

Root Exudates of Legume Plants and Their Involvement in Interactions with Soil Microbes

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Abstract Plants secrete both high- and low-molecular weight compounds from their roots, and these root exudates function not only as nutrients for soil microbes but as signal molecules in plant–microbe interactions. Legume plants establish symbiotic interactions with rhizobia and arbuscular mycorrhizal fungi to obtain several nutrients such as nitrogen and phosphate. In these interactions, flavonoids and strigolactones in root exudates serve as signal molecules to establish the symbiotic interactions. Root exudates from some legume plants also function to acidify surrounding soils to acquire phosphate. Here, we provide an overview of the functions of legume root exudates with emphasis on the interaction between legume plants and soil microbes and also on the acquisition of nutrients from surrounding soil.

1 Introduction

The legume family (Fabaceae) is composed of more than 700 genera containing approximately 20,000 species (Doyle and Luckow 2003). It represents the third largest plant family next to Orchidaceae and Asteraceae. Legume plants have

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a significant agricultural importance because many crop plants such as soybean (*Glycine max*), pea (*Pisum sativum*), and bean (*Phaseolus vulgaris*) belong to this family, and legume crops are cultivated on 12–15% of the arable land throughout the world. Legume species, including soybean and peanut (*Arachis hypogaea*), provide more than one third of the processed vegetable oil throughout the world's market (Graham and Vance 2003). In addition to these crop legumes, nongrain legumes, such as licorice (*Glycyrrhiza galbra*) provide glycyrrhizin, which is a useful phytotherapeutic and sweating agent, while legume plants such as indigo (*Indigofera tinctoria* and *I. suffruticosa*) and logwood (*Haematoxylon campechianum*) provide natural dyes. Forage legumes such as alfalfa (*Medicago sativa*) and birdsfoot trefoil (*Lotus corniculatus*) are widely used for livestock feed, and tree legume such as various Acacia and Robinia species supply large-scale biomaterials like tannin and pulp (Dixon and Sumner 2003; Downs et al. 2003; Christie 2007).

A hallmark feature of legume plants is their ability to establish a mutualistic symbiosis with bacteria belonging to Rhizobiaceae family for the utilization of atmospheric nitrogen as a nitrogen source. It is estimated that approximately 40–60 million metric tons of atmospheric nitrogen is fixed by cultivated legume plants annually (Smil 1999), which is important not only for agriculture, but also for the environment because nitrogen fixation can supplement the use of synthetic nitrogen fertilizers which require a large amount of energy input during production that can contribute to environmental pollution. Most of the legume plants also establish a symbiotic interaction with arbuscular mycorrhizal fungi which help to obtain mineral nutrients, as well as water.

In addition to these mutualistic interactions, legume plants interact with diverse soil microbes both positively and negatively. Both culture-dependent and culture-independent methods were employed to analyze the microbial communities and unique communities were found depending on the plant species, cultivars, and ecotypes (Priha et al. 1999; Yang and Crowley 2000; Narasimhan et al. 2003; Innes et al. 2004; Mazzola et al. 2004; Batten et al. 2006; Kowalchuka et al. 2006; Mougel et al. 2006; Micallef et al. 2009). It has also been reported that symbiotic mutants (i.e., hypernodulating mutants, mutants defective in *Rhizobium* symbiosis, and mutants defective in both rhizobial and arbuscular mycorrhizal symbiosis) have different microbial communities contained in the rhizosphere soil as well as the roots than do wild type plants (Offre et al. 2007; Ikeda et al. 2008). Among the various methods plants employ to interact with soil microbes, root exudates are a key factor that influences the microbial communities (Uren 2007; Badri and Vivanco 2009; Badri et al. 2009).

This chapter provides an overview of the literature on the root exudates of legume plants with emphasis on the interaction between legume plants and soil microbes. We first describe the characteristics of legume root exudates and then discuss the functions of these root exudates in the interaction with soil microbes such as rhizobia and arbuscular mycorrhizal fungi. Finally, we describe some features of root exudates in the acquisition of nutrients from soil.

2 Characteristics of Root Exudates from Legume Plants

Metabolites in legume plants have been intensively studied due to biological interests such as symbiotic nitrogen fixation, as well as their importance to human health. Recently, metabolomic analyses have been performed on various legume species such as *M. truncatula*, *L. japonicus*, soybean, and the common bean (Desbrosses et al. 2005; Farag et al. 2008; Suzuki et al. 2008; Farag et al. 2009; Hernandez et al. 2009; Brechenmacher et al. 2010; Risipail et al. 2010). Many of these studies are focused, at least in part, on flavonoids and other phenolic compounds. Profiling of flavonoids in *M. truncatula* roots and cell suspension culture showed *M. truncatula* contains at least 40 flavonoids, most of which are glycosides (glucoside or glucoside malonate), and that methyl jasmonate induced the accumulation of the phytoalexin medicarpin and decreased the isoflavone glucosides, while a yeast elicitor coordinately increased isoflavonoid precursors and medicarpin (Farag et al. 2008). In *L. japonicus*, 61 flavonoid compounds have been identified from leaves, stems, flowers, and seeds. Most of these are glycosides and about half of them are flower specific (Suzuki et al. 2008). Another group recently reported that *M. loti* inoculation induced quantitative rather than qualitative changes of the phenolic compounds in roots of *L. japonicus* and that phytoalexin vestitol and sativan were not found in the inoculated roots (Risipail et al. 2010).

In contrast to the metabolite profiling in plants, metabolite profiling of root exudates are limited to the targeted analysis of particular compounds such as organic acids, flavonoids, and fatty acids (Smit et al. 1992; Lucas Garcia et al. 2001). There are several reports on the metabolome and proteome analyses of *Arabidopsis* root exudates identifying various metabolites such as sugars, amino acids, fatty acids, as well as various classes of proteins (De-la-Pena et al. 2008, 2010; Badri and Vivanco 2009; Badri et al. 2009). Proteome and metabolome analyses of root exudates in legume species will be of particular interest to target compounds that function in plant–microbe interactions, such as the recruitment of rhizobia, arbuscular mycorrhizal fungi, and PGPR (plant growth promoting rhizobacteria) species that potentially improve crop yields. It was shown that *L. japonicus* secreted strigolactone for the establishment of arbuscular symbiosis and more recently was reported that *L. japonicus* secretes a phytoalexin vestitol as a chemical barrier against parasitic weeds (Ueda and Sugimoto 2010). We also performed metabolite profiling of the root exudates of *L. japonicus* and found that methyl jasmonate induced several unknown compounds in the exudates (unpublished results). It would be interesting to characterize the function of these induced compounds in response to pathogens. The following chapters provide the functions of legume root exudates in plant–microbe interactions in soils.

