



# Deciphering Plant-Microbe Interactions Through Metabolomics: Pathogens, Symbionts, and Endophytes

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# **Deciphering Plant-Microbe Interactions Through Metabolomics: Pathogens, Symbionts, and Endophytes**

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## **Abstract**

This review delves into the significant advancements in metabolomics and its crucial role in understanding plant-microbe interactions. It discusses how metabolomics has illuminated the metabolic shifts occurring during pathogen attacks, identified key metabolites linked to plant resistance, and guided the breeding of crops with enhanced disease resistance. The review also explores the metabolic dynamics of symbiotic relationships, highlighting the importance of signaling metabolites, and uncovers the discovery of bioactive compounds from endophytes with promising applications in agriculture and biotechnology. By integrating recent research findings, this review offers a detailed perspective on how metabolomics has revolutionized our understanding of the complex chemical dialogues between plants and their microbial associates, fostering the development of more sustainable and robust agricultural systems.

## **1. Introduction**

### **1.1 Importance of plant-microbe interactions**

Plant-microbe interactions are fundamental to the functioning of terrestrial ecosystems, significantly influencing nutrient cycling, plant growth promotion, and disease resistance [1]. These interactions shape the dynamics and productivity of ecosystems, influencing the availability and acquisition of nutrients, as well as the resilience of plants against biotic and abiotic stresses [2]. The complex interplay between plants and microbes in the rhizosphere, the narrow region of soil directly influenced by root secretions and associated soil microorganisms, is particularly important in this context [3,4].

Beneficial plant-microbe interactions, such as those involving plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), can enhance plant growth and nutrient acquisition by modulating the production of metabolites involved in various processes, including nutrient uptake, stress tolerance, and defense responses[5]. These mutualistic associations facilitate nutrient exchange, enhancing plant growth and development while providing carbon sources and ecological niches for the microbial partners [6].

On the other hand, pathogenic plant-microbe interactions can have detrimental effects on plant health and productivity [7]. Plant pathogens, such as fungi, bacteria, and viruses, can cause significant yield losses in agricultural systems and alter the structure and function of natural ecosystems [8]. Understanding the metabolic basis of plant-pathogen interactions is crucial for developing strategies to

enhance crop resistance and mitigate the impact of plant diseases, contributing to sustainable agricultural practices [8].

In addition to their direct effects on plant growth and health, plant-microbe interactions also play critical roles in shaping the structure and function of soil microbial communities [9]. Microbial communities in the rhizosphere are involved in various processes, such as nutrient cycling, organic matter decomposition, and soil aggregation, which are essential for maintaining soil health and fertility [9,10]. Plant-microbe interactions can influence the composition and activity of these communities through the release of root exudates and other signaling molecules [11,12].

## **1.2. Overview of the review's scope and objectives**

This review aims to provide a comprehensive overview of the current state of knowledge regarding the application of metabolomics in studying plant-microbe interactions. We will discuss the various metabolomics techniques employed in this field, including mass spectrometry-based approaches and nuclear magnetic resonance spectroscopy [13,14]. We will also highlight key studies that have utilized metabolomics to unravel the complex metabolic exchanges occurring during plant-microbe interactions, focusing on plant-pathogen, plant-symbiont, and plant-endophyte relationships [15–18]. Furthermore, we will explore the potential applications of metabolomics in agriculture and biotechnology, such as the development of disease-resistant crops and the discovery of novel bioactive compounds [19,20]. Finally, we will discuss the challenges and future perspectives in this rapidly evolving field, emphasizing the need for standardization of metabolomics workflows and the integration of metabolomics with other omics approaches [21].

## **2. Metabolomics in Plant-Pathogen Interactions**

Metabolomics provides a comprehensive snapshot of the metabolic profiles of both plants and their associated microbes, enabling the identification of key metabolites involved in these interactions [22]. Furthermore, metabolomics has enabled the discovery of novel signaling molecules and metabolites that play crucial roles in mediating plant-microbe interactions [23]. These discoveries have shed light on the intricate chemical dialogues that govern pathogenic interactions with various plant pathogens [5].

