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Root exudates: the hidden part of plant defense

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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 past 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 has become evident that the profile of defense root exudates is not only diverse in its composition, but also strikingly dynamic. In this review, we discuss current knowledge of the nature and regulation of root-secreted defense compounds and the role of transport proteins in modulating their release.

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

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-106507>

Journal Article

Accepted Version

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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First Author:	Enrico Martinoia, Dr
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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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Opposed Reviewers:	

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

1 **Root exudates - the hidden part of plant defense**

2

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12 **Keywords**

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

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1 **Abstract**

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

12

13 **The diverse chemistry of belowground plant defense**

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

1 beneficial interactions between plants and microorganisms, plant-plant communication and
2 tripartite interactions mediated by root exudates [1-3].

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

14

15 **Dynamics in the composition of defense root exudates**

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

21 By definition, low-molecular weight antimicrobial chemicals that are present in the plant prior
22 biotic stress are named phytoanticipins [4]. In a recent study, the diterpene rhizathalene A was
23 found to be constitutively produced and released by non-infected *Arabidopsis* roots [5]. Since
24 plants that are deficient in rhizathalene A formation were more susceptible to insect
25 herbivory, this diterpene was suggested to be part of the constitutive direct defense system of
26 roots. It should be noted that plants secrete a wide array of other high and low molecular mass

1 defense compounds in the absence of pathogen elicitation [6-14]. Hence, the rhizosphere does
2 not simply represent the infection court on which pathogens encounter the plant, but is also a
3 preventive microbial buffer zone that protects against infection.

4 Together with the constitutive root exudation, the synthesis, accumulation and release of
5 defense-related compounds can be stimulated upon the establishment of pathogen
6 interactions. Inducible, low-molecular weight antimicrobial compounds that are not detectable
7 in healthy plants are called phytoalexins [4]. When investigating the release of root-derived
8 aromatic exudates in barley attacked by the soil-borne pathogen *Fusarium*, Lanoue and co-
9 workers found an induction of five phenylpropanoids exhibiting antifungal activity [15].
10 Amongst them, labeling experiments highlighted the *de novo* biosynthesis and secretion of *t*-
11 cinnamic acid [15]. Besides this ‘phytoalexin prototype’, pathogen infection can increase the
12 quantities of certain constitutively exuded phytoanticipins. Momilacton A is an antimicrobial
13 diterpene, which is produced and secreted from roots of rice seedlings into the rhizosphere [6,
14 7]. In addition, rice challenged with the blast fungus shows targeted leaf-accumulation of
15 Momilacton A [16]. Thereby, this and other root exudates feature properties of both,
16 constitutively produced and secreted phytoanticipins, as well as pathogen-elicitable
17 phytoalexins [4, 17, 18].

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

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

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

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

1

2 **The release of low molecular weight compounds**

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

19 Phenylpropanoids are ubiquitous plant phenolics, which occur in defense root exudates [15,
20 17]. In line, resistance to *Fusarium* attack in barley is based on the rapid accumulation and
21 secretion of phenylpropanoids (cinnamic acid derivatives) after fungal infection [15]. Moreover,
22 flavonoids represent one of the largest class of phenylpropanoid-derived secondary
23 metabolites in plants and constitute a large proportion of root exudates [32]. Derivatives of
24 isoflavonoids such as the pea phytoalexin pisatin are a crucial class of compounds with potent
25 antimicrobial properties in legumes [33, 34]. Its tissue-specific release from the root tip can be
26 stimulated by pathogen elicitation [35].

1 Terpenoids form the largest class of plant defense chemicals above- and belowground and
2 contribute to root exudates [5-7, 18, 36]. It has been known for some time that nonvolatile
3 terpenoid phytochemicals such as momilactones can be secreted into the rhizosphere [6, 7].
4 However, it was only recently that volatile organic compounds (VOCs) were also shown to be
5 emitted from roots as a direct defense mechanism. Plant-derived volatiles have been
6 previously described to function in tritrophic interactions by attracting natural enemies of
7 herbivores upon herbivore attack to provide indirect plant defense [37-40]. In contrast, an
8 example of a direct belowground volatile defense compound is the monoterpene 1,8-cineole,
9 which is released from hairy-root cultures of *Arabidopsis* during pathogen interaction [26,
10 41]. Furthermore, a semivolatile diterpene hydrocarbon designated rhizathalene A is
11 implicated in the belowground resistance towards root-feeding insects as a local antiherbivore
12 metabolite of *Arabidopsis* [5]. A new role was discovered for another class of terpenoids, the
13 strigolactones. An extensive body of literature has been published on strigolactones as
14 phytohormones and, when released into the rhizosphere, as a compound involved in plant
15 symbiosis with arbuscular mycorrhizal fungi and in plant infection by root parasitic plants
16 [42]. The synthetic strigolactone analog GR24 intriguingly inhibits the growth of an array of
17 phytopathogenic fungi when present in the growth medium, indicating that secreted
18 strigolactones can directly affect natural fungal enemies and contribute to belowground plant
19 biotic stress responses [43].

