There is strong evidence that beneficial microorganisms, through direct (e.g., improving nutrient availability, producing phytohormones and enzymes) and indirect (e.g., inducing systemic resistance and suppressing pathogens) mechanisms, direct plant metabolic pathways in a way that not only enhances plant growth and yield but also promotes the accumulation of valuable secondary metabolites associated with quality, flavor, aroma, medicinal properties, and stress resistance.

The practical application of this knowledge in the form of biofertilizers and microbial consortia has shown great potential to move towards sustainable horticultural production systems with reduced dependence on chemical inputs. However, the successful transfer of this technology from controlled laboratory conditions to the field requires overcoming challenges such as developing a better understanding of complex plant-microbiome interactions under different environmental conditions, creating stable formulations, and establishing appropriate regulatory frameworks.

Future advances in this field will depend on the integration of holo-omics approaches, new imaging and tracking technologies, and artificial intelligence to understand the causality of these interactions and engineer targeted microbial communities. A deeper understanding of microbial interactions and their communication with plants will enable the future design of more effective microbial consortia for field applications. Furthermore, leveraging multi-omics (hologenomics) data, artificial intelligence technologies, and predictive modeling to analyze the complex plant–microbe–environment nexus will pave the way for developing tailored microbiome-based solutions for specific plant genotypes and environmental conditions. Overall, the application of microbiome-based strategies is considered a promising solution and an inevitable necessity to ensure food security, produce high-quality products, and realize sustainable agriculture in the current century. The synthesis of more secondary metabolites under the influence of the microbiome will lead to the production of products with unique aroma, flavor, and medicinal properties. This will ultimately improve the profitability of horticultural production by enabling access to target markets and reducing the costs of chemical inputs. Finally, economic considerations must also be taken into account. These will vary across different production systems and require region-specific analysis.

Conflict of Interests

The authors have no conflicts of interest.

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