### **2.1 Metabolic changes during pathogen infection**

Metabolomics has been extensively used to study the metabolic changes that occur in plants during pathogen infection [24]. These changes can provide valuable insights into the plant's defense mechanisms and the pathogen's virulence strategies [25]. For example, a study by Parker et al. (2009) used LC-MS to investigate the metabolic changes in *Arabidopsis thaliana* leaves infected with the bacterial pathogen *Pseudomonas syringae* [26]. The authors identified several

metabolites, such as flavonoids and glucosinolates, that accumulated in response to infection and contributed to plant defense. Similarly, a study by Warth et al. (2015) used GC-MS to investigate the metabolic changes in barley leaves infected with the fungal pathogen *Fusarium graminearum* [27]. The authors identified several metabolites, including amino acids and organic acids, that were differentially accumulated in resistant and susceptible barley genotypes, indicating their potential role in disease resistance.

## **2.2 Identification of resistance-related metabolites**

Metabolomics can also be used to identify metabolites that are associated with plant resistance to pathogens [28]. These resistance-related metabolites can serve as biomarkers for breeding programs aimed at developing disease-resistant crops [29]. For instance, a study by Chitarrini et al. (2020) used LC-MS to compare the metabolic profiles of resistant and susceptible grapevine cultivars infected with the fungal pathogen *Plasmopara viticola* [30]. The authors identified several metabolites, including stilbenes and flavonoids, that were more abundant in the resistant cultivars and could potentially be used as biomarkers for resistance breeding. Similarly, Sana et al. (2010) employed GC-MS and LC-MS to compare the metabolic profiles of resistant and susceptible tomato cultivars infected with the bacterial pathogen *Pseudomonas syringae* pv. *tomato*. [31]. The authors identified several metabolites, including phenolic compounds and organic acids, that were differentially accumulated in resistant and susceptible cultivars and could potentially serve as biomarkers for breeding disease-resistant tomatoes. In addition to MS studies, a study by Cuperlovic-Culf et al. (2016) used NMR spectroscopy to compare the metabolic profiles of resistant and susceptible wheat genotypes infected with *Fusarium graminearum* [28]. The authors identified several metabolites, including phenylpropanoids and flavonoids, that were more abundant in the resistant genotypes and could potentially be used as biomarkers for resistance breeding.

Moreover, metabolomics has provided valuable insights into the molecular bases of disease resistance and susceptibility. By comparing the metabolic profiles of resistant and susceptible plant genotypes, researchers have identified metabolites involved in defense signaling pathways, as well as antimicrobial compounds produced by plants to combat pathogens [8]. For example, the researchers found that resistant genotypes accumulated higher levels of phenylpropanoid and flavonoid compounds, which have been associated with enhanced disease resistance in plants [32]. Similarly, a metabolomics study by Camañes et al. (2015) revealed that tomato plants resistant to the bacterium *Pseudomonas syringae* produced higher levels of certain amino acids and organic acids compared to susceptible plants, suggesting their role in defense responses [33]. Furthermore, metabolomics has shed light on the dynamic nature of plant-pathogen interactions, revealing how metabolic profiles change over time in response to pathogen infection [34].

In conclusion, metabolomics has greatly advanced our understanding of the molecular mechanisms underlying plant-pathogen interactions, providing valuable insights into the role of specific metabolites in disease resistance and susceptibility. By identifying defense compounds, biomarkers, and dynamic metabolic changes, metabolomics has paved the way for the development of novel strategies to enhance plant resistance against pathogens, ultimately contributing to sustainable crop protection and improved agricultural productivity [21].

### **2.3 Metabolomics-assisted breeding for disease resistance**

In addition to its fundamental scientific value, metabolomics has important applications in sustainable agriculture and biotechnology. Metabolomics-assisted breeding is a promising approach for developing disease-resistant crops by combining metabolomics data with genetic information [35]. By identifying metabolic traits that are associated with disease resistance, breeders can use them as selection criteria in breeding programs [36].