3 Functions of Root Exudates in Symbiosis with Rhizobia

3.1 Signaling Molecules from Legume Plants to Rhizobia

Interaction between legume plants and rhizobia in the soil is of particular importance in agriculture and ecology and many studies have been performed to characterize the molecular mechanisms on how this species-specific interaction is established (Werner 2007). In soils, *Rhizobium* spp. can find its host legume plant from a distance because of the chemotactic nature of rhizobia. Rhizobia have been shown to be attracted to the root exudates of legume plants (Currier and Strobel 1976). It was reported that flavonoids present in root exudates are responsible for the attraction of rhizobia in alfalfa (Parke et al. 1985; Caetano-Anolles et al. 1988; Dharmatilake and Bauer 1992), and a *Rhizobium* mutant defective in chemotaxis was shown to be less competitive in forming the functional nodules (Yost et al. 1998). Because phytochemicals such as flavonoids may not diffuse for long distances in soils, it is presumed that root volatiles are also involved in the attraction of rhizobia to host plants. In *Medicago*–*Sinorhizobium* interaction, root volatiles of *Medicago*, especially dimethylsulfide, were shown to attract nematodes which bring *Sinorhizobium meliloti* to the proximity of plant roots (Horiuchi et al. 2005). In a similar way, it may also be possible that other volatile organic compounds attract rhizobia from a distance.

In the 1980s, many genes and proteins involved in the recognition of plant signal molecules were identified in rhizobia: such as NodD (a LysR-type regulator which acts as a transcriptional activator for the *nod* operon), NodA (*N*-acetyltransferase), NodB (de-*N*-acetylase), and NodC (UDP-Gluc *N*-acetyltransferase), which together synthesize the backbone of a lipochitooligosaccharide called Nod factor (Rossen et al. 1984; Egelhoff et al. 1985; Egelhoff and Long 1985; Mulligan and Long 1985; Rossen et al. 1985; Fisher and Long 1992). To identify the signaling molecules from plant roots, a nod promoter–*LacZ* fusion reporter system was employed. With this reporter system, signaling molecules from legume plants were identified to be luteolin from alfalfa (*M. sativa*), 7,4'-dihydroxyflavone and geraldone from white clover (*Trifolium repens*), and daidzein and genistein from soybean (*G. max*) (Peters et al. 1986; Redmond et al. 1986; Djordjevic et al. 1987; Kossak et al. 1987) (Table 1), whereas formononetin and umbelliferone were shown to exhibit inhibitory effects on the *nod* gene expression (Djordjevic et al. 1987). Beside these flavones and isoflavonoids, a chalcone (4,4'-dihydroxy-2'-methoxychalcone) from alfalfa (Maxwell et al. 1989), anthocyanidins (petunidin and malvidin) from the common bean (*P. vulgaris*) (Hungria et al. 1991), betains (trigonelline and stachydrine) from alfalfa (Phillips et al. 1992), and aldonic acids (erythronic acid and tetronic acid) from white lupine (*Lupinus albus*) (Gagnon and Ibrahim 1998) were also reported to be *nod* gene inducers (Table 1). These various data suggest that a structurally diverse variety of phytochemicals can function as signal molecules. Signaling molecules from a legume tree species, black locust (*Robinia pseudoacacia*), were also identified to be flavonoids (7,4'-dihydroxyflavone,

Table 1 Nod-gene-inducing compounds of root exudates of legume plants

Plant	Compound	Reference
Alfalfa (<i>Medicago sativa</i>)	Luteolin	Peters et al. (1986)
	7,4'-Dihydroxyflavone	Maxwell et al. (1989)
	7,4'-Dihydroxyflavanone	
	4,4'-Dihydroxy-2'-methoxychalcone	
	Chrysoeriol	Hartwig et al. (1990)
	Trigonelline	Phillips et al. (1992)
Barrel Medic (<i>Medicago truncatula</i>)	Stachydrine	
	7,4'-Dihydroxyflavone	Zhang et al. (2006)
Black Locust (<i>Robinia pseudoacacia</i>)	7,4'-Dihydroxyflavone	Scheidemann and Wetzel (1997)
	Apigenin	
	Naringenin	
	Chrysoeriol	
	Isoliquiritigenin	
Common bean (<i>Phaseolus vulgaris</i>)	Eriodictyol	Hungria et al. (1991a)
	Naringenin	
	Genistein 7-O-glycoside	
	Delphinidin	Hungria et al. (1991b)
	Petunidin	
	Malvidin	
	Myricetin	
	Quercetin	
	Kaempferol	
Common vetch (<i>Vicia sativa</i>)	3,5,7,3'-Tetrahydroxy-4'-methoxyflavanone	Zaat et al. (1989)
	7,3'-Dihydroxy-4'-methoxyflavanone	
Cowpea (<i>Vigna unguiculata</i>)	Daidzein	Dakora et al. (2000)
	Genistein	
	Coumestrol	
Miyakogusa (<i>Lotus japonicus</i>)	Unknown	
Pea (<i>Pisum sativum</i>)	Apigenin	Firmin et al. (1986)
	Eriodictyol	
Rostrate sesbania (<i>Sesbania rostrata</i>)	7,4'-Dihydroxyflavanone	Messens et al. (1991)
Soybean (<i>Glycine max</i>)	Daidzein	Kossalak et al. (1987)
	Genistein	
	Coumestrol	Bassam et al. (1988)
White clover (<i>Trifolium repens</i>)	7,4'-Dihydroxyflavone	Redmond et al. (1986)
	Geraldone	
White lupine (<i>Lupinus albus</i>)	Erythronic acid	Gagnon and Ibrahim (1998)
	Tetronic acid	

apigenin, naringenin, chrysoeriol, isoliquiritigenin) (Scheidemann and Wetzel 1997). The synthesis of these signaling molecules was induced under nitrogen deficiency (Cho and Harper 1991), and the application of signaling molecules to the legume plants was shown to increase the number of nodules in the roots (Begum et al. 2001; Novak et al. 2002).

