Root exudates: the hidden part of plant defense

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DOI: https://doi.org/10.1016/j.tplants.2013.11.006

Originally published at:
Baetz, Ulrike; Martinoia, Enrico (2014). Root exudates: the hidden part of plant defense. Trends in Plant Science, 19(2):90-98.
DOI: https://doi.org/10.1016/j.tplants.2013.11.006

Trends in Plant Science

Root exudates - the hidden part of plant defense

--Manuscript Draft--

Manuscript Number:	
Article Type:	Review
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Abstract:	The significance of root exudates as belowground defense substances has long been underestimated, presumably due to being buried out of sight. Nevertheless, this chapter of root biology has been progressively addressed within the last decade through the characterization of novel constitutively secreted and inducible phytochemicals that directly repel, inhibit or kill pathogenic microorganisms in the rhizosphere. In addition, the complex transport machinery involved in their export has been considerably unraveled. It became evident that the profile of defense root exudates is not just diverse in its composition, but is also strikingly dynamic. In this review we will discuss the current knowledge about the identity and regulation of root-secreted defense compounds and the role of transport proteins in modulating their release.
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Dear Editor

Herewith we submit our review "**Root exudates - the hidden part of plant defense"** which we would like to have considered for publication in Trends in Plant Biology.

Several reviews on root exudates have been published during the last years, but they addressed different topics such as aluminum tolerance, phosphate and iron nutrition or the beneficial interaction with microorganism. However, to our knowledge there is no review focusing on the root exudates that are involved in plant defense belowground. This may be due to the fact that this topic includes a very broad range of compounds, which ranges from volatiles to low molecular compounds and enzymes. Therefore, we think that reviewing this topic could help the scientific community to get informed efficiently about this topic where the publications are quite dispersed and are not easy to be assembled.

We hope that the referees will also see the importance of such a review and like it but at the same time we are looking forward for getting their comments to improve the manuscript.

Best regards

Ulrike Baetz and Enrico Martinoia

Root exudates - the hidden part of plant defense

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Keywords

Root Exudates; Rhizosphere; Defense; Pathogens; Border Cells; ABC Transporter

Abstract

The significance of root exudates as belowground defense substances has long been underestimated, presumably due to being buried out of sight. Nevertheless, this chapter of root biology has been progressively addressed within the last decade through the characterization of novel constitutively secreted and inducible phytochemicals that directly repel, inhibit or kill pathogenic microorganisms in the rhizosphere. In addition, the complex transport machinery involved in their export has been considerably unraveled. It became evident that the profile of defense root exudates is not just diverse in its composition, but is also strikingly dynamic. In this review we will discuss the current knowledge about the identity and regulation of root-secreted defense compounds and the role of transport proteins in modulating their release.

The diverse chemistry of belowground plant defense

Plants are constantly exposed to a large variety of natural enemies including pathogenic fungi, oomycetes, bacteria, viruses and nematodes. These organisms engross the soil surrounding the root system, which is designated the rhizosphere. The fact that roots anchor plants in the soil, account for a substantial storage reservoir and perform water and nutrient uptake forces plants to efficiently defend them against detrimental microorganisms. To counteract infection and confer tissue-specific resistance, plants release a wide variety of biologically active compounds into the rhizosphere. Indeed, such root exudates are known to have a multitude of functions in ecological interactions with the microbial soil communities, for example by acting as signaling molecules, attractants, and stimulants, but also as inhibitors or repellents. In this review, we focus mainly on compiling the information available on various secreted natural compounds that were demonstrated to confer direct defense against soil-borne pathogens. The reader is referred to other recent reviews for detailed information on mutually beneficial interactions between plants and microorganisms, plant-plant communication and tripartite interactions mediated by root exudates [1-3].

The tremendous metabolic diversity of root exudates is gradually being elucidated through the identification and characterization of numerous novel antimicrobial compounds and previously undescribed groups of defense chemicals. Concurrently, genes and biosynthetic pathways involved in the production of these phytochemicals are being deciphered. Besides discussing examples of how and which of these chemical weapons contribute to the constitutive and targeted local belowground defense of plants, we will review recent advances on the complex transport machinery that mediates export processes into the rhizosphere. The nature and relative abundance of components in root exudate blends have a profound effect on shaping the soil environment including pathogen levels. Therefore, the root-secreted defense system and their fine-tuned regulation are essential for plant performance and make up a major field of interest in root biology research.

Dynamics in the composition of defense root exudates

Up to 40 % of the photosynthetically fixed carbon can be released by plants as root exudates, including antimicrobial compounds [3]. A continuous secretion of defense-related phytochemicals is thereby *prima facie* an immense carbon cost for the plant. To prevent excessive energy loss, the biosynthesis of phytochemicals and their rhizodeposition requires tight regulation and adjustment towards necessity in heterogeneous environments.

By definition, low-molecular weight antimicrobial chemicals that are present in the plant prior biotic stress are named phytoanticipins [4]. In a recent study, the diterpene rhizathalene A was found to be constitutively produced and released by non-infected *Arabidopsis* roots [5]. Since plants that are deficient in rhizathalene A formation were more susceptible to insect herbivory, this diterpene was suggested to be part of the constitutive direct defense system of roots. It should be noted that plants secrete a wide array of other high and low molecular mass defense compounds in the absence of pathogen elicitation [6-14]. Hence, the rhizosphere does not simply represent the infection court on which pathogens encounter the plant, but is also a preventive microbial buffer zone that protects against infection.