20 Other highly potent antifungal or antimicrobial root exudates are tryptophan-derived
21 secondary metabolites, such as some glucosinolates or the indole derivate camalexin - the
22 only characterized phytoalexin in *Arabidopsis* [30, 44-51]. Several molecular players
23 involved in camalexin biosynthesis are identified at the genetic level. Their transcriptional
24 activation after infection leads to the intrinsic production, accumulation [44] and exudation
25 [46] of camalexin from *Arabidopsis* roots, whereas their genetic disruption results in lower
26 secretion levels [46], accompanied by enhanced pathogen disease symptoms and fungal

1 growth [44]. In line with these observations, the ectopic overexpression of an *Arabidopsis*
2 gene that modulates camalexin and SA biosynthesis confers disease resistance to soybean
3 against nematodes [52].

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

11

12 **Border cells function as a defensive barrier of roots**

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

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

23 Besides the presence of antimicrobial enzymes long known to be associated with plant
24 defense, the root cap secretome astonishingly contains histone H4 [53]. In mammals, histone-
25 linked extracellular DNA (exDNA) is anticipated to have a critical role in defense against
26 microbial pathogens [65-67]. A similar mechanism was suggested in plants when exDNA

1 linked to histone proteins was discovered to be synthesized and exuded from root border cells
2 [8]. The specific mechanism of how exDNA inhibits pathogen growth needs to be determined.
3 However, recent research suggests that exDNA is probably similar to various structural
4 proteins a fundamental scaffold to trap, immobilize and subsequently kill root-infecting
5 pathogens in the mucilage matrix, since degradation of either component in root tip exudates
6 using a protease or an nuclease respectively results in an abolishment of root tip resistance to
7 fungal infection [8]. Because border cells secrete a mucilage layer that contains proteins and
8 exDNA to protect the root tip by adhesion and aggregation of pathogens, they function
9 analogously to white blood cells in the mammalian innate immune response [8, 59, 68].
10 Taken together, the penetration of physiologically independent border cells, the root cap and
11 border cell exudation of the mucilage layer including proteins and exDNA that immobilize
12 pathogens, as well as the secretion of, for instance, antimicrobial enzymes and secondary
13 metabolites co-operatively provide root tip resistance towards pathogens.

14

15 **The transport machinery that modulates the release of defense phytochemicals**

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

1 Evidence exists that members of both MATE and ABC transporter families are capable of
2 releasing constituents of the root phytochemical cocktail into the rhizosphere. In case of
3 MATE transporter proteins, a subclade that can be found in all plants analyzed so far is
4 implicated in the release of citrate into the rhizosphere to confer aluminum resistance to
5 plants. Since citrate is a nutrient for many microorganisms this exudation may also have an
6 impact on the microflora at the root tip [71-75]. Recently, a MATE transporter in the stele of
7 rice roots was found to facilitate efflux of phenolic compounds into the xylem [76]. It was
8 speculated that similar transporters might be responsible for phenolic secretion into the soil.
9 In Table 1, we highlight the few genes out of 56 members of the MATE family that are
10 promising candidates to encode transport proteins that are involved in such processes because
11 they exhibit a strong or predominant expression in the outer cell layers of root caps and were
12 not previously shown to be localized in another intracellular membrane than the plasma
13 membrane (Table 1) [69, 77]. However, to our knowledge to date no MATE transporter has
14 been identified to export root-derived antimicrobial compounds into the rhizosphere. In
15 contrast, members of the ABC transporter family were demonstrated to be fundamentally
16 involved in root exudation and the defense system [78-81], and recently, putative substrates
17 were attributed to particular transporter proteins [82, 83]. Initially, indirect pharmacological
18 approaches were deployed to demonstrate that the root exudate profile of *Arabidopsis*
19 *thaliana* is quantitatively and qualitatively dependent on ATP hydrolysis [84], indicating that
20 the secretion process of certain phytochemicals is mediated by active transport systems such
21 as ABC-type proteins [85]. Subsequently, among the >120 genes encoding ABC transporter
22 proteins in *Arabidopsis*, 25 candidates were identified to have a potential role in
23 rhizosecretion based on their high expression in root cells [86]. Exudate [79, 86, 87] and
24 microbial [87] composition differs significantly between knock-out lines of several root-
25 expressed ABC transporters and the corresponding wild-type, providing evidence that this
26 protein family is implicated in root secretion, also of antimicrobial compounds. Furthermore,

1 these studies revealed that multiple ABC transporters can be used for the release of a given
2 phytochemical, and a specific ABC transporter can be capable of mediating the export of
3 several structurally and functionally unrelated substrates. For instance, *AtPDR9* is suspected
4 to transport phenolic compounds in order to bind and acquire iron in aerobic soil systems [88]
5 but was shown previously to also transport auxinic compounds [89, 90]. Interestingly, to our
6 knowledge, the only direct link between a defined transporter, its substrate and an effect on
7 soil microorganisms has been demonstrated for *PhPDR1*, a petunia ABC transporter that
8 catalyzes the release of strigolactones from root cells [83].