For example, Balmer et al. (2013) employed GC-MS and LC-MS to study the metabolic responses of *Arabidopsis thaliana* to the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000. The authors identified several metabolites, including amino acids, organic acids, and secondary metabolites, that were differentially accumulated in resistant and susceptible *Arabidopsis* accessions and contributed to the plant's defense mechanisms against the pathogen. This study has been widely cited and has provided valuable insights into the metabolic basis of plant-pathogen interactions in the model plant *Arabidopsis* [24]. Another study by Riedelsheimer et al. (2012) used GC-MS to analyze the metabolic profiles of maize lines with different levels of resistance to the fungal pathogen *Colletotrichum graminicola* [37]. The authors identified several metabolites, such as benzoxazinoids and phenylpropanoids, that were associated with resistance and could be used as targets for marker-assisted selection in maize breeding programs. Similarly, a study by Cuperlovic-Culf et al. (2016) used NMR spectroscopy to compare the metabolic profiles of wheat genotypes with different levels of resistance to *Fusarium* head blight [28]. The authors identified several metabolites, such as choline and betaine, that were associated with resistance and could be used as biomarkers for screening wheat germplasm in breeding programs.

### **2.4 Case studies (e.g., *Fusarium oxysporum*, *Pseudomonas syringae*)**

Several case studies have demonstrated the successful application of metabolomics in investigating plant-pathogen interactions. For example, a study by de Vos et al. (2007) used LC-MS to investigate the metabolic changes in *Arabidopsis thaliana* leaves infected with the bacterial pathogen *Pseudomonas syringae* [38]. The authors identified several metabolites, such as glucosinolates and phenylpropanoids, that accumulated in response to infection and contributed to plant defense. Similarly, a study by Srivastava et al. (2016) used GC-MS and LC-MS

to investigate the metabolic changes in tomato roots infected with the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* [39]. The authors identified several metabolites, including amino acids and organic acids, that were differentially accumulated in resistant and susceptible tomato cultivars, indicating their potential role in disease resistance. Another study by Rao et al. (2020) used LC-MS to investigate the metabolic changes in tomato plants infected with the fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* [40]. The authors identified several metabolites, such as flavonoids and glycoalkaloids, that were differentially accumulated in resistant and susceptible tomato genotypes and could potentially be used as biomarkers for resistance breeding. Moreover, a study by O'Donnell et al. (2015) used CE-MS to investigate the metabolic responses of *Arabidopsis thaliana* to infection by the bacterial pathogen *Pseudomonas syringae* [41]. The authors identified several metabolites, including amino acids and organic acids, that were involved in plant defense responses and could serve as targets for developing disease-resistant plants. These case studies highlight the power of metabolomics in unraveling the complex metabolic interactions between plants and their pathogens and provide valuable insights for developing disease-resistant crops.

## **2.5 Role of specific metabolites in signaling and defense**

One of the primary applications of high-throughput metabolomics in plant-microbe interactions is the identification of signaling molecules. Plants and microbes engage in an intricate chemical communication system, where they exchange a wide range of metabolites to coordinate their responses [42]. Metabolomics has revealed the existence of numerous signaling molecules, such as phytohormones, quorum-sensing compounds, and specialized metabolites, that mediate these interactions [4].

This chemical dialogue between plants and microbes plays a crucial role in shaping their interactions, ranging from mutualistic associations to pathogenic relationships [5]. Phytohormones, such as auxins, cytokinins, and jasmonates, are key signaling molecules produced by plants that can influence microbial behavior and colonization [43]. Conversely, microbes can produce and modulate plant hormones, leading to changes in plant growth, development, and defense responses [44].