Together with the constitutive root exudation, the synthesis, accumulation and release of defense-related compounds can be stimulated upon the establishment of pathogen interactions. Inducible, low-molecular weight antimicrobial compounds that are not detectable in healthy plants are called phytoalexins [4]. When investigating the release of root-derived aromatic exudates in barley attacked by the soil-borne pathogen *Fusarium*, Lanoue and co-workers found an induction of five phenylpropanoids exhibiting antifungal activity [15]. Amongst them, labeling experiments highlighted the *de novo* biosynthesis and secretion of *t*-cinnamic acid [15]. Besides this 'phytoalexin prototype', pathogen infection can increase the quantities of certain constitutively exuded phytoanticipins. Momilacton A is an antimicrobial diterpene, which is produced and secreted from roots of rice seedlings into the rhizosphere [6, 7]. In addition, rice challenged with the blast fungus shows targeted leaf-accumulation of Momilacton A [16]. Thereby, this and other root exudates feature properties of both, constitutively produced and secreted phytoanticipins, as well as pathogen-elicitable phytoalexins [4, 17, 18].

An alteration in the defense exudate composition can be stimulated by various biotic and abiotic factors besides pathogen infection under laboratory conditions. For instance, the ectopic expression of the oomycetal elicitor *ß*-cryptogein in hairy roots of the flowering plant *Coleus blumei* mimics pathogen attack, and increases significantly the concentration of rosmarinic acid in the culture medium [19], which was previously demonstrated to exhibit antimicrobial activity [20]. In contrast, the defense compound is reduced in the root hair tissue when ß-cryptogein is expressed, indicating that this elicitor functions as a regulator of phenolic secretion into the rhizosphere [19]. Furthermore, following exogenous application the defense signaling molecules salicylic acid (SA), nitric oxide (NO) and methyl jasmonate

(MeJA) independently elicit a differential genome-wide transcription profile in roots, resulting in an enhanced rhizosecretion of root exudates [21] in *Arabidopsis thaliana* [22] and hairy roots of *Catharanthus roseus* [23].

Astonishingly, compositional shifts in root exudates do not only occur in response to exogenous stimuli such as the above mentioned pathogenic elicitors or defense signals, but are also controlled by endogenous developmental programs. In maize, benzoxazinoids form a class of defense molecules [24] that are released during the emergence of lateral and crown roots [25]. These benzoxazinoids present a genetically regulated, protective chemical barrier, which is thought to prevent pathogenic attack at sites that are temporally more susceptible, or in developmental stages at which infection is more deleterious for the plant. Accordingly, the peak of defense-related protein exudation into the rhizosphere can be observed just before flowering [9]. Similarly, plants adopt a tighter defensive strategy towards later stages of their life cycle as evidenced by an increased amount of putative antimicrobial phenolic compounds in their root exudate profile [11].

Breakthroughs in genome sequencing, extensive collections of mutant lines and tools available for the model plant *Arabidopsis thaliana*, a combination of '-omics' approaches, new sampling methods and higher-resolution detection systems have been indispensable in facilitating comparative large-scale analysis of root exudate blends in response to different pathogens. Specifically, the compatible interaction of bacterial or fungal pathogens, or root-feeding insects with *Arabidopsis* roots but not mechanical wounding stimulates the rapid secretion of the antimicrobial 1,8-cineole [26]. However, incompatible interactions do not influence the exudation of this monoterpene. In agreement with this result, the secretion of biotic stress-responsive proteins from *Arabidopsis thaliana* roots is induced during compatible but not incompatible interaction [27]. These results circumstantiate that the secretion of defense compounds into the rhizosphere is a tightly controlled, temporally dynamic process that is dependent on the identity of the microbial neighbor.

The release of low molecular weight compounds

Various low molecular weight phytochemicals such as amino acids, organic acids, sugars, phenolics and other secondary metabolites comprise the majority of root exudates, synergistically protecting plants against pathogenic microbe invasion. In general, root-secreted compounds belonging to the chemical class of phenolics and terpenoids have strong external antibacterial and antifungal qualities [15, 17-20]. Notably, phenolic metabolites also function efficiently in attracting some soil-borne microorganisms and can beneficially influence the native soil microbial community [28]. It was also observed that molecules like the amino acid canavanine can act as a stimulator for one group of microbes while being a suppressor for many other soil bacteria [29]. Thus, compounds of the same chemical class can differentially affect the soil environment, and certain substances can be considered as microorganism specific in their biological activity and toxicity. Similarly, a phytoalexin derived from *Arabidopsis* root exudates is required for conferring resistance to *Phytophthora capsici* [30], however resistance to *Phytophthora cinnamomi* does not rely on this phytochemical [31]. The finding of differential compound activities may be explained in part by variations in the tolerance to specific defense molecules based on the efficiency of active detoxification and efflux processes between different pathogens [17].

Phenylpropanoids are ubiquitous plant phenolics, which occur in defense root exudates [15, 17]. In line, resistance to *Fusarium* attack in barley is based on the rapid accumulation and secretion of phenylpropanoids (cinnamic acid derivates) after fungal infection [15]. Moreover, flavonoids represent one of the largest class of phenylpropanoid-derived secondary metabolites in plants and constitute a large proportion of root exudates [32]. Derivatives of isoflavonoids such as the pea phytoalexin pisatin are a crucial class of compounds with potent antimicrobial properties in legumes [33, 34]. Its tissue-specific release from the root tip can be stimulated by pathogen elicitation [35].

