Feature Review

What lies beneath: belowground defense strategies in plants

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Diseases caused by soil-borne pathogens result worldwide in significant yield losses in economically important crops. In contrast to foliar diseases, relatively little is known about the nature of root defenses against these pathogens. This review summarizes the current knowledge on root infection strategies, root-specific preformed barriers, pathogen recognition, and defense signaling. Studies reviewed here suggest that many commonalities as well as differences exist in defense strategies employed by roots and foliar tissues during pathogen attack. Importantly, in addition to pathogens, plant roots interact with a plethora of non-pathogenic and symbiotic microorganisms. Therefore, a good understanding of how plant roots interact with the microbiome would be particularly important to engineer resistance to root pathogens without negatively altering root-beneficial microbe interactions.

Importance of soil-borne pathogens

Plants are crucial for life because they convert sunlight into simple sugars and oxygen through their chlorophyll-containing aboveground organs such as leaves and stems, whose perceived roles often outshine those of 'the hidden half' represented by the roots [1]. However, the importance of roots for plant functioning can hardly be overestimated as these belowground organs are essential for a myriad of physiological processes such as water and nutrient uptake, storage of assimilates, anchoring of plants, and mechanical support of the aboveground organs. Because anchoring of plants by roots in the soil is inevitably linked to a sessile existence, plants evolved to have a highly efficient defense system to defend themselves against pests and pathogens. Importantly, roots are constantly in contact with the soil microbial community consisting of mostly beneficial but also pathogenic organisms. An exponentially growing number of reports focus on how roots cope with the soil microbiome and which mechanisms are involved in microbial recognition by plant roots.

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Diseases caused by root-infecting pathogens routinely cause significant reduction in yield and quality of many crop plants [2,3]. Under favorable environmental conditions (e.g., wet and cool soil conditions), root infections can

Glossary

Biotroph, hemibiotroph, necrotroph: different life styles of pathogens. Broadly, biotrophs can live and multiply only on another living organism, thereby establishing an intimate and often specific interaction using specialized infection structures (e.g., haustoria). By contrast, necrotrophs, infecting multiple plant species, kill their hosts and extract nutrients from death cells. Hemibiotrophs are those that show a mixed life style often biotrophic during initial stages of disease development and necrotrophic during later stages of disease development.
Callose: β-1,3-glucan polymer produced in the plant cell wall often in response to wounding and pathogen attack.
Damping-off: phenomenon of seedlings that collapse or break near the soil line, often due to infection by soil-borne pathogens.
Effector-triggered immunity (ETI): immune response in plants resulting from recognition of pathogen effectors by the plant. This response is in general faster and stronger than MTI and often results in a hypersensitive cell death response.
Flagellin 22 (flg22): conserved 22-amino acid-long N-terminal part of the principal protein of the bacterial flagellum, flagellin; recognized as a MAMP by the plasma membrane localized FLAGELLIN SENSING 2 (FLS2) receptor.
Haustoria: specialized hyphae of biotrophic fungi/oomycetes responsible for nutrient uptake from the (living) host cell and for delivery of effectors. Haustoria penetrate the cell wall and invaginate the host membrane.
Hypersensitive response (HR): rapid local cell death of host cells following the recognition of an incompatible pathogen and characterized by the generation of an oxidative burst (production of reactive oxygen species, ROS). A hypersensitive response is efficient to restrict the growth and spread of invading infection by biotrophs but is believed to facilitate the invasion of necrotrophs.
MAMP-triggered immunity (MTI): immune response in plants resulting from recognition of MAMPs/DAMPs by pattern recognition receptors (PRRs). This response is characterized by the activation of a mitogen-activated protein kinase (MAPK)-dependent signaling cascade, production of reactive oxygen species (ROS), transcription of defense-related genes, and formation of cell wall appositions (CWA).
Oospores, zoospores, chlamydospores, sclerotia: reproductive survival structures produced by fungi or oomycetes. Oospores are thick-walled sexual spores produced by oomycetes. Zoospores are motile flagellated spores from oomycetes. Chlamydospores are thick-walled asexual spores formed from hyphae. A sclerotium is a very resistant vegetative body consisting of a compact mass of hyphae.
Pathogenesis-related proteins (PR proteins): inducible defense-related proteins found in many plant species. Based on sequence similarity, enzymatic activity, and/or biologic activity, these proteins are currently subdivided into 17 protein families.
Phytoalexin: heterogenous group of low molecular mass secondary metabolites with antimicrobial activity that are induced by stress. The main phytoalexin of *Arabidopsis thaliana* is camalexin (3-thiazol-2′-yl-indole) and the main phytoalexin of *Pisum sativum* is pisatin (derivative of isoflavonoid).
Rhizosphere: the soil region surrounding plant roots in which roots and soil microbes directly and/or indirectly interact with each other.
Tylose: outgrowth of the protoplast of a parenchyma cell in the neighboring xylem vessel.

cause damping-off in seedlings (see Glossary), result in inhibition of root development, wilting, stunted growth, and plant death (Figure 1A,B). Effective control of soil-borne pathogens is hampered by their persistence in soil through the formation of survival structures (e.g., oospores, chlamydospores, and sclerotia), often wide host-range, and the inefficiency of chemical controls due to poor soil accessibility. However, crop losses due to soil-borne pathogens are often underestimated and their economic importance is expected to significantly rise due to the implementation of reduced or no-tillage farming practices, which are increasingly being adopted in many regions of the world [4] to conserve soil moisture in view of climate change [5].

