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Current Opinion in Plant Biology

Spatiotemporal control of root immune responses during microbial colonization

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Abstract

The entire evolutionary trajectory of plants towards large and complex multi-cellular organisms has been accompanied by incessant interactions with omnipresent unicellular microbes. This led to the evolution of highly complex microbial communities, whose members display the entire spectrum of pathogenic to mutualistic behaviors. Plant roots are dynamic, fractally growing organs and even small *Arabidopsis* roots harbor millions of individual microbes of diverse taxa. It is evident that microbes at different positions on a root surface could experience fundamentally different environments, which, moreover, rapidly change over time. Differences in spatial scales between microbes and roots compares to humans and the cities they inhabit. Such considerations make it evident that mechanisms of root-microbe interactions can only be understood if analyzed at relevant spatial and temporal scales. This review attempts to provide an overview of the rapid recent progress that has been made in mapping and manipulating plant damage and immune responses at cellular resolution, as well as in visualizing bacterial communities and their transcriptional activities. We further discuss the impact that such approaches will have for a more predictive understanding of root-microbe interactions.

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Current Opinion in Plant Biology 2023, **74:**102369

This review comes from a themed issue on **Biotic interactions 2023**

Edited by **Li Yang** and **Eunyoung Chae**

For complete overview of the section, please refer the article collection - Biotic interactions 2023

Available online 2 May 2023

https://doi.org/10.1016/j.pbi.2023.102369

Keywords

Arabidopsis root, Restricted immune responses, Cell damage, Microbial colonization, Spatiotemporally resolved next generation technologies.

Introduction

Plants and animals alike cannot avoid exposure to large numbers of microorganisms. Instead, both attempt to cultivate a community of beneficial or innocuous microorganisms around their exposed surfaces that can assist at suppressing the establishment of pathogenic microbes. To cope with pathogenic microbes, plants have evolved a two-tiered innate immune system, which consists of microbe-associated molecular pattern (MAMP)-triggered immunity (MTI) and effector-triggered immunity (ETI) [1]. In general, MTI is initiated upon the recognition of MAMPs by cell surface-localized pattern-recognition receptors (PRRs), and ETI is triggered by the recognition of pathogen effector proteins by nucleotide-binding, leucine-rich repeat receptors (NLRs) [2]. Despite the substantial variations in the strength and duration of immune responses activated by conserved microbial patterns or polymorphic pathogen effectors, MTI and ETI trigger similar, convergent downstream molecular events. Critical components involved in the two immune signaling pathways are jointly required for the activation and potentiation of each other to boost stronger plant defense responses against pathogens [3,4].

In contrast to the prevalence of virulence-assisting effectors in pathogenic microbes, most commensal/beneficial microbes lack the genes encoding structural and regulatory components of secretion systems, for example the type III secretion system (T3SS), which are widespread and essential for effector action and pathogenesis of many pathogenic bacteria [5,6]. Thus, a range of alternative strategies to interfere with MTI-mediated immunity are present for non-pathogenic microbes to achieve host colonization. MTI therefore appears to be a major protective immune layer that needs to be negotiated for engaging in cooperative plant–microbe interactions [7]. Intriguingly, PRRs

indistinguishably recognize identical microbial patterns from commensals and pathogens. Hence, the activation of innate immune system needs to be controlled, incorporating spatial and temporal dynamics to accommodate non-pathogenic "friends" while keeping the vast majority of pathogenic "foes" at bay [8,9].

Our present understanding of plant immunity predominantly came from studying the response of leaves on a whole-tissue scale, often lacking a meaningful degree of spatiotemporal resolution. The *Arabidopsis* root has well-characterized cell types, developmentally defined stages, established cell-type specific markers, and abundant molecular genetic resources, and therefore is an exceptional model for investigating spatiotemporal regulation of immune responses. Accumulated publications have shown that roots are capable of mounting strong cellular and physiological immune responses during microbial recognition [10,11]. Immunity in roots is intricate and variable due to the compartmentalized structure of a root that consists of different cell types with distinct roles and features. Hence understanding the spatial and temporal dynamics of immunity is critical to advance our knowledge about the complex interactions that shape structure and function of rhizosphere communities. With the rapid development of state-of-the-art microscopic visualization, simple-to-use histological stains and high-throughput single-cell sequencing technologies, sufficient temporal and spatial resolutions can now be achieved. Here, we outline current advances on the spatial regulation of innate immune responses in *Arabidopsis* roots at cellular resolution. We also discuss latest visualizable, highly-resolved approaches that would be invaluable for understanding microbial colonization within the context of root immunity and development.