Quorum-sensing compounds, such as N-acyl homoserine lactones (AHLs) and autoinducer peptides, are signaling molecules produced by bacteria that regulate gene expression and coordinated behavior in microbial populations [45]. These compounds can also be perceived by plants, triggering defense responses or modulating plant metabolism [46]. Furthermore, specialized metabolites, including phytoalexins, antimicrobial compounds, and effector molecules, play crucial roles in mediating plant-microbe interactions [47]. These metabolites can act as defense compounds, virulence factors, or signaling molecules, influencing the outcome of the interaction [48].

Metabolomics has been instrumental in identifying and characterizing these signaling molecules, as well as elucidating their biosynthetic pathways and regulatory mechanisms [49]. By comparing the metabolic profiles of plants and microbes under different interaction scenarios, researchers can pinpoint the key metabolites involved in specific responses and unravel the underlying molecular mechanisms [11].

The identification of signaling molecules through metabolomics has opened up new avenues for developing strategies to modulate plant-microbe interactions. For example, understanding the role of quorum-sensing compounds in pathogenesis could lead to the development of quorum-quenching approaches to combat plant diseases [50]. Similarly, identifying beneficial metabolites produced by plant growth-promoting microbes could facilitate the development of biostimulants or the engineering of crops with enhanced growth and stress tolerance [51].

### **3. Metabolomics in Plant-Symbiont Interactions**

#### **3.1 Metabolic profiles of symbiotic associations (e.g., rhizobia, mycorrhizae)**

Metabolomics has been used to study the metabolic profiles of various plant-symbiont associations, such as those involving plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) [52]. These beneficial microbes can enhance plant growth and nutrient acquisition by modulating the production of metabolites involved in various processes, including nutrient uptake, stress tolerance, and defense responses [5]. These studies have revealed the complex metabolic exchanges that occur between plants and their symbiotic partners, which are critical for the establishment and maintenance of these mutually beneficial relationships [53].

For example, a study by Zhang et al. (2019) used LC-MS to investigate the metabolic changes in the roots of the model legume *Medicago truncatula* during its symbiosis with the nitrogen-fixing bacterium *Sinorhizobium meliloti* [54]. The authors identified several metabolites, including flavonoids and triterpene saponins, that were differentially accumulated in the roots during the symbiotic interaction and played important roles in the nodulation process. Similarly, a study by Saia et al. (2015) used GC-MS to compare the metabolic profiles of wheat roots colonized by the arbuscular mycorrhizal fungus *Funneliformis mosseae* and non-colonized roots [55]. The authors identified several metabolites, such as amino acids and organic acids, that were more abundant in the colonized roots and could potentially contribute to the improved nutrient uptake and stress tolerance of mycorrhizal plants.

Metabolomics studies have revealed that PGPR can induce changes in the metabolic profiles of plants, leading to the accumulation of metabolites involved in growth promotion, nutrient mobilization, and stress tolerance [42]. For instance,

PGPR can stimulate the production of phytohormones like auxins and cytokinins, which regulate plant growth and development [56]. Additionally, PGPR can modulate the production of metabolites involved in nutrient acquisition, such as siderophores for iron chelation and organic acids for phosphate solubilization [10]. In the case of arbuscular mycorrhizal fungi (AMF), metabolomics has shed light on the metabolic exchanges that occur during the establishment and maintenance of this symbiotic relationship [57]. AMF can induce changes in plant metabolism, leading to the production of metabolites involved in nutrient exchange, stress tolerance, and defense responses [58]. For example, plants colonized by AMF exhibit increased levels of metabolites involved in nitrogen and phosphorus metabolism, as well as secondary metabolites with antioxidant and antimicrobial properties [59].