These signaling compounds were secreted from root tissues into the rhizosphere using an energy-dependent transport system, and therefore diffuse around plant roots. They bind to the NodD receptor in the rhizobial cell surface and induce the expression of *nod* genes leading to the synthesis of Nod factors in a species-specific manner. These signaling flavonoids were also shown to induce the type III secretion systems (TTSS) and the excretion of proteins (Viprey et al. 1998; Fauvart and Michiels 2008). TTSS, which is composed of ca. 20 proteins, is a molecular machine found in both symbiotic (such as *Bradyrhizobium japonicum*, *Mesorhizobium loti*, *Rhizobium fredii*) and pathogenic bacteria to deliver effector, translocator, and regulator proteins to eukaryotic cells. It was reported that a mutation in TTSS affects symbiosis in a host-specific manner, i.e., the mutation in TTSS of *Rhizobium* sp. NGR234 resulted in increased number of nodules in yam bean (*Pachyrhizus tuberosus*), less number of nodules in fish bean (*Tephrosia vogelii*), and had no effect on nodulation in cowpea (*Vigna unguiculata*) and white leadtree (*Leucaena leucocephala*) (Viprey et al. 1998). It was also shown that the TTSS mutant of *Rhizobium* sp. NGR234 formed small and nonnitrogen fixing nodules that seemed to be devoid of meristematic cells in sunhemp (*Crotalaria juncea*) (Marie et al. 2003). The precise function of TTSS and its secreted proteins (called Nops; nodulation outer proteins) in the nodulation process is not yet well understood, but three Nops (NopL, NopP, and NopT) are proposed to be involved in modulating the host signaling pathways such as the down regulation of host plant defenses, and two other Nops (NopD and NopM) may be involved in the interference of host protein regulation in the nuclei (Krishnan 2002; Krishnan et al. 2003; Ausmees et al. 2004; Fauvart and Michiels 2008). It is noteworthy that TTSS is not common machinery conserved in all *Rhizobia*, for instance, *S. meliloti* does not contain TTSS genes in its genome (Galibert et al. 2001).

3.2 Signaling Molecules from *Rhizobia* to Legume Plants

Upon the recognition of plant-derived signal molecules, rhizobia induce the expression of *nod* genes, which are responsible for the synthesis of a lipochitooligosaccharide, Nod factor. Each *Rhizobium* species contains both common *nod* genes such as *nod A*, *nod B*, and *nod C*, as well as other species-specific *nod* genes. The common *nod* genes are responsible for the synthesis of the backbone structure of Nod factors, while species-specific *nod* genes are responsible for the modification of that structure, especially the side chain of the oligosaccharide. Functions and properties of species-specific *nod* genes were summarized previously (Fisher and Long 1992; Werner and Muller 2002). These species-specific modifications are

necessary for the divergence of the structures of various Nod factors, which is indispensable in the specific host–*Rhizobium* interactions. The secretion of Nod factors from rhizobia is an energy-dependent process, and several genes involved in the secretion of the Nod factor have been identified; *nodI*, *nodJ*, *nodT*, *nodFGHI* (Rivilla et al. 1995; Spaink et al. 1995; Cardenas et al. 1996; Fernandez-Lopez et al. 1996). The products of these genes comprise a bacterial type ATP-binding cassette (ABC) transporter which consists of two transmembrane proteins and two nucleotide binding proteins.

Nod factors secreted into the rhizosphere are received by the Nod receptors located at the plasma membrane of the host legume root cells. Upon the recognition of Nod factors, plant roots undergo a series of drastic physiological changes: (1) Nod factors induce the formation of a transient subcellular gradient of chloride, potassium, and calcium ions as well as pH, followed by sharp oscillations of the cytoplasmic calcium ion concentration, called “calcium spiking” in root hair cells; (2) Nod factors induce the curling of root hairs, which entrap rhizobia; (3) rhizobia begin to penetrate plant roots by forming an infection thread; (4) cortical cells of the roots begin to divide to form the nodule primordia; (5) rhizobia eventually enter the plant cell via endocytosis, resulting in the formation of symbiosomes in which rhizobia are surrounded by the plasma-membrane-derived peribacteroid membrane (Verma and Hong 1996).

It is also noteworthy that the backbone structure of Nod factors is chitin (chitooligosaccharides), which is a major component of fungal cell walls and induces various defense responses in plants. Chitooligosaccharides have been shown to induce various defense responses in plant cells, such as the induction of defense-related genes, synthesis of phytoalexin, and the production of reactive oxygen species (Baier et al. 1999; Stergiopoulos and De Wit 2009).

Screening of legume mutants defective in the nodulation identified genes involved in the recognition of Nod factors. LysM domain-containing receptor-like kinases (NFR1, NFR5, SYM2) in legumes were shown to be crucial for the perception of Nod factors in the legume–*Rhizobium* symbiosis (Limpens et al. 2003; Madsen et al. 2003; Radutoiu et al. 2003). As Nod factors are structurally similar to chitooligosaccharides, it has been presumed that plants possess a specific receptor to perceive chitooligosaccharides leading to defense gene induction. In fact, Arabidopsis LysM type receptor-like kinase, which has an amino acid similarity to Nod factor receptors, was shown to be involved in the recognition of chitinoligosaccharides (Miya et al. 2007; Wan et al. 2008). Interestingly, it was very recently shown that Arabidopsis LysM receptor kinase can transmit signals leading to the symbiosis in *L. japonicus* when a part of the kinase domain was modified to match the sequence of *L. japonicus* (Nakagawa et al. 2011).

When *L. japonicus* was inoculated with *M. loti*, many genes involved in the defense mechanism, such as those responsible for phenylalanine ammonia-lyases, 4-coumarate, CoA ligase, chalcone reductase, chitinase, β -1,3-glucanase, and peroxidase, were induced in the early stage of nodulation and then suppressed to normal levels (Kouchi et al. 2004). Considering the similarity between the Nod factor receptor and chitin receptor, this phenomenon indicates that when legume

plants recognize rhizobia, plants first treat them as “potential pathogens” with the induction of defense genes and the production of phytoalexins and do not automatically accept the foreign bacteria into root cells until the plant further recognizes their symbiotic partners with different machineries such as extracellular lipopolysaccharides (EPS) and lipo-polysaccharides (LPS), as well as effector proteins secreted by TTSS.

3.3 *Transporters Involved in the Secretion of Root Exudates*

The mechanism by which plant roots secrete phytochemicals was thought to be a passive process mediated by diffusion and channels. However, recent advances suggest that both primary and secondary transporters are involved in the secretion of phytochemicals into the rhizosphere. In plants, there are two major transporter families which are able to transport organic substrates, the ABC transporter family and multidrug and toxic compound extrusion (MATE) transporter family, which consist of 123 and 56 genes in *Arabidopsis*, respectively (Omote et al. 2006; Verrier et al. 2008; Yazaki et al. 2008). It has been shown that some transporters in these families are also involved in the secretion of root exudates (Loyola-Vargas et al. 2007; Sugiyama et al. 2007; Badri et al. 2008; Yazaki et al. 2008, 2009). For example, a MATE transporter of barley was shown to secrete citrate into the rhizosphere, protecting plant roots from aluminum toxicity (Furukawa et al. 2007; Wang et al. 2007).