Terpenoids form the largest class of plant defense chemicals above- and belowground and contribute to root exudates [5-7, 18, 36]. It has been known for some time that nonvolatile terpenoid phytochemicals such as momilactones can be secreted into the rhizosphere [6, 7]. However, it was only recently that volatile organic compounds (VOCs) were also shown to be emitted from roots as a direct defense mechanism. Plant-derived volatiles have been previously described to function in tritrophic interactions by attracting natural enemies of herbivores upon herbivore attack to provide indirect plant defense [37-40]. In contrast, an example of a direct belowground volatile defense compound is the monoterpene 1,8-cineole, which is released from hairy-root cultures of *Arabidopsis* during pathogen interaction [26, 41]. Furthermore, a semivolatile diterpene hydrocarbon designated rhizathalene A is implicated in the belowground resistance towards root-feeding insects as a local antiherbivore metabolite of *Arabidopsis* [5]. A new role was discovered for another class of terpenoids, the strigolactones. An extensive body of literature has been published on strigolactones as phytohormones and, when released into the rhizosphere, as a compound involved in plant symbiosis with arbuscular mycorrhizal fungi and in plant infection by root parasitic plants [42]. The synthetic strigolactone analog GR24 intriguingly inhibits the growth of an array of phytopathogenic fungi when present in the growth medium, indicating that secreted strigolactones can directly affect natural fungal enemies and contribute to belowground plant biotic stress responses [43].

Other highly potent antifungal or antimicrobial root exudates are tryptophan-derived secondary metabolites, such as some glucosinolates or the indole derivate camalexin - the only characterized phytoalexin in *Arabidopsis* [30, 44-51]. Several molecular players involved in camalexin biosynthesis are identified at the genetic level. Their transcriptional activation after infection leads to the intrinsic production, accumulation [44] and exudation [46] of camalexin from *Arabidopsis* roots, whereas their genetic disruption results in lower secretion levels [46], accompanied by enhanced pathogen disease symptoms and fungal growth [44]. In line with these observations, the ectopic overexpression of an *Arabidopsis* gene that modulates camalexin and SA biosynthesis confers disease resistance to soybean against nematodes [52].

Collectively, defense root exudate blends build a diverse and flexible protective layer of chemical compounds in the rhizosphere. In addition to low molecular weight metabolites, high molecular weight root exudates also contribute to the local belowground resistance. In particular, the repelling and inhibiting role of previously unrecognized molecules such as secreted proteins and extracellular DNA emerged in the past years [8, 53]. We will discuss these two components of defense root exudates in the context of border cells, since their examination was predominantly conducted using this specialized ‘front line’ cell layer.

Border cells function as a defensive barrier of roots

Root tips display local resistance to various infections, whereas more vulnerable parts of roots such as the elongation zone are more susceptible [54, 55]. This phenomenon of spatially different susceptibility is correlated with the highly controlled, inducible formation and release of metabolically active border cells at the root periphery that originate and detach from the root cap meristem [56-59]. Phytochemicals in the rhizosphere largely derive from cap and border cells, hence these cells account for a protective shield against pathogen invasion with a vital impact on plant health [59-61]. Besides developmental and environmental signals, the invasion of pathogenic microorganisms initiates border cell production as a plant defense mechanism [35, 59, 62]. It was recently shown that the formation of root border cells and exudation of the isoflavonoid phytoalexin pisatin is stimulated in pea when root tips were challenged with a plant pathogen [35]. Moreover, exogenous pisatin can feedback on the plant by up-regulating the production of border cells *in vitro* [62].

Border cells and their exudates can account for root tip resistance by coping with pathogens via at least three mechanisms that act in concert (Figure 1). Firstly, peripheral border cells can attract pathogenic microorganisms to get infected, a strategy that confers transitory protection to the root tip. In fact, after the removal of the border cell layer, the physiologically independent root tip remains uninfected, defense gene expression is not elicited and root growth proceeds indistinguishably from non-treated roots [54, 63]. Besides providing a sustainable substitute for deleterious pathogenic root tip invasion, border cells can act as chemical and physical barriers towards pathogens by secreting not only antipathogenic low molecular weight metabolites, but also a mucilaginous matrix of up to 95 % high molecular weight polysaccharides and 5 % extracellular proteins [53, 64]. Proteolytic solubilization of protein exudates derived from progenitor root cap and border cells (referred to as 'root cap secretome') in pea results in the disintegration of the mucilage, the release of bacteria as well as the loss of root tip resistance towards infection by a pea pathogen [53]. Therefore, despite its minor physical fraction proteins are engaged in the binding, trapping and aggregation of pathogenic bacteria [53, 64]. Moreover, secreted antimicrobial proteins can serve as a direct external defense mechanism by repelling, inhibiting or killing pathogenic microorganisms. Proteomic analysis of the root exudates of root cap and border cells confirmed that the complex mixture of approximately >100 proteins contains mostly stress and defense-related proteins, besides structural components such as actin [9, 10, 13, 14, 27, 53]. Upon encountering pathogenic interactions, the protein composition alters dynamically as antimicrobial compounds (e.g. hydrolases, peptidases, and peroxidases) accumulate in the rhizosphere [9, 10, 27, 53].

Besides the presence of antimicrobial enzymes long known to be associated with plant defense, the root cap secretome astonishingly contains histone H4 [53]. In mammals, histone-linked extracellular DNA (exDNA) is anticipated to have a critical role in defense against microbial pathogens [65-67]. A similar mechanism was suggested in plants when exDNA linked to histone proteins was discovered to be synthetized and exuded from root border cells [8]. The specific mechanism of how exDNA inhibits pathogen growth needs to be determined. However, recent research suggests that exDNA is probably similar to various structural proteins a fundamental scaffold to trap, immobilize and subsequently kill root-infecting pathogens in the mucilage matrix, since degradation of either component in root tip exudates using a protease or an nuclease respectively results in an abolishment of root tip resistance to fungal infection [8]. Because border cells secrete a mucilage layer that contains proteins and exDNA to protect the root tip by adhesion and aggregation of pathogens, they function analogously to white blood cells in the mammalian innate immune response [8, 59, 68].