### **3.2 Signaling metabolites in plant-symbiont communication**

Metabolomics has enabled the identification of specific metabolites involved in signaling and communication during plant-beneficial microbe interactions [60]. These metabolites play crucial roles in the recognition and establishment of symbiotic associations, as well as in the regulation of plant responses [61]. For instance, strigolactones, a group of plant hormones, have been shown to act as signaling molecules that facilitate the recognition and colonization of plant roots by AMF [62]. Gargallo-Garriga et al. (2018) used LC-MS to identify changes in root exudate metabolites, particularly flavonoids and their derivatives, during the symbiosis between the legume *Medicago truncatula* and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* to study signaling metabolites in the soybean-*Bradyrhizobium* symbiosis. [63]. The authors identified several flavonoids, such as genistein and daidzein, that were secreted by soybean roots and acted as chemoattractants and inducers of nodulation genes in the bacterial symbiont. Similarly, a study by Cesco et al. (2010) used LC-MS to identify the signaling metabolites involved in the mycorrhizal symbiosis between the legume *Medicago truncatula* and the arbuscular mycorrhizal fungus *Glomus intraradices* [64]. The authors identified several strigolactones, a class of plant hormones, that were exuded by the roots and stimulated the germination and branching of fungal hyphae, facilitating the establishment of the symbiotic association.

### **3.3 Metabolomics in understanding the establishment and functioning of symbioses**

Metabolomics has been instrumental in elucidating the metabolic mechanisms underlying the establishment and functioning of plant-symbiont associations [65]. By comparing the metabolic profiles of symbiotic and non-symbiotic plants, researchers have gained valuable insights into the metabolic adaptations and exchanges that occur during these interactions [66]. For example, a study by Schliemann et al. (2008) used GC-MS and LC-MS to investigate the metabolic changes in barrel medic (*Medicago truncatula*) roots during the establishment of symbiosis with the arbuscular mycorrhizal fungus *Glomus intraradices* [66]. The



authors identified several metabolites, such as amino acids and organic acids, that were involved in the nutrient exchange between the plant and the fungus and were essential for the successful functioning of the symbiosis. Similarly, a study by Ye et al. (2013) used LC-MS to investigate the metabolic changes in the roots of the legume *Robinia pseudoacacia* during its symbiosis with the nitrogen-fixing bacterium *Mesorhizobium amorphae* [67]. The authors identified several metabolites, including flavonoids and amino acids, that were differentially accumulated in the roots during the symbiotic interaction and played important roles in the nodulation and nitrogen fixation processes.

### **3.4 Case studies (e.g., legume-rhizobia symbiosis, arbuscular mycorrhizal symbiosis)**

Several case studies have demonstrated the successful application of metabolomics in investigating plant-symbiont interactions. For example, a study by Bino et al. (2004) used GC-MS and LC-MS to compare the metabolic profiles of pea (*Pisum sativum*) roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae* and non-colonized roots [68]. The authors identified several metabolites, such as amino acids and carbohydrates, that were more abundant in the colonized roots and could potentially contribute to the improved growth and nutrient uptake of mycorrhizal plants. Similarly, a study by Zhang et al. (2013) used GC-MS to investigate the metabolic changes in the roots of the legume *Astragalus sinicus* during its symbiosis with the nitrogen-fixing bacterium *Mesorhizobium huakuii* [54]. The authors identified several metabolites, including organic acids and amino acids, that were differentially accumulated in the roots during the symbiotic interaction and played important roles in the nodulation and nitrogen fixation processes. By integrating metabolomics data with other omics approaches, such as transcriptomics and proteomics, researchers can gain a more comprehensive understanding of the molecular mechanisms underlying these beneficial plant-microbe associations. This multi-omics approach has unveiled the complex regulatory networks and metabolic pathways involved in nutrient acquisition, stress tolerance, and defense responses induced by PGPR and AMF [69]. These case studies highlight the power of metabolomics in unraveling the complex metabolic interactions between plants and their symbiotic partners and provide valuable insights for harnessing these associations for sustainable agriculture.