In legume–*Rhizobium* interaction, an ABC-type transporter was shown to be involved in the secretion of the isoflavonoid genistein, a signal molecule from soybean to *B. japonicum* (Sugiyama et al. 2007) (Fig. 1). The genistein transport activity of the soybean root plasma membrane was inhibited by sodium orthovanadate, which is a general inhibitor for ABC transporters and P-type ATPases, but not by other inhibitors of electrochemical gradients such as nigericin, valinomycin, or gramicidin D which act as ionophores. It has been known that the synthesis and secretion of genistein is up-regulated under nitrogen deficiency, but the genistein transport activity of the soybean root plasma membrane remained unchanged even under nitrogen deficiency. This suggests that transport activity is constitutively active while the genistein secretion is regulated by the control of the biosynthesis level (Sugiyama et al. 2007). Furthermore, a pharmacological approach using subfamily-specific inhibitors such as verapamil and cyclosporine A, which are often used as general inhibitors of ABCB (P-glycoprotein)-type ABC transporters, and glybenclamide, a sulfonylurea derivative that acts as an effective inhibitor of several ABCC-type members, suggested that these transporter members are unlikely to be responsible for the genistein secretion in soybean. Bioinformatic analysis was indicative that a PDR (pleiotropic drug resistance)-type (full-size ABCG-type) ABC transporter is a primary candidate in the secretion of genistein (Sugiyama et al. 2008).

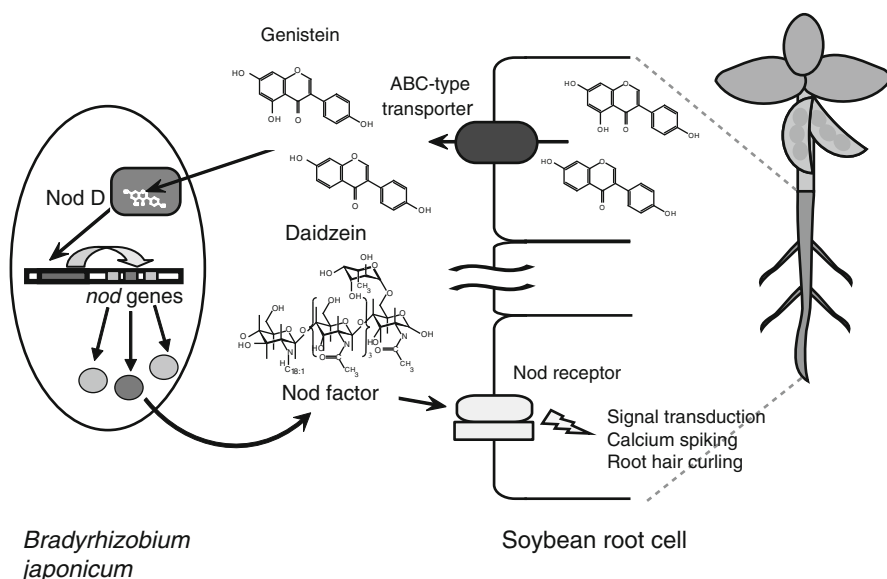


Fig. 1 A model of flavonoid secretion from soybean roots and the interrecognition between soybean and *B. japonicum*

4 Functions of Root Exudates in Symbiosis with Mycorrhiza

4.1 Symbiosis with Arbuscular Mycorrhizal Fungi

Mycorrhizal fungi are major components of the soil microbial community, aiding in the transfer of nutrients from the soil to the plants. They are divided into two groups: endomycorrhiza, such as arbuscular, ericoid, and orchid mycorrhiza, and ectomycorrhiza, which are found especially in temperate forests. These heterogeneous fungi colonize the roots of about 240,000 plant species in a wide range of terrestrial ecosystems. Among the mycorrhiza, the arbuscular mycorrhiza fungi symbiotically interact with more than 80% of plant species widely distributed throughout the plant kingdom (Parniske 2008), but some plant families such as Brassicaceae and Chenopodiaceae are nonhost plants (Smith and Read 1997). Interestingly, lupins are also a nonhost for arbuscular mycorrhizal fungi, although they belong to Leguminosae. Symbiosis with plants results in the formation of tree-shaped subcellular structures, called arbuscules, within the plant cells. These structures are thought to be the main site of nutrient exchange between the fungi and plants. Because the arbuscular mycorrhizal hyphal network reaches to more than 100 m per cubic centimeter of soil and is positioned to efficiently take up various nutrients and water from the surrounding soil (Miller et al. 1995), plants can obtain nutrients such as phosphate and various micronutrients, as well as water, by

utilizing this hyphal network. Fossil records have revealed that the origin of arbuscular mycorrhizal symbiosis occurred at least 420–460 million years ago, which coincides with the appearance of the first terrestrial plants, suggesting that the colonization of land by plants from the water was assisted by ancestral arbuscular mycorrhizal fungi (Simon et al. 1993; Remy et al. 1994; Redecker et al. 2000).

4.2 Signal Molecules Between Legume Plants and Arbuscular Mycorrhizal Fungi

Because arbuscular mycorrhizal fungi are obligate biotrophs and depend on a living photoautotrophic host to complete their life cycle, a critical developmental step is hyphal branching which enables them to make contact with the host's roots and establish the symbiosis. In a similar way that flavonoids function as a signaling molecule for rhizobia, the branching factor is hypothesized to be a plant signal molecule to trigger hyphal branching (Buee et al. 2000), and these authors have ruled out flavonoids as candidates for the branching factor because root exudates of maize mutants deficient in chalcone synthase show branching activity similar to those of the wild type (Buee et al. 2000). It was also reported that root exudates of plants grown under phosphate deficient conditions have higher activity than those under sufficient phosphate nutrition (Tawaraya et al. 1995, 1998). Using root exudates of *L. japonicas*, the chemical structure of the branching factor was identified to be a strigolactone (Akiyama et al. 2005). Strigolactones have been previously isolated from the root exudates of a variety of plants (Bouwmeester et al. 2003) as a seed germination factor for parasitic weeds such as *Striga* and *Orobanche* (see Sect. 4.3).

It was shown that strigolactones were derived from a carotenoid pathway that is also induced under phosphate deficiency (Lopez-Raez et al. 2008). Strigolactones are found in the root exudates of tomato, sorghum, and pea, as well as *L. japonicus*, but not in carrot, tobacco, or alfalfa (Garcia-Garrido et al. 2009), suggesting the presence of other compounds in root exudates that activate hyphal branching. It should also be mentioned that strigolactones were identified in the root exudates of both *Arabidopsis* and lupin which are nonhost plants to arbuscular mycorrhizal fungi (Goldwasser et al. 2008; Yoneyama et al. 2008). There will be divergent phytochemicals that function as signals to arbuscular mycorrhizal fungi depending on the plant species, as is the signals to rhizobia. It was recently shown that lupin secretes pyranosylflavones which inhibit hyphal development in arbuscular mycorrhizal fungi (Akiyama et al. 2010).