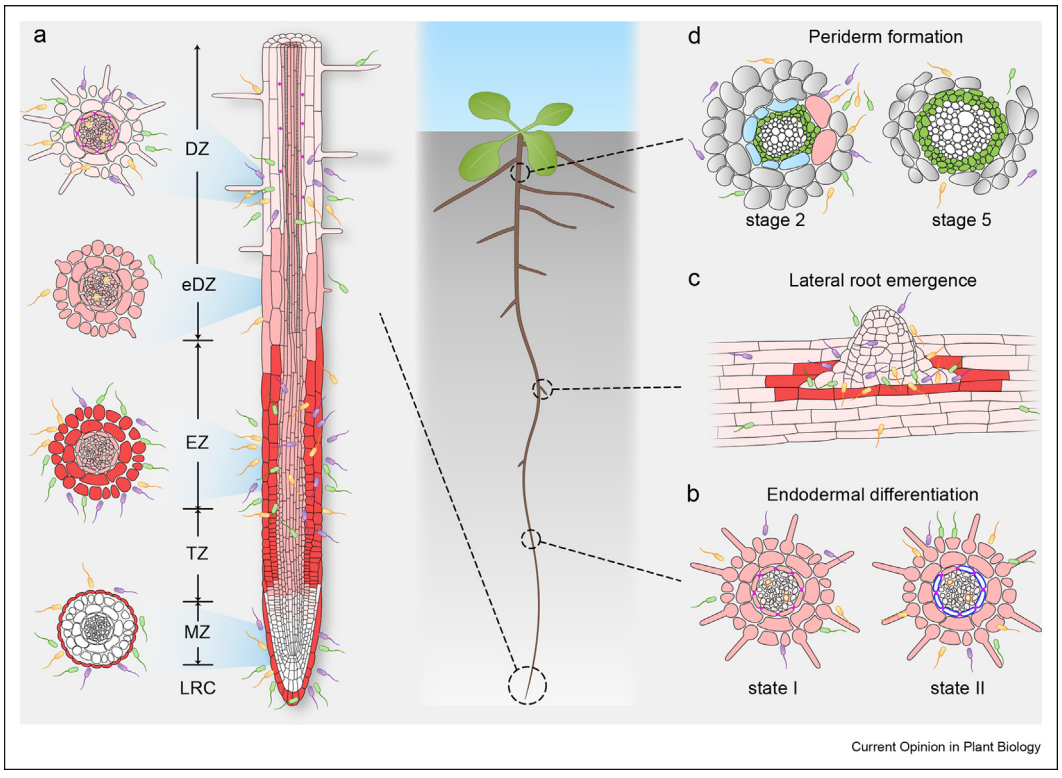
Compartmentalization of root immune responses

Plant roots fulfill functions analogous to animal intestines, providing large surfaces that allow for active and selective accumulation of nutrients. Yet, plants have an inverted topology, compared to animals, such that the processes happening within the lumen in the gut are occurring at the surface of plant roots, allowing for a much easier visualization. As a model system, the study of *Arabidopsis* root development and cellular architecture has seen great advances over the past few decades and the *Arabidopsis* root possibly represent the best understood organ system in plants. The *Arabidopsis* root builds a developmental gradient along the longitudinal axis where three successive zones lay on top of each other, which include the meristematic zone, the transition/elongation zone and the differentiation or maturation zone. Radially, three concentric "outer" cell layers comprising epidermis, cortex and endodermis surround the central "inner" vascular system. Subsequent branching and radial thickening, that is, lateral root formation and periderm development are both crucial for establishing root architecture. It is generally agreed that this strict organization of root cell layers and stages is associated with and determines the compartmentalization and diversity of root immune responses (Figure 1).

In order to obtain cellular resolution readouts of immune responses, a number of elegant tools for visualizing root responses have been developed. A pioneering work by Millet et al. revealed a restricted immune response to high concentrations of two bacterial MAMPs, the flagellar peptide flg22 and peptidoglycan, mostly at the elongation zone of the *Arabidopsis* roots [10] (Figure 1a). However, the methods that were used, β-glucuronidase (GUS) reporter assay and callose staining, are destructive to the root tissues, and therefore did not allow for temporal monitoring or live-imaging of MAMP-elicited responses. By generating transcriptional fluorescent markers with strict nuclear localization signals for obtaining single cell resolutions, it was confirmed that flg22 responses are not only confined to the peripheral root cap and transition/elongation zone, but are also present at specific differentiated cells close to lateral root emergence sites (Figure 1a, c), as well as sites of sporadic damage [9]. By using the intensity-based reporter R-GECO1 for tracing calcium waves, it was shown that MAMPs such as flg22 or chitin induce a spatially restricted onset of Ca^{2+} responses in the root elongation zone, which then spreads toward the root tip and base, illustrating that there is a clear non-cell-autonomous branch of MAMP-triggered immune responses [12,13]. MAMP responses are known to lead to cell wall modifications, such as lignification. Recent whole-mount lignin staining revealed strong lignification induced by flg22 application to root overexpressing the PRR FLS2 in young, epidermal cells of the root [13]. Interestingly, while a weak basal expression of PRR such as FLS2 was not necessarily predictive of immune responsiveness, strong PRR expression or upregulation coincided well with the observed pattern of MAMP responsiveness. This suggests a thresholded relationship between PRR expression and the respective microbial pattern-dependent immune responses [9,14]. This fits with the complex layers of negative regulation of PRR signaling that have been described [15]. Overall, it is now evident that immune responses in roots are strictly confined, and that this spatial regulation is necessary to balance root development and defense [13].