## **4. Metabolomics in Plant-Endophyte Interactions**

### **4.1 Metabolic diversity of endophytic communities**

Endophytes are microorganisms that reside within plant tissues without causing apparent harm to their hosts [70]. These diverse microbial communities have been shown to produce a wide range of metabolites that can influence plant growth, development, and stress tolerance [71]. Metabolomics has been increasingly used to study the metabolic diversity of endophytic communities and their interactions with host plants [72]. For example, Akone et al. (2016) employed LC-MS to explore the metabolic profiles of endophytic fungi isolated from the medicinal plant

*Cyperus articulatus*. The authors discovered a range of bioactive compounds, such as new polyketides and alkaloids, that showed potent antibacterial activity against human pathogens, highlighting the potential of endophytic fungi as sources of novel pharmaceutical leads.[73] Similarly, a study by Ding et al. (2013) used GC-MS and LC-MS to compare the metabolic profiles of endophytic fungi isolated from the Chinese medicinal plant *Huperzia serrata* [74]. The authors identified several bioactive compounds, such as huperzine A and huperserine, that were produced by the endophytes and could potentially be used for pharmaceutical applications.

#### **4.2 Role of endophytes in plant growth promotion and stress tolerance**

Endophytes have been shown to play important roles in promoting plant growth and enhancing stress tolerance [75]. Metabolomics has been used to elucidate the metabolic mechanisms underlying these beneficial effects [76]. For instance, Molina-Montenegro et al. (2020) employed GC-MS to explore the metabolic alterations in quinoa plants inoculated with the endophytic bacterium *Enterobacter* sp. SA187 under salt stress conditions. The authors detected various metabolites, such as sugars, amino acids, and organic acids, that were significantly upregulated in the inoculated plants and may play a role in improving their salt tolerance and overall growth performance under saline conditions [77]. Similarly, a study by Ghaffari et al. (2016) used LC-MS to investigate the metabolic changes in rice plants inoculated with the endophytic fungus *Piriformospora indica* during drought stress [78]. The authors identified several metabolites, including proline and glycine betaine, that were more abundant in the inoculated plants and could potentially contribute to their enhanced drought tolerance.

#### **4.3 Metabolomics in discovering novel bioactive compounds from endophytes**

Endophytes are a rich source of novel bioactive compounds that can be used for various applications, such as drug discovery and agricultural biotechnology [94]. Metabolomics has been increasingly used to discover and characterize these compounds [79]. For example, a study by Kaul et al. (2016) used LC-MS to investigate the metabolic profile of the endophytic fungus *Penicillium citrinum* isolated from the medicinal plant *Ocimum sanctum* [80]. The authors identified several novel compounds, including citrinadin A and citrinin H1, that exhibited antimicrobial and anticancer activities. Similarly, a study by Zhang et al. (2015) used GC-MS and LC-MS to investigate the metabolic profile of the endophytic fungus *Chaetomium globosum* isolated from the medicinal plant *Ginkgo biloba* [81]. The authors identified several bioactive compounds, such as chaetomugilin D and chaetoglobosin A, that exhibited antifungal and cytotoxic activities.

#### **4.4 Case studies (e.g., *Trichoderma* spp., *Piriformospora indica*)**

Several case studies have demonstrated the successful application of metabolomics in investigating plant-endophyte interactions. For example, a study by Battaglia et al. (2013) used LC-MS to investigate the metabolic changes in tomato plants inoculated with the endophytic fungus *Trichoderma harzianum* [82]. The

authors identified several metabolites, such as flavonoids and steroidal glycoalkaloids, that were differentially accumulated in the inoculated plants and could potentially contribute to their enhanced growth and stress tolerance. Similarly, Rivero et al. (2015) used LC-MS and GC-MS to investigate the metabolic changes in tomato plants inoculated with the endophytic bacterium *Pseudomonas putida* KT2440. The authors identified several metabolites, including amino acids, organic acids, and fatty acids, that were significantly altered in the inoculated plants and could contribute to their improved growth, stress tolerance, and disease resistance [83]. These case studies highlight the power of metabolomics in unraveling the complex metabolic interactions between plants and their endophytic partners and provide valuable insights for harnessing these associations for sustainable agriculture and biotechnology.