Taken together, the penetration of physiologically independent border cells, the root cap and border cell exudation of the mucilage layer including proteins and exDNA that immobilize pathogens, as well as the secretion of, for instance, antimicrobial enzymes and secondary metabolites co-operatively provide root tip resistance towards pathogens.

The transport machinery that modulates the release of defense phytochemicals

As our knowledge about the synthesis of defense metabolites and their function in the rhizosphere has improved progressively, the complex mechanisms of regulated rhizosecretion and the critical transport components have also started to be unraveled. Traditionally, root exudation has been suspected to be a passive process mediated by diffusion, channels and vesicle transport. However, recent studies elucidated a pivotal role of tightly regulated primary and secondary active transport processes across the root plasma membrane in the export and accumulation of defense phytochemicals in the rhizosphere. Two protein families shown to be involved in mediating the transport of a wide array of organic substances, namely MATE (multidrug and toxic compound extrusion) and ABC (ATP-binding cassette) transporters [69, 70], have attracted particular interest. Nevertheless, there is a large number of uncharacterized transporters which might participate in the belowground defense system.

Evidence exists that members of both MATE and ABC transporter families are capable of releasing constituents of the root phytochemical cocktail into the rhizosphere. In case of MATE transporter proteins, a subclade that can be found in all plants analyzed so far is implicated in the release of citrate into the rhizosphere to confer aluminum resistance to plants. Since citrate is a nutrient for many microorganisms this exudation may also have an impact on the microflora at the root tip [71-75]. Recently, a MATE transporter in the stele of rice roots was found to facilitate efflux of phenolic compounds into the xylem [76]. It was speculated that similar transporters might be responsible for phenolic secretion into the soil. In Table 1, we highlight the few genes out of 56 members of the MATE family that are promising candidates to encode transport proteins that are involved in such processes because they exhibit a strong or predominant expression in the outer cell layers of root caps and were not previously shown to be localized in another intracellular membrane than the plasma membrane (Table 1) [69, 77]. However, to our knowledge to date no MATE transporter has been identified to export root-derived antimicrobial compounds into the rhizosphere. In contrast, members of the ABC transporter family were demonstrated to be fundamentally involved in root exudation and the defense system [78-81], and recently, putative substrates were attributed to particular transporter proteins [82, 83]. Initially, indirect pharmacological approaches were deployed to demonstrate that the root exudate profile of *Arabidopsis thaliana* is quantitatively and qualitatively dependent on ATP hydrolysis [84], indicating that the secretion process of certain phytochemicals is mediated by active transport systems such as ABC-type proteins [85]. Subsequently, among the >120 genes encoding ABC transporter proteins in *Arabidopsis*, 25 candidates were identified to have a potential role in rhizosecretion based on their high expression in root cells [86]. Exudate [79, 86, 87] and microbial [87] composition differs significantly between knock-out lines of several root-expressed ABC transporters and the corresponding wild-type, providing evidence that this protein family is implicated in root secretion, also of antimicrobial compounds. Furthermore, these studies revealed that multiple ABC transporters can be used for the release of a given phytochemical, and a specific ABC transporter can be capable of mediating the export of several structurally and functionally unrelated substrates. For instance, *At*PDR9 is suspected to transport phenolic compounds in order to bind and acquire iron in aerobic soil systems [88] but was shown previously to also transport auxinic compounds [89, 90]. Interestingly, to our knowledge, the only direct link between a defined transporter, its substrate and an effect on soil microorganisms has been demonstrated for *Ph*PDR1, a petunia ABC transporter that catalyzes the release of strigolactones from root cells [83].

Only few studies addressed the connection of transport proteins, defense-related phytochemical rhizosecretion and soil-borne pathogen susceptibility. The transporter *Np*PDR1of *Nicotiana plumbaginifolia* is directly involved in plant defense against pathogen invasion [78, 91]. Silencing this ABC transporter results in enhanced sensitivity of roots and petals towards various soil-borne pathogens, possibly due to diminished secretion of antifungal compounds, such as the diterpene sclareol [91]. In general, the appearance of a phytochemical in the rhizosphere can be genetically and biochemically regulated by various factors, including ABC-type protein abundance, transport activity, substrate concentration and specificity, but also by pleiotropic effects mediated by ABC transporters. The gene expression of the transporter *Nt*PDR1 positively correlates with export rates of antipathogenic diterpenes into the extracellular medium, and the expression can be modified by microbial elicitation [82, 92]. On the other hand, another study showed that nitrogen deficiency can elicit the increased biosynthesis of the flavonoid signaling molecule, genistein, resulting in its secretion from soybean roots to initiate rhizobium symbiosis [93], whereby the transport machinery involved in genistein export is constitutively active regardless of the nitrogen availability [85]. The further twist to that story is the fact that ABC transporter proteins themselves exhibit a regulatory function in modulating the synthesis and exudation of defense phytochemicals. For instance, the *Arabidopsis* mutant *abcg30* exhibits lower levels of several compounds in the rhizosphere, whereas other defense exudates show a higher secretion in the mutant plants [87]. This finding provides evidence that *At*ABCG30 mediates the transport of compounds, but also that the lack of the protein directly and indirectly influences various metabolic processes such as biosynthesis of secondary metabolites or the expression of other transporters. Another recent study showed that roots of *Medicargo truncatula* are rapidly infected by *Fusarium* when *MtABCG10*, a gene which encodes a close homologue of *Nt*PDR1 [82, 92], is silenced. Concomitantly, this silencing results in a reduction of the phenylpropanoid pathway-derived phytoalexin, medicarpin, as well as its precursors in root tissue and exudates [94]. It was therefore proposed that *Mt*ABCG10 may modulate isoflavonoid levels during the belowground biotic stress response associated with the *de novo* biosynthesis of phytoalexins. Furthermore, the mutation of *At*ABCG37 and *At*ABCC5 results in the accumulation of the phytoalexin, camalexin, in the rhizosphere [79]. This elevated secretion was suggested to be a pleiotropic effect of the mutations, which induce an increased expression of genes involved in the indolic metabolite biosynthesis as previously observed in other mutants [47]. Similarly, the dysfunction of *At*ABCG36 results in higher basal defense levels, since flavonoid glycosides and defense proteins accumulated in root tissue supposedly due to a salicylic acid over-production even when roots are grown under sterile conditions [79].