Recently, a cell-type-specific transcriptome analysis based on fluorescence-activated cell sorting (FACS) of epidermis, cortex, and pericycle cells of *Arabidopsis* roots revealed distinct immune responses in different cell types [16]. In line with this result, one study reported that early immune response markers are largely tethered

Figure 1



Spatially distributed immune responses and microbial colonization in *Arabidopsis* root. (**a**) Schematic representation of restricted MAMP responses and microbial colonization, presented as transversal (left panel) and longitudinal views (right panel) for the young part of an *Arabidopsis* root. Root cells in different colors indicate the intensity of immune responses. LRC, lateral root cap; MZ, meristematic zone; TZ, transition zone; EZ, elongation zone; eDZ, early differentiation zone. (**b**) Endodermal barriers compartmentalize MAMP responses in differentiated states I and II of the endodermis. In state I endodermal differentiation, the apoplastic diffusion of MAMPs can be blocked by the Casparian strip (dark pink). In state II, suberin lamellae (blue) prevents direct MAMP signal perception on the cell surface. The unsuberized endodermal cell is indicative of a passage cell. (**c**) MAMP responsiveness and microbial attraction during lateral root formation. Cells indicated in red color surrounding the lateral root emergence site show a strong immune response. (**d**) Possible immune response (pink) patterns and bacterial localization at different stages of periderm formation illustrated by schematics of cross sections. Phellem layer (green) division and expansion causes the surrounding endodermis (blue), cortex and epidermis (gray) to rupture and eventually completely detach from the root once the phellem is suberized.

to the young parts and mostly activated in "outer" cell layers of the root, while immune response markers associated to the defense-related hormone ethylene (ET) were specifically induced in the vascular cell types from the onset of early differentiated zone when exposed to flg22. Surprisingly, no significant root responses for the specific jasmonic acid (JA) and salicylic acid (SA) markers used were observed following perception of flg22 and chitin [17]. Furthermore, flg22 was only able to trigger suberin deposition in the endodermis when FLS2 was expressed in different cell layers. Suberization is a well-described part of the endodermal differentiation program, suggesting that developmental programs have influence on the quality of the immune response [13]. In *Arabidopsis* roots that had undergone extensive secondary growth, the periderm (phellogen) initiation originally arises from cell divisions in the pericycle, which is followed by endodermal cell breakdown, and subsequent cortical and epidermal cell detachment [18] (Figure 1d). It will be fascinating to see whether and how this secondary growth process and its associated loss of cellular integrity translates into immune responsiveness in adjacent cell layers.