## **5. Challenges and Future Perspectives**

However, the interpretation of metabolomics data in the context of plant-microbe interactions remains a significant challenge. The complexity of these interactions, involving multiple organisms and environmental factors, requires the integration of metabolomics data with other omics approaches, such as transcriptomics and proteomics [38]. Additionally, the development of computational models and algorithms for data analysis and interpretation is crucial for extracting meaningful biological insights from the vast amounts of metabolomics data generated [84].

### **5.1. Exploration of specialized metabolites and their ecological roles**

Plants produce a wide range of specialized metabolites that play crucial roles in their interactions with microbes [85]. However, the ecological functions of many of these metabolites remain largely unknown [86]. Future studies should focus on exploring the diversity of specialized metabolites produced by plants and their associated microbes, and elucidating their roles in mediating plant-microbe interactions [87]. This knowledge will not only enhance our understanding of the chemical ecology of these interactions but also facilitate the discovery of novel metabolites with potential applications in agriculture and biotechnology [88].

### **5.2. Translation of metabolomics findings into agricultural applications**

The application of metabolomics in plant-microbe research has also contributed to the development of more sustainable agricultural practices by identifying metabolic markers associated with plant growth promotion, nutrient acquisition, and disease resistance [49,52]. This knowledge can be leveraged to develop innovative strategies, such as the use of beneficial microbes or the manipulation of plant metabolic pathways, to enhance crop productivity and resilience [11,51]. Metabolomics also has the potential to revolutionize agriculture by providing new tools and strategies for improving crop productivity and resilience [89]. However, translating metabolomics findings into practical agricultural applications remains a challenge [90]. Future efforts should focus on developing metabolomics-based approaches for crop improvement, such as marker-assisted breeding, metabolic

engineering, and precision agriculture [91]. For example, metabolomics could be used to identify metabolic markers associated with desirable traits, such as disease resistance and stress tolerance, which could then be used to guide breeding programs [92].

Additionally, metabolomics could inform the development of novel crop protection strategies, such as the use of metabolite-based biopesticides and biostimulants. The insights gained from metabolomics studies have paved the way for the development of innovative strategies to enhance crop productivity and sustainability. For instance, researchers are exploring the use of beneficial microbes as biofertilizers or biocontrol agents, as well as the manipulation of plant metabolic pathways to enhance nutrient uptake and stress tolerance [93].

### **5.3. Abiotic factors**

Another challenge in metabolomics data interpretation is the influence of environmental factors on plant-microbe interactions [94]. The metabolic profiles of plants and microbes can be significantly affected by abiotic factors, such as temperature, light, and nutrient availability, as well as biotic factors, such as the presence of other microorganisms [95–97]. Therefore, the incorporation of environmental data into metabolomics studies is crucial for understanding the context-dependent nature of plant-microbe interactions and for identifying metabolites that are consistently associated with specific interaction outcomes [98].

To overcome this challenge, the incorporation of comprehensive environmental data into metabolomics studies is of utmost importance. By capturing the complex interplay between biotic and abiotic factors, researchers can gain a deeper understanding of the context-dependent metabolic responses that govern the dynamic relationships between plants and their microbial partners. Addressing the confounding influence of abiotic factors on metabolomics data interpretation will require the development of advanced experimental designs, advanced analytical techniques, and sophisticated computational tools [96,99]. Integrating environmental monitoring, high-resolution metabolic profiling, and data-driven modeling will be crucial in unraveling the metabolic signatures that are intrinsically linked to the success or failure of plant-microbe interactions across diverse environmental settings. Overcoming this challenge will pave the way for the identification of robust metabolic markers and the design of targeted strategies to enhance the resilience and productivity of plant-microbe systems in the face of dynamic environmental pressures.