Hence, in the future, it will be challenging not only to identify the transport machinery of defense compounds and their substrates, but also to examine the distinct pathways that are modified in transporter mutants. This will deepen our understanding of the effects on root exudate patterns mediated by ABC-type proteins.

Concluding remarks and future perspectives

Despite its importance in ensuring tissue protection and optimizing plant performance, deciphering the belowground defense system has been neglected for a long time- a result of difficulties in accessing the undisturbed, natural rhizosphere communication, which includes symbiotic and pathogenic interactions between plants and microorganisms. Exploring the profiles of secreted metabolites that exhibit a defensive function outside the plant in close proximity to the roots presents a more pronounced technical challenge compared to aboveground or endogenously sequestered antimicrobial compounds. The chemical ensemble released by roots is majorly shaping native microbial community structures. Notably, alterations of single root exudates in the rhizosphere or single genes in the biosynthetic pathway of phytochemicals or transporters can influence the composition and activity of the soil microbiome [28, 48, 52, 87, 95, 96]. Hence, the complex network of root exudation and pathogen defense needs to be decoded comprehensively to integrate the regulation of rhizosecretion with direct and indirect physiological effects on plants and the entire microbial ecosystem. Understanding the ecological impact of valuable defense molecules will open up novel opportunities to engineer a protective rhizosphere. Some studies aimed to generate roots releasing artificial exogenous bioactive molecules in order to create plants with increased resistance to pathogens [19, 48, 52, 97, 98]. For instance, pathogen infection is significantly inhibited in tomato roots secreting selected antimicrobial peptides fused to a maize cytokinin/ dehydrogenase protein scaffold [97]. Such peptide-delivery agents for plant defense molecules or targeted ectopic expression systems of secondary metabolites or transporters are auspicious candidates for manipulating the formation and secretion of root exudates and enhancing their natural defense properties. However, the potentially large impact at the ecological and environmental level from minor compositional changes in root exudation needs to be carefully taken into account to avoid possible repercussions on microbial communities and non-target organisms.

Acknowledgments

We thank the journal editors for providing us the opportunity to address this topic in *Trends in Plant Science*. Furthermore, we are grateful to the Forschungskredit, UZH, for financial support of UB and to Robert Dudler, Cornelia Eisenach and David Seung for critical reading and fruitful discussion on the manuscript.

References

1. Bais, H.P. *et al.* (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233-266
2. Badri, D.V. *et al.* (2009) Rhizosphere chemical dialogues: plant-microbe interactions. *Curr. Opin. Biotechnol.* 20, 642-650
3. Doornbos, R.F. *et al.* (2012) Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agron. Sustain. Dev.* 32, 227-243
4. VanEtten, H.D. *et al.* (1994) Two Classes of Plant Antibiotics: Phytoalexins versus "Phytoanticipins". *Plant Cell* 6, 1191-1192
5. Vaughan, M.M. *et al.* (2013) Formation of the Unusual Semivolatile Diterpene Rhizathalene by the Arabidopsis Class I Terpene Synthase TPS08 in the Root Stele Is Involved in Defense against Belowground Herbivory. *Plant Cell* 25, 1108-1125
6. Kato-Noguchi, H. *et al.* (2008) Secretion of momilactone A from rice roots to the rhizosphere. *J. Plant Physiol.* 165, 691-696
7. Toyomasu, T. *et al.* (2008) Diterpene phytoalexins are biosynthesized in and exuded from the roots of rice seedlings. *Biosci. Biotechnol. Biochem.* 72, 562-567

8. Wen, F. *et al.* (2009) Extracellular DNA is required for root tip resistance to fungal infection. *Plant Physiol.* 151, 820-829

9. De-la-Peña, C. *et al.* (2010) Root secretion of defense-related proteins is development-dependent and correlated with flowering time. *J. Biol. Chem.* 285, 30654-30665

10. Shinano, T. *et al.* (2011) Proteomic analysis of secreted proteins from aseptically grown rice. *Phytochemistry* 72, 312-320

11. Chaparro, J.M. *et al*. (2013) Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One* 8, e55731

12. Badri, D.V. *et al.* (2010) Root secretion of phytochemicals in Arabidopsis is predominantly not influenced by diurnal rhythms. *Mol. Plant* 3, 491-498

13. Liao, C. *et al.* (2012) Comparative analyses of three legume species reveals conserved and unique root extracellular proteins. *Proteomics* 12, 3219-3228

14. Ma, W. *et al.* (2010) The mucilage proteome of maize (Zea mays L.) primary roots. *J. Proteome Res.* 9, 2968-2976

15. Lanoue, A. *et al.* (2010) De novo biosynthesis of defense root exudates in response to Fusarium attack in barley. *New Phytol.* 185, 577-588

16. Hasegawa, M. *et al.* (2010) Phytoalexin accumulation in the interaction between rice and the blast fungus. *Mol. Plant Microbe Interact.* 23, 1000-1011

17. Bais, H.P. *et al.* (2005) Mediation of pathogen resistance by exudation of antimicrobials from roots. *Nature* 434, 217-221