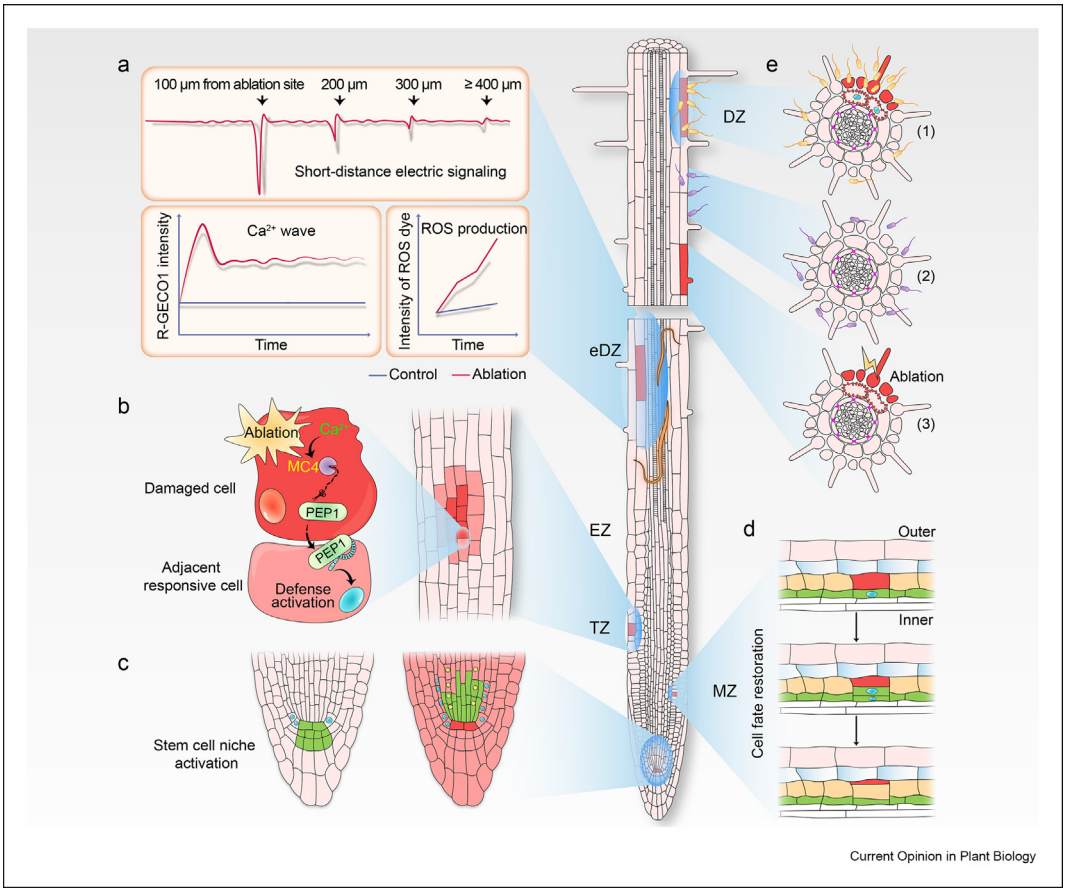
Beyond the diverse functions of root cell types in immune responsiveness, some specialized structures of root cells also play a role in the restriction and compartmentation of defense responses. The root cap cuticle, for example, was shown to facilitate lateral root emergence and protect root meristem from abiotic stresses upon germination [19]. While it has been shown that flg22-induced immune responses are restricted to peripheral root cap cells [13], not enough evidence is present to indicate the direct involvement of root cap cuticle in biotic stress interaction. It would be intriguing to further investigate whether targeted disruption of root cap cuticle can cause MAMP hypersensitivity in otherwise protected meristematic cells. In the endodermis, Casparian strips as extracellular diffusion

barriers can efficiently block the penetration of MAMPs into the stele, which therefore compartmentalizes flg22-dependent transcriptional outputs between inner and outer root cell layers [9]. In addition, suberin lamellae, surrounding the entire endodermis, are thought to prevent molecules from the cell wall to reach the endodermal plasma membrane. Indeed, this hydrophobic layer inhibits the association between plasma membrane-localized PRRs and corresponding MAMP peptides, thus abrogating an intracellular immune response [9]. Interestingly, specific unsuberized endodermal cells, so called "passage cells," show a distinct developmental expression profile and are thought to fulfill unique physiological functions in nutrition uptake and stress resistance [20]. It is speculated that passage cells should also have an immune responsiveness that is distinct from their suberized neighbors (Figure 1b).

Restricted immune responses by localized cell damage

Cell death is the catastrophic, irreversible cessation of fundamental cellular functions. As in animals, plants can undergo active or programmed cell death during development [21,22]. During immune responses, this occurs mainly in the form of the hypersensitive response. Passive cell death can occur as a result of wounding or damage caused by biotic or abiotic stresses. In general, the death of a cell has two types of impact: it can restore

Figure 2



Damage in different parts of the root triggers multiple local and regional immune responses. (**a**) Restricted, single-cell wounding in the early differentiated zone (eDZ) by laser ablation elicits non-systemic, regional surface potential changes, calcium waves, and ROS production. These responses can restrict nematode invasion. (**b**) Wounding in epidermal cells at the transition zone (TZ) induces a prolonged influx of Ca^{2+} in the cytosol, triggering META-CASPASE4 (MC4) to cleave PROPEP1 and to release AtPEP1. It can then diffuse to neighboring cells to activate a localized defense response. Red and pink-color cells indicate damaged and their adjacent responsive cells, respectively. (**c**) Ablations in the stem cell niche trigger JA induction (pink cells) and promote auxin signaling-dependent tissue regeneration (green cells). Dots with different colors show wounding-induced transcriptional regeneration responses. (**d**) Damage healing process after single cell death in the meristematic zone (MZ). Ablation triggers restorative cell divisions in inner adjacent cells (green) to replace the eliminated one (red). (**e**) Schematic model of localized PRR activation and damage-gated local immune responses during root–bacteria interaction in the differentiated zone (DZ). Damaged cells are indicated in red color. In the presence of non-pathogenic bacteria (purple), differentiated root cells have a relatively low *PRR* expression (2). In the presence of pathogenic/damage-inducing bacteria (yellow), the expression of *PRRs* is induced in neighboring cells (red dots on the cell surface), which subsequently activate transcriptional MAMP responses (shown as blue, nuclear-shaped appearance within the cells) (1). The same activation of PRR is observed when damage is elicited using cell-specific ablation (3).

and increase regenerative competence of surviving neighboring cells, and it can trigger or inhibit immune responses [23].