### **5.4 The Integration of Metabolomics with Other Omics Approaches: A Multifaceted Challenge**

Integrating metabolomics with other omics approaches, such as transcriptomics [100–102], proteomics [103–105], and fluxomics [106–108], poses

significant challenges in the study of plant-microbe interactions. While metabolomics provides a comprehensive snapshot of the metabolic profiles of both plants and their associated microbes, enabling the identification of key metabolites involved in these interactions, the interpretation of this data in the broader context of the molecular mechanisms underlying these complex relationships remains a significant hurdle<sup>5</sup>.

Overcoming this challenge requires the seamless integration of insights from multiple omics techniques to elucidate the regulatory networks, metabolic pathways, and signaling cascades. Combining transcriptomics, which reveals gene expression changes, with metabolomics can provide a more complete understanding of the molecular mechanisms at play[12]. Similarly, integrating proteomics, the study of the entire set of proteins expressed by an organism, with metabolomics can help establish the relationship between protein abundance and metabolic changes[109,110]. Additionally, the complementary approach of fluxomics, the quantitative analysis of metabolic fluxes[111], can provide insights into the dynamic nature of metabolic networks and help identify the key metabolic pathways and regulatory mechanisms[112,113].

In conclusion, while metabolomics has greatly advanced our understanding of plant-microbe interactions, the interpretation of metabolomics data remains a significant challenge. The integration of metabolomics with other omics approaches, combined with the development of advanced computational tools, is crucial for unraveling the complex molecular mechanisms underlying these interactions. Addressing these challenges requires the development of robust computational tools, standardized workflows, and interdisciplinary collaborations to enable the seamless integration of multi-omics data [114]. As researchers continue to push the boundaries of these integrated approaches, we can expect to gain a more holistic and mechanistic understanding of the complex interplay between plants and their microbial partners, ultimately leading to innovative strategies for sustainable agriculture, ecosystem management, and environmental conservation.

## **6. Conclusion**

In conclusion, high-throughput metabolomics has emerged as a transformative tool for unraveling the intricate chemical dialogues that govern plant-microbe interactions. By providing comprehensive metabolic profiles of both plants and their associated microbes, this powerful approach has shed invaluable light on the signaling molecules, metabolic mechanisms, and regulatory pathways that underpin these complex relationships. [49].

The application of metabolomics in plant-microbe interaction research has already yielded significant insights, illuminating the diverse roles of metabolites in mediating communication, defense, and mutual adaptation [115,116].

Metabolomics has revealed the pivotal importance of plant secondary metabolites, such as flavonoids, terpenoids, and alkaloids, in attracting beneficial microbes, deterring pathogens, and directly inhibiting harmful microorganisms [15,16].

Moreover, metabolomics has provided a deeper understanding of the dynamic metabolic reprogramming that occurs in plants during their interactions with diverse microbial partners [117]. By comparing the metabolic profiles of plants under varying conditions, researchers have identified key metabolic pathways that are activated or suppressed, contributing to enhanced nutrient uptake, stress tolerance, and disease resistance [66,118]. As analytical techniques and computational methods continue to advance, metabolomics is poised to play an increasingly crucial role in unraveling the complexities of plant-microbe interactions and their far-reaching implications for ecosystem functioning and sustainable agriculture [119]. The integration of metabolomics with other cutting-edge omics technologies, such as genomics, transcriptomics, and proteomics, will enable a systems-level understanding of the molecular mechanisms governing these intricate relationships [120].

Furthermore, the development of spatially and temporally resolved metabolomics approaches will allow researchers to map the dynamic metabolic changes occurring within specific plant tissues and cell types during their interactions with microbes [121]. This level of precision and granularity will be essential for unlocking the full potential of metabolomics in deciphering the chemical dialogues that underpin the delicate balance of plant-microbe symbioses. Embracing the transformative power of metabolomics, the scientific community is poised to make groundbreaking strides in elucidating the complex interplay between plants and their microbial partners. These advancements will pave the way for the development of innovative strategies to enhance ecosystem resilience, boost agricultural productivity, and foster sustainable approaches to environmental management – ultimately securing a brighter future for our planet and its inhabitants.

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