9 Only few studies addressed the connection of transport proteins, defense-related
10 phytochemical rhizosecretion and soil-borne pathogen susceptibility. The transporter
11 *NpPDR1* of *Nicotiana plumbaginifolia* is directly involved in plant defense against pathogen
12 invasion [78, 91]. Silencing this ABC transporter results in enhanced sensitivity of roots and
13 petals towards various soil-borne pathogens, possibly due to diminished secretion of
14 antifungal compounds, such as the diterpene sclareol [91]. In general, the appearance of a
15 phytochemical in the rhizosphere can be genetically and biochemically regulated by various
16 factors, including ABC-type protein abundance, transport activity, substrate concentration and
17 specificity, but also by pleiotropic effects mediated by ABC transporters. The gene expression
18 of the transporter *NtPDR1* positively correlates with export rates of antipathogenic diterpenes
19 into the extracellular medium, and the expression can be modified by microbial elicitation
20 [82, 92]. On the other hand, another study showed that nitrogen deficiency can elicit the
21 increased biosynthesis of the flavonoid signaling molecule, genistein, resulting in its secretion
22 from soybean roots to initiate rhizobium symbiosis [93], whereby the transport machinery
23 involved in genistein export is constitutively active regardless of the nitrogen availability [85].
24 The further twist to that story is the fact that ABC transporter proteins themselves exhibit a
25 regulatory function in modulating the synthesis and exudation of defense phytochemicals. For
26 instance, the *Arabidopsis* mutant *abcg30* exhibits lower levels of several compounds in the

1 rhizosphere, whereas other defense exudates show a higher secretion in the mutant plants
2 [87]. This finding provides evidence that *AtABCG30* mediates the transport of compounds,
3 but also that the lack of the protein directly and indirectly influences various metabolic
4 processes such as biosynthesis of secondary metabolites or the expression of other
5 transporters. Another recent study showed that roots of *Medicago truncatula* are rapidly
6 infected by *Fusarium* when *MtABCG10*, a gene which encodes a close homologue of *NtPDR1*
7 [82, 92], is silenced. Concomitantly, this silencing results in a reduction of the
8 phenylpropanoid pathway-derived phytoalexin, medicarpin, as well as its precursors in root
9 tissue and exudates [94]. It was therefore proposed that *MtABCG10* may modulate
10 isoflavonoid levels during the belowground biotic stress response associated with the *de novo*
11 biosynthesis of phytoalexins. Furthermore, the mutation of *AtABCG37* and *AtABCC5* results
12 in the accumulation of the phytoalexin, camalexin, in the rhizosphere [79]. This elevated
13 secretion was suggested to be a pleiotropic effect of the mutations, which induce an increased
14 expression of genes involved in the indolic metabolite biosynthesis as previously observed in
15 other mutants [47]. Similarly, the dysfunction of *AtABCG36* results in higher basal defense
16 levels, since flavonoid glycosides and defense proteins accumulated in root tissue supposedly
17 due to a salicylic acid over-production even when roots are grown under sterile conditions
18 [79].

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

23

24 **Concluding remarks and future perspectives**

25 Despite its importance in ensuring tissue protection and optimizing plant performance,
26 deciphering the belowground defense system has been neglected for a long time- a result of

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

24

25

26

1 **Acknowledgments**

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

6

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1 **Figure legends**

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

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1 **Table 1. Expression profiles of genes coding for *Arabidopsis* MATE transporters which**
 2 **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

3

4 ^aGene ontology

5 ^bRoot stage I represents cells of the root cap, stage II of the elongation zone and stage III of
 6 the root hair zone.

7 ^cThe stele is the most inner cell layer of the root, the lateral root cap the most outer cell layer.
 8 Abbreviations are Endo, endodermis; Endo+cortex, endodermis and cortex; Epi, epidermal
 9 artrichoblasts; LRC, lateral root cap.

10 ^dFold changes are calculated as the ratio of the gene expression in a given tissue to the mean
 11 expression level in the entire plant. Consequently, this value indicates the homogeneity of the
 12 gene expression and the maximal fold change occurs in the tissue with the highest absolute
 13 expression levels.

1 **Highlights**

- 2 Defense root exudates are chemically diverse.
- 3 Plants constitutively secrete root exudates to prevent pathogen attack.
- 4 Stimuli such as microbial elicitors trigger compositional changes in root exudates.
- 5 ABC transporters are involved in releasing and regulating defense root exudates.

Figure
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