Although damage-induced long-distance responses have been well described in foliar organs, damage is usually inflicted in ways that encompasses many tissue layers and larger areas, thus lacking cellular resolution and precision. Recently, the easily accessible and observable *Arabidopsis* root system has been used for a precise, laser-assisted ablation approach. This minimal wounding technique with spatial and temporal precision allowed the observation of damage perception and responses at the single-cell level in plants [24]. Two subsequent studies reported that localized cell damage by laser ablation in the root transition and elongation zone causes propagating calcium waves, surface potential changes and oxidative bursts in adjacent cells [25,26] (Figure 2a, b). Different from leaves, however, these responses appear much more restricted and non-systemic in roots. Roots have the capacity for inducing and transmitting systemic responses, but this apparently necessitates more drastic and less localized stresses [27]. Apart from being reported to elicit transient, long-distance responses, wounding has also been involved in increasing the production of defense-related phytohormones [28]. More work has confirmed that restricted cell damage in root meristematic zones triggers JA signaling and promotes stem cell activation and regeneration through wounding-responsive genes [29–31] (Figure 2c, d). Surprisingly, contrasting meristem damage, single-cell ablation of the root elongation zone does not trigger a robust JA response, but instead activates ethylene production in a relatively large region around the wounding site [25]. This seemingly contradictory finding might again be explained by the specificities of signaling outputs within different root cell types. Yet in both cases, localized, wound-induced JA or ET accumulation are important for the resistance against infestation by root nematodes [25,29].

Beyond the non-cell-autonomous responses, whether damage can activate very localized, damage-associated molecular pattern (DAMP) or MAMP responses in roots has remained unclear. One study reported that loss of cellular integrity by laser ablation activates rapid processing of AtPEP1, a putative DAMP, and releases it into the apoplast, where it could inform wound signal to the neighbors and initiate signaling through the extracellular PEP receptors to trigger an immune-like response in the immediate vicinity of a damaged cell [26] (Figure 2b). By mapping MAMP responses at high resolution in *Arabidopsis* roots, it was demonstrated that cellular damage is indeed a prerequisite for mounting a strong, localized MAMP response. Through a currently unknown mechanism, cells subjected to injury warn its neighbors, resulting in highly increased PRR expression levels in the immediate neighbors, thus unlocking their ability to sense microbial signals. It was concluded that a combination of wounding and presence of MAMPs, acts as a true danger signal. This mechanism might be widely employed by roots, allowing to restrain immune responses in the presence of non-damage-inducing commensal or beneficial microbes [9] (Figure 2e).