There is a large amount of interest in the molecular identification of signaling molecules from fungi to plants that induce symbiosis-specific responses in the host root. These hypothetical compounds are called Myc factors, and the existence of such factors became evident by using an ENOD11-promoter GUS (β -glucuronidase)

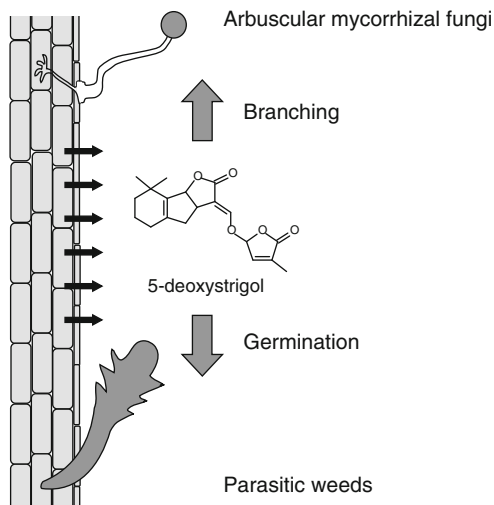
reporter gene fusion in the roots of *M. truncatula* (Kosuta et al. 2003), because hyphae from germinating spores produced a diffusible factor that was perceived by Medicago roots separated by a physical barrier which prevented direct physical contact. Calcium spiking, which occurs in root hairs within a few minutes after the Nod factor application, is also observed following the recognition of Myc factors by plant roots (Navazio et al. 2007). Rapid and transient elevations in cytosolic calcium ions were observed in response to the culture medium of spores of *Gigaspora margarita*, indicating that diffusible molecules released by arbuscular mycorrhizal fungi are perceived by plant cells. The fungal molecules were found to be heat stable with a molecular weight of less than 3,000 and partially lipophilic (Navazio et al. 2007). It is still unknown whether the production of Myc factor is induced by strigolactones or other factors of plants.

4.3 Diverse Functions of Strigolactones

Strigolactones are a group of apocarotenoids and at least nine strigolactones (strigol, strigyl acetate, 5-deoxystrigol, orobanchol, orobanchyl acetate, sorgolactone, epi-orobanchol, solanacol, and sorgomol) have been structurally characterized from the root exudates of various plant species (Rani et al. 2008). The function of strigolactones in the rhizosphere had been identified to stimulate seed germination of harmful parasitic weeds such as witchweed (*Striga*) and broomrape (*Orobanche*) before the identification as a hyphal branching factor for arbuscular mycorrhizal fungi. Parasitic weeds are noxious root parasites on many crop species and cause devastating losses of crop yield in many parts of the world including Africa, India, and the Middle East (Rani et al. 2008). The first step in the life cycle is the germination of the seeds of these parasitic plants, which resemble the hyphal branching induced by strigolactones, and both parasitic weeds and arbuscular mycorrhizal fungi are obligate biotrophs. Strigolactones are short-lived in the rhizosphere because of a labile ether bond which spontaneously hydrolyses in water. The fragility of these compounds forms a steep concentration gradient from the plant roots, and this concentration can be a conscientious indicator for the distance to the plant roots (Parniske 2005), thereby enabling these obligate biotrophs to find living plant roots. Considering the fact that the origin of arbuscular mycorrhizal symbiosis occurred far before the appearance of parasitic weeds, it can be concluded that they have evolved to utilize this ancient signal of living plants to parasitize their roots (Fig. 2).

Besides being the signal molecule to arbuscular mycorrhizal fungi and parasitic weeds, strigolactones have been shown to function as an endogenous phytohormone. It was shown that strigolactone levels were reduced in rice mutants that have enhanced shoot branching and that application of strigolactones inhibited shoot branching (Umehara et al. 2008). Another group reported that pea mutants which have reduced levels of strigolactones and altered axial bud growth have root exudates that were shown to exhibit a significantly reduced amount of activity in

Fig. 2 Strigolactone stimulates both hyphal branching of arbuscular mycorrhizal fungi and germination of parasitic weeds



fungus hyphae branching (Gomez-Roldan et al. 2008). There is no information on how strigolactone acts as a phytohormone or on the receptor for strigolactone, but arbuscular mycorrhizal fungi may use the ancestral receptor for strigolactones to perceive signals of strigolactones in the rhizosphere. Identification of receptors for strigolactones in plants, as well as in fungi, is an interesting topic for future research. There also remains an open question on the original physiological role of strigolactones, i.e., whether strigolactones evolved first as an endogenous phytohormone or as a signal for arbuscular mycorrhizal fungi.

5 Functions of Root Exudates in Mineral Acquisition

5.1 Mineral Requirements for Legume Plants

Plants require 17 essential elements to complete their life cycles, and beside C, H, and O, they absorb mineral nutrients from the soil through their roots. Legume plants are unique in that they form symbiotic interaction with rhizobia. Symbiosis with nitrogen-fixing rhizobia enables legume plants to grow under low nitrogen conditions; however, legume plants require some micronutrients in greater quantities to maintain the symbiosis. One of the examples of these micronutrients is molybdenum (Mo). Mo is a cofactor of a few, but important, enzymes such as nitrate reductase and sulfite oxidase which are involved in nitrogen assimilation and sulfur metabolism, respectively (Hansch and Mendel 2009). Aldehyde oxidase and xanthine dehydrogenase are also Mo-containing enzymes. Legume plants need Mo not only for these enzymes but also for nitrogenase, the most important rhizobial

enzyme for the fixation of nitrogen. Nitrogenase has a heterometal complex (FeMoCo) in its active site, and transport of Mo to the bacteroid is required for the proper function of nitrogenase. Application of molybdenum was shown to increase the yield and nitrogen content in legume crops in both laboratory and field conditions (Weeraratna 1980; Yanni 1992; Vieira et al. 1998). It is also reported that a *B. japonicum* strain deficient in molybdenum transport showed impaired nitrogen fixation activity when inoculated to soybean roots (Delgado et al. 2006).

During the reduction of atmospheric N_2 by nitrogenase, gaseous H_2 is produced. In order to utilize H_2 as an energy source, a limited number of rhizobia have hydrogenase that oxidizes H_2 and generates ATP. This hydrogen recycling was shown to increase productivity in symbiotic systems such as soybean. Hydrogenase is a nickel (Ni)-containing enzyme and consists of large and small subunits (HupL and HupS, respectively). It was reported that the exogenous application of Ni to pea roots increased the mature Hup proteins and hydrogenase activity in nodules (Brito et al. 1994), but it is not yet clear how Ni is transported from plant roots into bacteroids.