Major classes of root pathogens

In this review, we discuss recent advances made in understanding the molecular mechanisms underpinning the root-specific defense response against soil-borne pathogenic fungi and oomycetes, ranging from the formation of constitutive barriers to pathogen recognition and the activation of downstream signaling events. The most important soil-borne pathogens infecting roots (root pathogens) to be exemplified in this review are fungi such as *Fusarium oxysporum* [6], *Verticillium* spp. [7], and *Rhizoctonia solani* [8] as well as oomycetes such as *Pythium* spp. [9] and *Phytophthora* spp. [9,10] (Figure 1A,B). These pathogens are collectively responsible for multiple diseases in hundreds of plant species including economically important crops. In addition, a diverse and complex *Phytophthora* community occurs in forest ecosystems, causing destructive diseases around the world [11]. In contrast to the presence of many beneficial bacteria in soil, there are only a few soil-borne bacteria infecting roots, such as *Ralstonia solanacearum* [12]. Two other economically important root-infecting microorganisms that induce gall formation in roots are *Agrobacterium* spp. [13] and the protist *Plasmodiophora brassicae* [14]. Although nematodes [15] and herbivorous insects [16,17] also infest plant roots, only fungal and oomycete root pathogens will be the focus of this review.

Root infection strategies

Many root pathogens show a hemibiotrophic lifestyle typified by an initial biotrophic phase followed by a necrotrophic phase with the development of visible symptoms on roots, as well as senescence, chlorosis, and necrosis in the aboveground part of the plant. By contrast, *Pythium* spp. and *R. solani* are considered to have necrotrophic lifestyles [3].

In the vicinity of roots, resistant survival structures of root pathogens germinate and hyphae grow towards the roots. When conditions are favorable, oomycetes can produce zoospores [2]. Different root pathogens appear to use different root regions to penetrate plants, but reports regarding preferential infection sites often vary. For instance, while root tips and emerging lateral roots are the primary infection site for *F. oxysporum* in *Arabidopsis* (*Arabidopsis thaliana*) [18] and banana (*Musa acuminata*) [19], no specific infection sites were detected during the *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*)–tomato (*Solanum lycopersicum*) interaction [20]. In oilseed rape (*Brassica napus*), *Verticillium longisporum* enters mainly through lateral roots and root hairs while *Verticillium dahliae* infects via the main root [21]. Varying penetration structures have been reported for different root pathogens; from no specific structures (e.g., *Fol*–tomato) to swollen hyphae (e.g., *Pythium* spp.–rice and *V. dahliae*–cotton), infection cushions (*R. solani*) and clear appressoria (e.g., *V. dahliae*–lettuce and *Phytophthora parasitica*–tomato) [3,20,22–25]. Penetration of host cells can be followed by the formation of a penetration peg or haustoria. The latter has been reported in the interaction between roots and *Phytophthora* spp. during the initial biotrophic phase [26]. In addition, several fungi produce extracellular enzymes such as cutinases, pectinases, pectin lyases, and cellulases that can enzymatically break down the plant cell wall. Moreover, depending on the pathosystem, pathogen invasion of epidermal and cortical cells can potentially occur inter- or intra-cellularly through plasmodesmata [18,20,22] (Figure 1C).

After initial root penetration, the hemibiotrophs *F. oxysporum* and *Verticillium* spp., which are considered to be true vascular pathogens, enter the xylem vessels where they produce hyphae and/or conidia [6,7] (Figure 1C).

Some fungal pathogens that are normally considered as 'leaf-infecting' can also attack roots, although their subsequent colonization mechanisms might differ. The rice pathogen *Magnaporthe oryzae*, for example, exhibits a hemibiotrophic lifestyle in leaves, but behaves like a biotrophic pathogen in roots [27]. Similarly, while the anthracnose pathogen *Colletotrichum graminicola* is able to infect both above- and below-ground organs of maize, fungal proliferation in roots appears restricted to a few cells [28].

Preformed root defenses

Although plant roots lack a cuticle layer, they possess two specific physical protective barriers, the exodermis and the endodermis. Both of these layers can be surrounded by so-called 'Casparian strips', root-specific boundaries mainly composed of lignin [29] (Figure 1C). These apoplastic barriers not only control the radial flow of both water and ions but also constitute a hindrance for invading microorganisms. Additionally, endodermal cells contain suberin, an aliphatic polyester of fatty acids and alcohols that can play a role in preventing pathogen ingress [29]. For example, a higher suberin content of the endodermis of soybean roots reduces susceptibility against *Phytophthora sojae* by delaying pathogen penetration [30].

The preformed chemical compounds consist of secondary metabolites such as glucosinolates and flavonoids, as well as antimicrobial proteins. Glucosinolates are mainly present in Brassicaceae [31] and are generally stored in the vacuoles in order to spatially separate them from myrosinases, cytoplasmic enzymes that can hydrolyze glucosinolates into cytotoxic compounds upon tissue damage. In a study comparing glucosinolates constitutively present in roots and shoots for a number of plant species, the authors concluded that the variety of glucosinolates in roots exceeds that of leaves as well as their overall level [32]. Although most glucosinolate studies focused on the compounds' role in plant–herbivore interactions, indole