Spatiotemporal microbial colonization

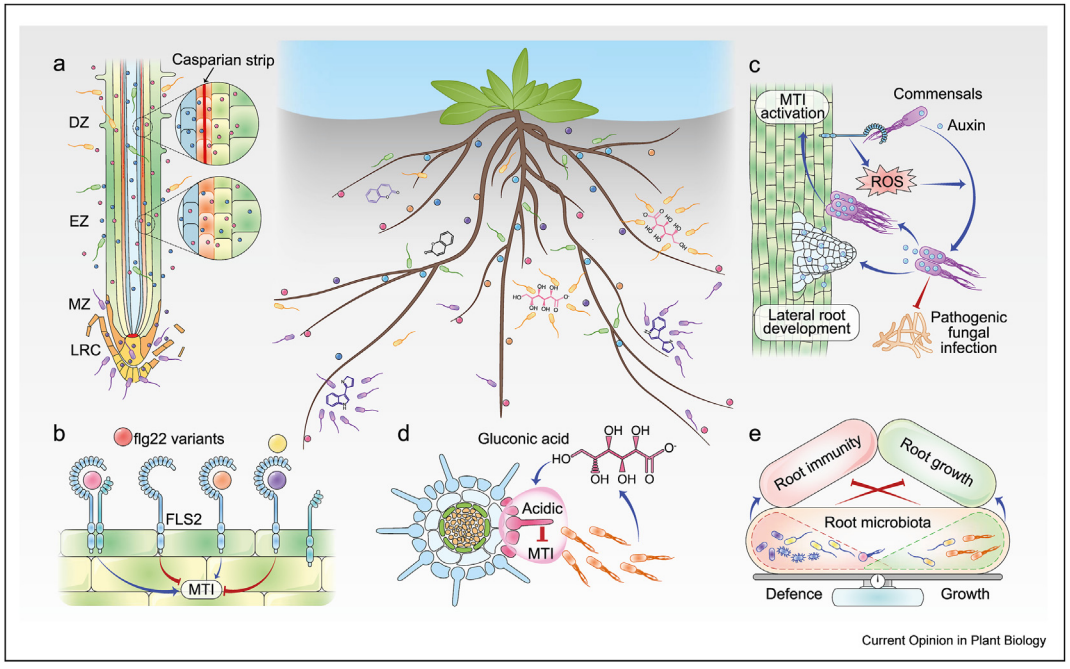
Microbes are known to preferentially associate with specific root regions during colonization. Since the root tips are the tissues that make first contact with its growth environment, root tips are usually enriched with active microbes. Attraction towards the root cap was shown to be an initial path for subsequent colonization of mature root epidermis [32]. During bacterial colonization of root surfaces, bacteria tend to accumulate in the grooves between epidermal cells [33]. By using the microfluidic device TRIS (tracking root interactions system), *Bacillus subtilis* was found to rapidly colonize and form biofilms around the root elongation zone in *Arabidopsis* [34]. Root hairs were also reported to be an important microbial niche, as the absence of root hair alters bacterial community [35]. Interestingly, a recent preprint reported that root hair cells activate immunity during bacterial colonization [36]. In the case of rhizobium, it colonizes and infects leguminous plants mainly through root hairs to form nodules. During this process, gene expression in both the host plant and the bacteria is precisely regulated in a spatiotemporal manner to ensure the exchange of signals between the two organisms is appropriate for the specific nodulation stages [37].

For most pathogens to successfully infect a plant, they penetrate the root radially and spread in the vasculature. Like many commensals, pathogenic bacteria also accumulate around the elongation zone, where endodermal barriers are not yet established, potentially making it a vulnerable zone and favorable entry point [38,39]. The elongation zone therefore appears to be crucial for initial microbial colonization, possibly due to higher diffusion of root exudates that are sensed by microbes as "attractants" for successful root colonization and/or invasion. Another microbial hotspot along the root axis are lateral root emergence sites where Casparian strips are temporarily damaged and suberin is remodeled [40,41]. These sites have been shown to be preferred colonization or entry points for different pathogenic and commensal/beneficial bacteria, such as *Ralstonia solanacearum* GMI1000, *Pseudomonas syringae* DC3000 and *Pseudomonas protegens* CHA0 [9,42,43]. Interestingly, these microbial hotspots coincide with an enhanced flg22 responsiveness, suggesting that roots have evolved adaptive mechanisms to precisely restrict their defense to vulnerable regions that are preferentially colonized by microbes [9] (Figure 1a). Furthermore, the integrity of endodermal diffusion barriers was shown to play an

important role in root-microbe interactions. Endodermal barrier-defective mutants showed modified root exudation profiles and exhibited distinct microbial populations [44]. Depending on the colonization strategies of different microbes, microbial colonization of roots can also influence the development of endodermal barriers [43,44].

One of the critical driving forces that shape the establishment and differential attraction of microbial communities along a root are rhizodeposits, which can broadly include carbohydrates, amino acids, organic acids, lipids, secondary metabolites, etc. [45,46]. Some rhizodeposits are known as specialized secondary metabolites that are involved in plant defense responses and signaling (Figure 3). Recent studies have indicated that several defense-related metabolites, such as coumarins, glucosinolates, benzoxazinoids, triterpenes and camalexin, can alter the root microbial community [47–52]. The exudation of coumarins into the rhizosphere was shown to have the ability to selectively inhibit soil pathogens, while promoting the colonization of growth-promoting bacteria that can activate induced systemic resistance [53]. Interestingly, it has been shown that the release of benzoxazinoids by maize roots influences growth and defense of the next plant generation, likely due to JA-mediated induced systemic resistance [49]. To what extent the modulation of these defense-related metabolites is influenced by the root immune response, or vice versa, is still unresolved, but it is speculated that root microbiome structure can be altered through differential root exudation in response to immune signaling activation and nutrient stresses [54,55]. A cell type-specific metabolomics analysis revealed that different cell types in *Arabidopsis* have distinct metabolic profiles [56]. While this does not directly indicate that different root zones exhibit unique root exudation, the composition of root exudates likely varies along the root axis and over the course of root development, which has been considered as the