Iron (Fe) is an important element in photosynthesis because up to 80% of the cellular iron is found in the chloroplasts, but in legume plants iron plays an essential role for leghemoglobin formation, which is the most abundant protein in the nodules. Leghemoglobin is a hemoprotein that has a high affinity for oxygen leading to low oxygen content in the bacteroids to protect the oxygen-sensitive nitrogenase in the nodules (Johnston et al. 2001). Fe is also required for nitrogenase (Fe-S cluster and FeMoCo active site) and nitrogenase reductase (Fe-S cluster) as well as cytochromes and other electron donors that have Fe centers. Legume plants, therefore, need to absorb Fe from soils to meet this high demand, but soluble Fe is very limited in soils because most of the Fe exists as insoluble Fe^{3+} . In fact, Fe deficiency causes a drastic effect on nodule development (O'Hara et al. 1988). Gramineae plants, for instance, secrete phytosiderophores into the rhizosphere for iron acquisition (Takagi 1976), and in legume nodules, rhizobia produce siderophores to aid the iron acquisition as well. Recently, it was reported that red clover promotes the growth of siderophore-producing microbes in the rhizosphere under iron deficient conditions, where phenolic exudates from the red clover roots were responsible for this phenomenon (Jin et al. 2010). Rhizobial genes involved in the biosynthesis of siderophores and related Fe uptake have been reported, but the functions of these genes were studied only in a free-living state (Johnston et al. 2001). In *R. leguminosarum*, it was shown that the mutation of genes involved in the synthesis of siderophores has no influence in the symbiotic nitrogen fixation in pea in which these genes are actually not expressed in the mature bacteroids (Carter et al. 2002). In contrast, *B. japonicum* mutant defective in the uptake of the siderophore have a drastically divergent phenotype in planta, i.e., soybean nodules without siderophore do not fix nitrogen (Benson et al. 2005), suggesting that rhizobial siderophores have functions in Fe uptake at least in the soybean-*B. japonicum* interaction, while the functions of siderophores in nodules are still to

be elucidated. It would be the future target of molecular breeding that genes of rhizobia are modified so that they produce siderophores to help legume plants to acquire more Fe from soils.

Contrary to Mo, Ni, and Fe that have symbiosis-specific functions in nodules, the specific relevance of zinc (Zn) for symbiosis is not known; although legume plants do contain a higher concentration of Zn. Zn is an important trace element for humans as well as plants, necessary for DNA replication, protein synthesis, and oxidative stress reduction. In fact, Zn malnutrition affects more than one third of world's population (Hess et al. 2009; Hirschi 2009). To overcome this huge problem, the legume crop lentil has been used for biofortification to increase Zn concentration in planta (Thavarajah et al. 2009).

5.2 *Root Exudates for Phosphorus Acquisition*

Phosphorus (P) is one of the major macronutrients in plants, but its availability is very limited because most P in soil is in insoluble forms such as organic phosphate or insoluble mineral phosphate. Organic phosphate, which can account for ca. 80% of the total phosphorus in soil (Li et al. 1997), has to be mineralized to inorganic phosphate in order to be absorbed by plant roots. As a strategy to acquire phosphate, plants secrete phosphohydrolases into the rhizosphere, which convert organic phosphate into inorganic soluble phosphate, e.g., acid phosphatase is secreted in response to phosphorus deficiency (Lefebvre et al. 1990; Duff et al. 1994). Another strategy is that plants also secrete protons to acidify the rhizosphere which increases the phosphates solubility (Staunton and Leprince 1996).

There are some plant species that can survive on infertile soil containing only limited amounts of available phosphorus: one example of these species is white lupin, a legume plant that has been extensively studied. In response to phosphorus starvation, white lupin develops special bottlebrush-like root clusters, called cluster roots, and secrete protons and a large amount of carboxylates, such as malate and citrate, which solubilizes the phosphates (Neumann and Martinoia 2002). Secretion of organic acids follows a spatial and temporal release pattern in the cluster roots of white lupin: cluster roots secrete low amounts of organic acids, mainly malate in early stage, while the roots secrete a larger amount of organic acids, mainly citrate in the mature stage, which is then accompanied by acidification of the rhizosphere (Neumann et al. 2000). It was also shown that the cluster roots of white lupin secrete isoflavonoids such as genistein and hydroxygenistein (Weisskopf et al. 2006b), which may act as antimicrobials by inhibiting the growth of soil microbes to suppress the biodegradation of citrate. In addition, cluster roots secrete antifungal enzymes, such as glucanase and chitinase, which is thought to prevent the biodegradation of the carboxylates by fungi (Weisskopf et al. 2006a).

6 Conclusions

This chapter provides an overview of literatures on the functions of root exudates in plant–microbe interactions and the acquisition of mineral nutrients from the soil. We have focused on the mutualistic interactions with rhizobia and arbuscular mycorrhizal fungi because recent research has identified genes and metabolites involved in these sophisticated interactions; however, there still remains many plant–microbe interactions in the soil to be analyzed in detail. For example, how plants recruit PGPR, how plants protect themselves from pathogens using phytoalexins, and how plants interfere with quorum sensing using *N*-acyl homoserine lactone mimics (Teplitski et al. 2000; Keshavan et al. 2005). There will also be underground multitrophic interactions, which involve both soluble and volatile root exudates. In maize, (*E*)- β -caryophyllene is emitted from insect-damaged roots that recruit entomopathogenic nematodes (Rasmann et al. 2005). It is possible that legume plants have a tri-interaction system mediated by root exudates including volatiles. Most studies of plant–microbe interactions so far have been focused on a particular microbe such as *Rhizobium* and arbuscular mycorrhizal fungi; however, in nature, legume plants have interactions with many microbes in the soil. Therefore, the interaction of legume plants with entire microbial communities is of particular interest for future research as we need to expand our understanding of the plant microbe interactions to the level that could help develop sustainable agriculture using the various functions of microbes on the soil. There is no argument that plants influence the soil microbial communities, but root exudates are not the only key player to the functions of those interactions. For instance, border cells and other rhizodeposits are nutrient sources and signals for microbes in soils as well (Dennis et al. 2010). The nature of root exudation enables plants to actively regulate the rhizosphere microbial communities, and further research on legume root exudates could open the door to the possibilities of sustainable agriculture practices utilizing legume crops that actively secrete metabolites to recruit beneficial microbes and prevent pathogens.

References

- Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435:824–827
- Akiyama K, Tanigawa F, Kashiwara T, Hayashi H (2010) Lupin pyranosylflavones inhibiting hyphal development in arbuscular mycorrhizal fungi. *Phytochemistry* 71:1865–1871
- Ausmees N, Kobayashi H, Deakin WJ, Marie C, Krishnan HB, Broughton WJ, Perret X (2004) Characterization of NopP, a type III secreted effector of *Rhizobium* sp. strain NGR234. *J Bacteriol* 186:4774–4780
- Badri DV, Vivanco JM (2009) Regulation and function of root exudates. *Plant Cell Environ* 32:666–681
- Badri DV, Loyola-Vargas VM, Broeckling CD, De-la-Pena C, Jasinski M, Santelia D, Martinoia E, Sumner LW, Banta LM, Stermitz F, Vivanco JM (2008) Altered profile of secondary