18. Wurst, S. *et al.* (2010) Microorganisms and nematodes increase levels of secondary metabolites in roots and root exudates of Plantago lanceolata. *Plant Soil* 329, 117–126

19. Vuković, R. *et al.* (2013) Genetic elicitation by inducible expression of β-cryptogein stimulates secretion of phenolics from Coleus blumei hairy roots. *Plant Sci.* 199-200, 18-28

20. Bais, H.P. *et al.* (2002) Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of Ocimum basilicum. *Plant Physiol. Biochem.* 40, 983–995

21. Badri, D.V. and Vivanco, J.M. (2009) Regulation and function of root exudates. *Plant Cell Environ.* 32, 666-681

22. Badri, D.V. *et al.* (2008) Transcriptome analysis of Arabidopsis roots treated with signaling compounds: a focus on signal transduction, metabolic regulation and secretion. *New Phytol.* 179, 209-223

23. Ruiz-May, E. *et al.* (2009) Differential secretion and accumulation of terpene indole alkaloids in hairy roots of Catharanthus roseus treated with methyl jasmonate. *Mol. Biotechnol.* 41, 278-285

24. Ahmad, S. *et al.* (2011) Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiol.* 157, 317-327

25. Park, W.J. *et al.* (2004) Release of the benzoxazinoids defense molecules during lateral- and crown root emergence in Zea mays. *J. Plant Physiol.* 161, 981-985

26. Steeghs, M. *et al.* (2004) Proton-transfer-reaction mass spectrometry as a new tool for real time analysis of root-secreted volatile organic compounds in Arabidopsis. *Plant Physiol.* 135, 47-58

27. De-la-Peña, C. *et al.* (2008) Root-microbe communication through protein secretion. *J. Biol. Chem.* 283, 25247-25255

28. Badri, D.V. *et al.* (2013) Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J. Biol. Chem.* 288, 4502-4512

29. Cai, T. *et al.* (2009) Host legume-exuded antimetabolites optimize the symbiotic rhizosphere. *Mol. Microbiol.* 73, 507-517

30. Wang, Y. *et al.* (2013) A novel Arabidopsis-oomycete pathosystem: differential interactions with Phytophthora capsici reveal a role for camalexin, indole glucosinolates and salicylic acid in defence. *Plant Cell Environ.* 36, 1192-1203

31. Rookes, J.E. *et al.* (2008) Elucidation of defence responses and signalling pathways induced in Arabidopsis thaliana following challenge with Phytophthora cinnamomi. *Physiol. Mol. Plant Pathol*. 72, 151–161

32. Cesco, S. *et al.* (2010) Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant Soil* 329, 1-25

33. Weisskopf, L. *et al.* (2006) White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. *Plant Cell and Environ.* 29, 919-927

34. Wu, Q. and VanEtten, H.D. (2004) Introduction of plant and fungal genes into pea (Pisum sativum L.) hairy roots reduces their ability to produce pisatin and affects their response to a fungal pathogen. *Mol. Plant Microbe Interact.* 17, 798-804

35. Cannesan, M.A. *et al.* (2011) Association between border cell responses and localized root infection by pathogenic Aphanomyces euteiches. *Ann. Bot.* 108, 459-469

36. Schmelz, E.A. *et al.* (2011) Identity, regulation, and activity of inducible diterpenoid phytoalexins in maize. *Proc. Natl. Acad. Sci. U.S.A.* 108, 5455-5460

37. Hiltpold, I. *et al.* (2011) Systemic root signalling in a belowground, volatile-mediated tritrophic interaction. *Plant Cell Environ.* 34, 1267-1275

38. Hiltpold, I. and Turlings, T.C. (2012) Manipulation of chemically mediated interactions in agricultural soils to enhance the control of crop pests and to improve crop yield. *J. Chem. Ecol.* 38, 641-650

39. Rasmann, S. *et al.* (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434, 732-737

40. Robert, C.A. *et al.* (2012) Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytol.* 194, 1061-1069

41. Chen, F. *et al.* (2004) Characterization of a root-specific Arabidopsis terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. *Plant Physiol.* 135(4), 1956-1966

42. Xie, X. and Yoneyama, K. (2010) The strigolactone story. *Annu. Rev. Phytopathol.* 48, 93-117

43. Dor, E. *et al.* (2011) The synthetic strigolactone GR24 influences the growth pattern of phytopathogenic fungi. *Planta* 234, 419-427

44. Iven, T. *et al.* (2012) Transcriptional activation and production of tryptophan-derived secondary metabolites in arabidopsis roots contributes to the defense against the fungal vascular pathogen Verticillium longisporum. *Mol. Plant* 5, 1389-1402

45. Millet, Y.A. *et al.* (2010) Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. *Plant Cell* 22, 973-990

46. Bednarek, P. *et al.* (2005) Structural complexity, differential response to infection, and tissue specificity of indolic and phenylpropanoid secondary metabolism in Arabidopsis roots. *Plant Physiol.* 138, 1058-1070

47. Consonni, C. *et al.* (2010), Tryptophan-derived metabolites are required for antifungal defense in the Arabidopsis mlo2 mutant. *Plant Physiol.* 152, 1544-1561

48. Bressan, M. *et al.* (2009) Exogenous glucosinolate produced by Arabidopsis thaliana has an impact on microbes in the rhizosphere and plant roots. *ISME J.* 3, 1243-1257

49. Schreiner, M. *et al.* (2011) Enhanced glucosinolates in root exudates of Brassica rapa ssp. rapa mediated by salicylic acid and methyl jasmonate. *J. Agric. Food Chem.* 59, 1400-1405

50. Schlaeppi, K. *et al.* (2010) Disease resistance of Arabidopsis to Phytophthora brassicae is established by the sequential action of indole glucosinolates and camalexin. *Plant J.* 62, 840-851