Figure 3



Strategies involved in spatiotemporal microbial colonization and the coordination of *Arabidopsis* root immunity and development. (**a**) Spatially secreted root exudates from different root regions influence the assembly and structure of root microbial community. Metabolite movement and exudation are controlled by the presence of Casparian strip (red line). The sloughing/collapse of "border cells," the outermost lateral root cap (LRC) layer, can secrete active compounds such as protein, polysaccharides, phytoalexins and extracellular DNA and promote mucilage production, which may influence the microbial population in the rhizosphere. (**b**) Interaction modes between root microbiota MAMP variants and the corresponding PRR, FLS2. Apart from immunogenic flg22 activating MTI, several different flg22 variants either evade/decrease FLS2-dependent MTI or antagonize perception of immunogenic flg22 peptides. (**c**) The colonization of the auxin-secreting commensal bacteria leads to a positive feedback loop between plant immune response and bacterial auxin secretion. Colonization of the bacteria triggers MTI-induced ROS production, which in turn elicits bacterial auxin production to resist ROS toxicity. Auxin promotes bacterial colonization and further induces plant immune response. Enhanced colonization of the bacteria inhibits pathogenic fungal infection and promotes lateral root development. (**d**) Rhizosphere-associated *Pseudomonas* can produce organic acids to acidify the rhizosphere. Lowering environmental pH can suppress root immunity, which facilitates root colonization by these beneficial microbes. (**e**) Inter-microbial interactions regulate root growth-defense trade-off. The root microbial community consisting of a set of microorganisms with different functions, integrates the balance between plant immunity and development, and buffers the system against pathogen challenge.

fundamental cause of differential microbial assembly and distribution [57,58] (Figure 3a). Studies using bacterial biosensors that respond to different rhizodeposition of pea, corn, black poplar and tomato roots revealed spatial and temporal dynamics in secreted metabolites [59,60]. Expanding such concept to the well-described and small root system of *Arabidopsis*, combined with multiplexed biosensor barcoding would be especially powerful in achieving unprecedented resolutions [61]. Novel approaches that adapt spatial metabolomics are likely to be employed in the coming years for root-microbe research to shed light on rhizodeposit-mediated communication and regulation during microbial colonization of roots [62]. Clearly, a much increased resolution of root exudate distribution and bacterial metabolic activities will be needed to understand the principles of root-microbe colonization [63].

While plants are able to make use of differential root exudation to actively attract or fend off specific microbial species, they also rely on spatiotemporal induction of immune responses. However, both pathogens and commensals have developed strategies to evade or suppress host immunity in order to successfully colonize roots in distinct regions. A direct and possibly pervasive strategy used by many pathogens and commensals to remain incognito is through MAMP modification to avoid plant cell recognition, and to antagonize or dysregulate plant immune responses [64,65] (Figure 3b). In a study that investigated the diversity of flg22 peptides in commensals, it was reported that most flg22 variants are not recognized by the flg22 receptor FLS2 [64]. As part of a strategy to limit microbial invasion in the root, plants downregulate auxin signaling to inhibit lateral root development to decrease the number of potential pathogen entry sites. However, the virulent pathogen *Pseudomonas* DC3000 has developed a mechanism to hijack this host defense strategy by producing auxin to induce lateral root formation [42]. Bacterial auxin secretion is also a strategy used by beneficial bacteria to colonize their hosts. The beneficial bacterium *Bacillus velezensis* secretes auxin in response to the activation of plant immunity, which in turn creates a positive feedback loop between plant immunity and bacterial auxin secretion to promote bacterial colonization and protection of the host from fungal infection [66] (Figure 3c). Salas-Gonzales et al. reported that commensal bacterial communities influence endodermal root barrier formation by interfering with signaling of the stress hormone ABA through a yet unknown mechanism [44]. Another strategy, described for rhizosphere-associated *Pseudomonas*, suppresses flg22-mediated root immune responses by producing organic acids to acidify the rhizosphere [67] (Figure 3d). Root microbial communities appear to have subsets of MTI-suppressive strains that modulate immune responses, which in turn helps other, non-suppressive commensals to colonize the root [68]. In a different study, Ma et al. categorized SynComs into root growth inhibition (RGI)-suppressive and RGI-non-suppressive strains, and showed that MTI activation alters the RGI non-suppressive community, but the presence of RGI-suppressive strains can help resist this alteration [69] (Figure 3e).