- metabolites in the root exudates of *Arabidopsis* ATP-binding cassette transporter mutants. *Plant Physiol* 146:762–771
- Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant-microbe interactions. *Curr Opin Biotechnol* 20:642–650
- Baier R, Schiene K, Kohring B, Flaschel E, Niehaus K (1999) Alfalfa and tobacco cells react differently to chitin oligosaccharides and *Sinorhizobium meliloti* nodulation factors. *Planta* 210:157–164
- Bassam BJ, Djordjevic MA, Redmond JW, Batley M, Rolfe BG (1988) Identification of a nodD-dependent locus in the *Rhizobium* strain NGR234 activated by phenolic factors secreted by soybeans and other legumes. *Mol Plant-Microbe Interact*. 1:161–168
- Batten K, Scow K, Davies K, Harrison S (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biol Invasions* 8:217–230
- Begum AA, Leibovitch S, Migner P, Zhang F (2001) Specific flavonoids induced nod gene expression and pre-activated nod genes of *Rhizobium leguminosarum* increased pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) nodulation in controlled growth chamber environments. *J Exp Bot* 52:1537–1543
- Benson HP, Boncompagni E, Guerinot ML (2005) An iron uptake operon required for proper nodule development in the *Bradyrhizobium japonicum*-soybean symbiosis. *Mol Plant Microbe Interact* 18:950–959
- Bouwmeester HJ, Matusova R, Zhongkui S, Beale MH (2003) Secondary metabolite signalling in host-parasitic plant interactions. *Curr Opin Plant Biol* 6:358–364
- Brechenmacher L, Lei Z, Libault M, Findley S, Sugawara M, Sadowsky MJ, Sumner LW, Stacey G (2010) Soybean metabolites regulated in root hairs in response to the symbiotic bacterium *Bradyrhizobium japonicum*. *Plant Physiol* 153:1808–1822
- Brito B, Palacios JM, Hidalgo E, Imperial J, Ruiz-Argueso T (1994) Nickel availability to pea (*Pisum sativum* L.) plants limits hydrogenase activity of *Rhizobium leguminosarum* bv. viciae bacteroids by affecting the processing of the hydrogenase structural subunits. *J Bacteriol* 176:5297–5303
- Buee M, Rossignol M, Jauneau A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. *Mol Plant Microbe Interact* 13:693–698
- Caetano-Anolles G, Crist-Estes DK, Bauer WD (1988) Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. *J Bacteriol* 170:3164–3169
- Cardenas L, Dominguez J, Santana O, Quinto C (1996) The role of the nodI and nodJ genes in the transport of Nod metabolites in *Rhizobium etli*. *Gene* 173:183–187
- Carter RA, Worsley PS, Sawers G, Challis GL, Dilworth MJ, Carson KC, Lawrence JA, Wexler M, Johnston AW, Yeoman KH (2002) The vbs genes that direct synthesis of the siderophore vicibactin in *Rhizobium leguminosarum*: their expression in other genera requires ECF sigma factor RpoI. *Mol Microbiol* 44:1153–1166
- Cho MJ, Harper JE (1991) Effect of inoculation and nitrogen on isoflavonoid concentration in wild-type and nodulation-mutant soybean roots. *Plant Physiol* 95:435–442
- Christie RM (2007) Why is indigo blue? *Biotech Histochem* 82:51–56
- Currier WW, Strobel GA (1976) Chemotaxis of *Rhizobium* spp. to plant root exudates. *Plant Physiol* 57:820–823
- Dakora FD (2000) Commonality of root nodulation signals and nitrogen assimilation in tropical grain legumes belonging to the tribe Phaseoleae. *Australian Journal of Plant Physiology* 27:885–892
- De-la-Pena C, Lei Z, Watson BS, Sumner LW, Vivanco JM (2008) Root-microbe communication through protein secretion. *J Biol Chem* 283:25247–25255
- De-la-Pena C, Badri DV, Lei Z, Watson BS, Brandao MM, Silva-Filho MC, Sumner LW, Vivanco JM (2010) Root secretion of defense-related proteins is development-dependent and correlated with flowering time. *J Biol Chem* 285:30654–30665
- Delgado MJ, Tresierra-Ayala A, Talbi C, Bedmar EJ (2006) Functional characterization of the *Bradyrhizobium japonicum* modA and modB genes involved in molybdenum transport. *Microbiology* 152:199–207

- Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiol Ecol* 72: 313–327
- Desbrosses GG, Kopka J, Udvardi MK (2005) *Lotus japonicus* metabolic profiling. Development of gas chromatography-mass spectrometry resources for the study of plant-microbe interactions. *Plant Physiol* 137:1302–1318
- Dharmatilake AJ, Bauer WD (1992) Chemotaxis of *Rhizobium meliloti* towards nodulation gene-inducing compounds from Alfalfa roots. *Appl Environ Microbiol* 58:1153–1158
- Dixon RA, Sumner LW (2003) Legume natural products: understanding and manipulating complex pathways for human and animal health. *Plant Physiol* 131:878–885
- Djordjevic MA, Redmond JW, Batley M, Rolfe BG (1987) Clovers secrete specific phenolic compounds which either stimulate or repress nod gene expression in *Rhizobium trifolii*. *EMBO J* 6:1173–1179
- Downs CT, McDonald PM, Brown K, Ward D (2003) Effects of Acacia condensed tannins on urinary parameters, body mass, and diet choice of an Acacia specialist rodent, *Thallomys nigricauda*. *J Chem Ecol* 29:845–858
- Doyle JJ, Luckow MA (2003) The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. *Plant Physiol* 131:900–910
- Duff SMG, Sarath G, Plaxton WC (1994) The role of acid phosphatases in plant phosphorus metabolism. *Physiol Plant* 90:791–800
- Egelhoff TT, Long SR (1985) *Rhizobium meliloti* nodulation genes: identification of nodDABC gene products, purification of nodA protein, and expression of nodA in *Rhizobium meliloti*. *J Bacteriol* 164:591–599
- Egelhoff TT, Fisher RF, Jacobs TW, Mulligan JT, Long SR (1985) Nucleotide sequence of *Rhizobium meliloti* 1021 nodulation genes: *nodD* is read divergently from *nodABC*. *DNA* 4: 241–248
- Farag MA, Huhman DV, Dixon RA, Sumner LW (2008) Metabolomics reveals novel pathways and differential mechanistic and elicitor-specific responses in phenylpropanoid and isoflavonoid biosynthesis in *Medicago truncatula* cell cultures. *Plant Physiol* 146:387–402
- Farag MA, Deavours BE, de Fatima A, Naoumkina M, Dixon RA, Sumner LW (2009) Integrated metabolite and transcript profiling identify a biosynthetic mechanism for hispidol in *Medicago truncatula* cell cultures. *Plant Physiol* 151:1096–1113
- Fauvert M, Michiels J (2008) Rhizobial secreted proteins as determinants of host specificity in the rhizobium-legume symbiosis. *FEMS Microbiol Lett* 285:1–9
- Fernandez-Lopez M, D’Haeze W, Mergaert P, Verplancke C, Prome JC, Van Montagu M, Holsters M (1996) Role of *nodI* and *nodJ* in lipo-chitoooligosaccharide secretion in *Azorhizobium caulinodans* and *Escherichia coli*. *Mol Microbiol* 20:993–1000
- Fisher RF, Long SR (1992) Rhizobium–plant signal exchange. *Nature* 357:655–660
- Firmin JL, Wilson KE, Rossen L, Johnston AWB (1986) Flavonoid activation of nodulation genes in *Rhizobium* reversed by other compounds present in plants. *Nature* 324:90–92
- Furukawa J, Yamaji N, Wang H, Mitani N, Murata Y, Sato K, Katsuhara M, Takeda K, Ma JF (2007) An aluminum-activated citrate transporter in barley. *Plant Cell Physiol* 48:1081–1091
- Gagnon H, Ibrahim RK (1998) Aldonic Acids: A Novel Family of *nod* Gene Inducers of *Mesorhizobium loti*, *Rhizobium lupini*, and *Sinorhizobium meliloti*. *Mol Plant Microbe Interact* 11:988–998
- Galibert F, Finan TM, Long SR, Puhler A, Abola P, Ampe F, Barloy-Hubler F, Barnett MJ, Becker A, Boistard P, Bothe G, Boutry M, Bowser L, Buhrmester J, Cadieu E, Capela D, Chain P, Cowie A, Davis RW, Dreano S, Federspiel NA, Fisher RF, Gloux S, Godrie T, Goffeau A, Golding B, Gouzy J, Gurjal M, Hernandez-Lucas I, Hong A, Huizar L, Hyman RW, Jones T, Kahn D, Kahn ML, Kalman S, Keating DH, Kiss E, Komp C, Lelaure V, Masuy D, Palm C, Peck MC, Pohl TM, Portetelle D, Purnelle B, Ramsperger U, Surzycki R, Thebault P, Vandenbol M, Vorholter FJ, Weidner S, Wells DH, Wong K, Yeh KC, Batut J (2001) The composite genome of the legume symbiont *Sinorhizobium meliloti*. *Science* 293:668–672