51. Schlaeppi, K. and Mauch, F. (2010) Indolic secondary metabolites protect Arabidopsis from the oomycete pathogen Phytophthora brassicae. *Plant Signal Behav.* 5, 1099-1101

52. Youssef, R.M. *et al.* (2013) Ectopic expression of AtPAD4 broadens resistance of soybean to soybean cyst and root-knot nematodes. *BMC Plant Biol.* 13, 67

53. Wen, F. *et al.* (2007) Extracellular proteins in pea root tip and border cell exudates. *Plant Physiol.* 143, 773-783

54. Gunawardena, U. and Hawes, M.C. (2002) Tissue specific localization of root infection by fungal pathogens: role of root border cells. *Mol. Plant Microbe Interact.* 15, 1128-1136

55. Gunawardena, U. *et al.* (2005) Tissue-specific localization of pea root infection by Nectria haematococca. Mechanisms and consequences. *Plant Physiol.* 137, 1363-1374

56. Stubbs, V.E. *et al.* (2004) Root border cells take up and release glucose-C. *Ann. Bot.* 93, 221-224

57. Vicré, M. *et al.* (2005) Root border-like cells of Arabidopsis. Microscopical characterization and role in the interaction with rhizobacteria. *Plant Physiol.* 138, 998-1008

58. Driouich, A. *et al.* (2007) Formation and separation of root border cells. *Trends Plant Sci.* 12, 14-19

59. Hawes, M.C. *et al.* (2012) Roles of root border cells in plant defense and regulation of rhizosphere microbial populations by extracellular DNA 'trapping'. *Plant Soil* 355, 1-16

60. Griffin, G. *et al.* (1976) Nature and quantity of sloughed organic matter produced by roots of axenic peanut plants. *Soil Biol.Biochem.* 8, 29-32.

61. Odell, R.E. *et al.* (2008) Stage-dependent border cell and carbon flow from roots to rhizosphere. *Am. J. Bot.* 95, 441-446

62. Curlango-Rivera, G. *et al.* (2010) Transient exposure of root tips to Primary and secondary metabolites: Impact on root growth and production of border cells. *Plant Soil* 332, 267-275

63. Hawes, M.C. *et al.* (2000) The role of root border cells in plant defense. *Trends Plant Sci.* 5, 128-133

64. Wen, F. *et al.* (2007) Proteins among the polysaccharides: a new perspective on root cap slime. *Plant Signal Behav.* 2, 410-412

65. von Köckritz-Blickwede, M. and Nizet, V. (2009) Innate immunity turned inside-out: antimicrobial defense by phagocyte extracellular traps. *J. Mol. Med.* 87, 775-783

66. Brinkmann, V. *et al.* (2004) Neutrophil extracellular traps kill bacteria. *Science* 303, 1532-1535

67. Medina, E. (2009) Neutrophil extracellular traps: a strategic tactic to defeat pathogens with potential consequences for the host. *J. Innate Immun.* 1, 176-180

68. Hawes, M.C. *et al.* (2011) Extracellular DNA: the tip of root defenses? *Plant Sci.* 180, 741-745.

69. Yazaki, K. *et al.* (2008) Secondary transport as an efficient Membrane transport mechanism for plant secondary metabolites. *Phytochem. Rev.* 7, 513-524

70. Kang, J. *et al.* (2011) Plant ABC Transporters. In *The Arabidopsis book/ American Society of Plant Biologists* 9, e0153

71. Furukawa, J. *et al.* (2007) An aluminum-activated citrate transporter in barley. *Plant Cell Physiol.* 48, 1081-1091

72. Fujii, M. *et al.* (2012) Acquisition of aluminium tolerance by modification of a single gene in barley. *Nat. Commun.* 3, 713

73. Magalhaes, J.V. *et al.* (2007) A gene in the multidrug and toxic compound extrusion (MATE) family confers aluminum tolerance in sorghum. *Nat Genet.* 39(9), 1156-1161

74. Liu, J. *et al.* (2009) Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer Arabidopsis aluminum tolerance. *Plant J.* 57, 389-399

75. Maron, L.G. *et al.* (2010) Two functionally distinct members of the MATE (multi-drug and toxic compound extrusion) family of transporters potentially underlie two major aluminum tolerance QTLs in maize. *Plant J.* 61, 728-740

76. Ishimaru, Y. *et al.* (2011) A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele. *J. Biol. Chem.* 286, 24649-24655

77. Birnbaum, K. *et al.* (2003) A gene expression map of the Arabidopsis root. *Science* 302, 1956-1960

78. Stukkens, Y. *et al.* (2005) NpPDR1, a pleiotropic drug resistance-type ATP-binding cassette transporter from Nicotiana plumbaginifolia, plays a major role in plant pathogen defense. *Plant Physiol.* 139(1), 341-352

79. Badri, D.V. *et al.* (2012) Influence of ATP-Binding Cassette Transporters in Root Exudation of Phytoalexins, Signals, and in Disease Resistance. *Front. Plant Sci.* 3, 149

80. Bienert, M.D. *et al.* (2012) A pleiotropic drug resistance transporter in Nicotiana tabacum is involved in defense against the herbivore Manduca sexta. *Plant J.* 72, 745-757

81. Meyer, S. *et al.* (2010) Intra- and extra-cellular excretion of carboxylates. *Trends Plant Sci.* 15, 40-47

82. Crouzet, J. *et al.* (2013) NtPDR1, a plasma membrane ABC transporter from Nicotiana tabacum, is involved in diterpene transport. *Plant Mol. Biol.* 82, 181-192