As described above, plants additionally rely on a damage-gated immune response that would target responses to sites of damage and might alleviate the need for commensals to suppress immunity. *Arabidopsis* differentiated roots appear to have low PRR expression for a number of receptors and thus respond poorly to their corresponding MAMPs, facilitating colonization of these regions by commensals such as *Pseudomonas* CHA0. Intriguingly, when cells were destroyed by pathogens, the expression of PRRs were induced only in neighboring cells, which subsequently allow strong and confined immune response [9] (Figure 2e). This strategy might prevent plants from constitutively activating immunity, and as a result, efficiently restrict defense responses to potential pathogen entry points. It appears that the combination of plant's ability to spatially regulate defense responses and the various strategies that microbes utilize to locally suppress and/or evade host immunity influences the structure of microbial assembly and root development.

Spatially resolved technologies for illuminating root-microbiota interactions

Plant-microbe interactions take place on a micro-scale spatial context, and it is thus crucially important to improve our ability to spatiotemporally resolve physical, chemical and biological processes that happen during microbial root colonization. A combination of advanced, spatially resolved-omics approaches, advanced imaging methods and machine-learning strategies will yield much deeper insights into the intricate relationships that define rhizosphere community structure and function. Combining fluorescently-labeled bacteria with growth systems that are accessible to microscopic observation, such as in microfluidics platforms or transparent soils, has thus far permitted us to observe some of the dynamics of root-microbe interactions [32,34]. However, the number of bacterial strains that can be simultaneously monitored by simple fluorescence separation is limited due to spectral overlaps. Recent advances in computational methods and the use of combinatorial labeling for fluorescence in situ hybridization (FISH) in mammalian systems has vastly improved the ability for multiplexing, and adaptation of such methods to root-microbiota studies will be of great value. The high-phylogenetic-resolution microbiome mapping by FISH (HiPR-FISH) was shown to be capable of distinguishing over 1000 bacterial taxonomies in mouse gut and human oral plaque [70]. Expanding on

this, Cao et al. reported on the use of sequential error-robust FISH (SEER-FISH) to identify taxonomies in microbial communities on *Arabidopsis* roots [71]. A similar concept was developed for parallel sequential FISH (par-seqFISH) to spatially map the gene expression profile of individual bacteria [72]. Alternatively, microbial single-cell genomics, which has recently become a rapidly emerging field, would be valuable in high-throughput gene expression studies to resolve heterogenous transcriptional states in a microbial community [73,74]. Microbe-seq, which was recently used to obtain strain-resolved genome of human gut microbiome, could be applicable to resolving the genomes of complex microbial communities in the rhizosphere [75]. Through Spatial metaTranscriptomics (SmT), it was shown that it is possible to spatially associate microbial identities to host transcriptional responses in *Arabidopsis* leaf tissue sections [76]. With these advancements in computational technology and experimental methods, integrative approaches hold promise to disentangle complex root-microbiota interactions at the required spatiotemporal resolutions.

Outlook

It has become evident that immune responses in roots cannot be explained without considering the differences between cell types, tissues, and developmental stages. Despite efforts in cell- and tissue-type-specific approaches in *Arabidopsis* roots that have unraveled some of the mechanisms underlying spatiotemporal regulation of root immune responses, many open questions remain due to our limited understanding of how roots respond during microbial colonization, and vice versa, in a spatial context. Future research needs to focus on developing spatial transcriptomic methods that enables the simultaneous measurement of both plant and microbe gene expression profiles. The host-microbe field in mammalian systems has rapidly been developing and employing innovative ways to improve multiplexity through advanced-omics approaches, imaging techniques and machine-learning strategies for interrogating the complex host-microbiota systems in unbiased ways. The same is not yet the case for plant systems, but related approaches are starting to be adopted. An exciting prospect would be to adapt and extend the concepts developed from current multiplexed FISH methods to capture both spatially resolved taxonomy and gene expression information, which would provide insights into the transient regulations of bacterial states at the community level in a rhizosphere. We envision that ongoing advancements in mammalian systems will continue to inspire host-microbiome research tools in plant systems, opening opportunities to explore uncharted questions to resolve the dynamics of microbiome structure and function, spatially and temporally.

Funding

This work was supported by funds from National Key R&D Program of China (2022YFF1001800) and CAS Pioneer Hundred Talents Program (2022000051) to F. Zhou, the European Research Council grant ROOBA-BAA to N. Geldner, the Taiwan Ministry of Science and Technology (111-2917-I-564-021) to H. Tsai and National Natural Science Foundation of China (32201818) to J. Wang.

Declaration of competing interest

The authors do not have any competing interests.

Data availability

No data was used for the research described in the article.

Acknowledgments

We apologize to authors whose relevant work we have not cited, either inadvertently or due to space restrictions. We would like to thank Xiu-Fang Xin, Aurélia Emonet and Ertao Wang for their constructive comments and suggestions on the manuscript.

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