83. Kretzschmar, T. *et al.* (2012) A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. *Nature* 483, 341-344

84. Loyola-Vargas, V.M. *et al.* (2007) Effect of transporters on the secretion of phytochemicals by the roots of Arabidopsis thaliana. *Planta* 225, 301-310

85. Sugiyama, A. *et al.* (2007) Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-Rhizobium symbiosis. *Plant Physiol.* 144, 2000-2008

86. Badri, D.V. *et al.* (2008) Altered profile of secondary metabolites in the root exudates of Arabidopsis ATP-binding cassette transporter mutants. *Plant Physiol.* 146, 762-771

87. Badri, D.V. *et al.* (2009) An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. *Plant Physiol.* 151, 2006-2017

88. Rodríguez-Celma, J. *et al.* (2013) Mutually Exclusive Alterations in Secondary Metabolism are Critical for the Uptake of Insoluble Iron Compounds by Arabidopsis and Medicago truncatula. *Plant Physiol.* 162, 1473-1485

89. Ito, H. and Gray, W.M. (2006) A gain-of-function mutation in the Arabidopsis pleiotropic drug resistance transporter PDR9 confers resistance to auxinic herbicides. *Plant Physiol.* 142, 63-74

90. Ruzicka, K. *et al.* (2010) Arabidopsis PIS1 encodes the ABCG37 transporter of auxinic compounds including the auxin precursor indole-3-butyric acid. *Proc. Natl. Acad. Sci. U.S.A.* 107, 10749-10753

91. Bultreys, A. *et al.* (2009) Nicotiana plumbaginifolia plants silenced for the ATP-binding cassette transporter gene NpPDR1 show increased susceptibility to a group of fungal and oomycete pathogens. *Mol. Plant Pathol.* 10, 651-663

92. Sasabe, M. *et al.* (2002) cDNA cloning and characterization of tobacco ABC transporter: NtPDR1 is a novel elicitor-responsive gene. *FEBS Lett.* 518, 164-168

93. Sugiyama, A. *et al.* (2008) Signaling from soybean roots to rhizobium: An ATP-binding cassette-type transporter mediates genistein secretion. *Plant Signal Behav.* 3, 38-40

94. Banasiak, J. *et al.* (2013) A Medicago truncatula ABC transporter belonging to subfamily G modulates the level of isoflavonoids. *J. Exp. Bot.* 64, 1005-1015

95. Weisskopf, L. *et al.* (2008) Spatio-temporal dynamics of bacterial communities associated with two plant species differing in organic acid secretion: A one-year microcosm study on lupin and wheat. *Soil Biol. Biochem.* 40, 1772-1780

96. Bulgarelli, D. *et al.* (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* 488, 91-95

97. Fang, Z.D. *et al.* (2006) Combinatorially selected defense peptides protect plant roots from pathogen infection. *Proc. Natl. Acad. Sci. U.S.A.* 103, 18444-18449

98. Fang, Z.D. *et al.* (2010) Combinatorially selected peptides for protection of soybean against Phakopsora pachyrhizi. *Phytopathology* 100, 1111-1117

Figure legends

Figure 1. Concerted action of root border cells and their exudates in root tip resistance against pathogens. Displayed microorganisms represent pathogenic fungi, oomycetes, bacteria, viruses and nematodes. (1) Pathogen attraction and penetration of physiologically independent border cells to prevent deleterious root tip infection. (2) The mucilage layer composed of mainly polysaccharides, proteins and extracellular DNA is secreted by border cells and represents a defensive matrix that binds, immobilizes and aggregates pathogens. (3) Root border cells release high and low molecular weight compounds that exhibit direct antimicrobial and/or antifungal properties to inhibit or kill microbes.

Table 1. Expression profiles of genes coding for *Arabidopsis* MATE transporters which are predominantly or highly expressed in root caps.

GO[a]	Gene ID	Root Stage I[b]						Max. FC[d]	Tissue of max. FC
		Stele[c]	Endo	Endo+cortex	Epi	LRC	FC LRC		
AtDTX5/6	At2g04090/ At2g04100	49.16	51.52	19.98	243.55	119.49	10.62	32.06	Root stage II Epi
AtDTX9	At1g66760	110.07	216.78	235.16	55.14	157.5	3.45	29.7	Mesophyll cells, with 100uM ABA
AtDTX12	At1g15170	40.01	53.91	61.21	220.75	184.89	5.88	7.1	Bicellular pollen
AtDTX33	At1g47530	109.4	232.04	159.19	179.31	162.65	0.59	5.86	Guard cells, with 100µM ABA, cordycepin and actinomycin added during protoplasting
AtDTX36	At1g11670	437.77	568.63	734.08	958.69	1847.14	39.76	52.16	Root stage III LRC
AtDTX37	At1g61890	882.33	745.87	1110.85	968.94	1144.92	2.57	16.39	Mesophyll cells
AtDTX39	At4g21910	37.86	28.42	39.09	96.92	197.76	1.88	6.56	Ovary tissue

[a]Gene ontology

[b]Root stage I represents cells of the root cap, stage II of the elongation zone and stage III of the root hair zone.

[c]The stele is the most inner cell layer of the root, the lateral root cap the most outer cell layer. Abbreviations are Endo, endodermis; Endo+cortex, endodermis and cortex; Epi, epidermal artrichoblasts; LRC, lateral root cap.

[d]Fold changes are calculated as the ratio of the gene expression in a given tissue to the mean expression level in the entire plant. Consequently, this value indicates the homogeneity of the gene expression and the maximal fold change occurs in the tissue with the highest absolute expression levels.

Highlights

Defense root exudates are chemically diverse.

Plants constitutively secrete root exudates to prevent pathogen attack.

Stimuli such as microbial elicitors trigger compositional changes in root exudates.

ABC transporters are involved in releasing and regulating defense root exudates.

Figure

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