- Garcia-Garrido JM, Lendzemo V, Castellanos-Morales V, Steinkellner S, Vierheilig H (2009) Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. *Mycorrhiza* 19:449–459
- Goldwasser Y, Yoneyama K, Xie X, Yoneyama K (2008) Production of Strigolactones by *Arabidopsis thaliana* responsible for *Orobancha aegyptiaca* seed germination. *Plant Growth Regul* 55:21–28
- Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF (2008) Strigolactone inhibition of shoot branching. *Nature* 455:189–194
- Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. *Plant Physiol* 131:872–877
- Hansch R, Mendel RR (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Curr Opin Plant Biol* 12:259–266
- Hartwig UA, Maxwell CA, Joseph CM, Phillips DA (1990) Chrysoeriol and Luteolin Released from Alfalfa Seeds Induce nod Genes in *Rhizobium meliloti*. *Plant Physiol* 92:116–122
- Hernandez G, Valdes-Lopez O, Ramirez M, Goffard N, Weiller G, Aparicio-Fabre R, Fuentes SI, Erban A, Kopka J, Udvardi MK, Vance CP (2009) Global changes in the transcript and metabolic profiles during symbiotic nitrogen fixation in phosphorus-stressed common bean plants. *Plant Physiol* 151:1221–1238
- Hess SY, Lonnerdal B, Hotz C, Rivera JA, Brown KH (2009) Recent advances in knowledge of zinc nutrition and human health. *Food Nutr Bull* 30:S5–S11
- Hirschi KD (2009) Nutrient biofortification of food crops. *Annu Rev Nutr* 29:401–421
- Horiuchi J, Prithiviraj B, Bais HP, Kimball BA, Vivanco JM (2005) Soil nematodes mediate positive interactions between legume plants and *rhizobium* bacteria. *Planta* 222:848–857
- Hungria M, Joseph CM, Phillips DA (1991) Anthocyanidins and flavonols, major *nod* gene Inducers from seeds of a black-seeded common bean (*Phaseolus vulgaris* L.). *Plant Physiol* 97:751–758
- Ikedo S, Rallos LE, Okubo T, Eda S, Inaba S, Mitsui H, Minamisawa K (2008) Microbial community analysis of field-grown soybeans with different nodulation phenotypes. *Appl Environ Microbiol* 74:5704–5709
- Innes L, Hobbs PJ, Bardgett RD (2004) The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. *Biol Fertil Soils* 40:7–13
- Jin CW, Li GX, Yu XH, Zheng SJ (2010) Plant Fe status affects the composition of siderophore-secreting microbes in the rhizosphere. *Ann Bot* 105:835–841
- Johnston AW, Yeoman KH, Wexler M (2001) Metals and the rhizobial-legume symbiosis—uptake, utilization and signalling. *Adv Microb Physiol* 45:113–156
- Keshavan ND, Chowdhary PK, Haines DC, Gonzalez JE (2005) L-Canavanine made by *Medicago sativa* interferes with quorum sensing in *Sinorhizobium meliloti*. *J Bacteriol* 187:8427–8436
- Kosslak RM, Bookland R, Barkei J, Paaren HE, Appelbaum ER (1987) Induction of *Bradyrhizobium japonicum* common nod genes by isoflavones isolated from *Glycine max*. *Proc Natl Acad Sci USA* 84:7428–7432
- Kosuta S, Chabaud M, Lounnon G, Gough C, Denarie J, Barker DG, Becard G (2003) A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. *Plant Physiol* 131:952–962
- Kouchi H, Shimomura K, Hata S, Hirota A, Wu GJ, Kumagai H, Tajima S, Suganuma N, Suzuki A, Aoki T, Hayashi M, Yokoyama T, Ohyama T, Asamizu E, Kuwata C, Shibata D, Tabata S (2004) Large-scale analysis of gene expression profiles during early stages of root nodule formation in a model legume, *Lotus japonicus*. *DNA Res* 11:263–274
- Kowalchuk GA, Hohn JHG, Van Veen JA (2006) Rhizosphere fungal communities are influenced by *Senecio jacobaea* pyrrolizidine alkaloid content and composition. *Soil Biol Biochem* 38:2852–2859
- Krishnan HB (2002) NodX of *Sinorhizobium fredii* USDA257, a type III-secreted protein involved in host range determination, is localized in the infection threads of cowpea (*Vigna unguiculata* [L.] Walp) and soybean (*Glycine max* [L.] Merr.) nodules. *J Bacteriol* 184:831–839

- Krishnan HB, Lorio J, Kim WS, Jiang G, Kim KY, DeBoer M, Pueppke SG (2003) Extracellular proteins involved in soybean cultivar-specific nodulation are associated with pilus-like surface appendages and exported by a type III protein secretion system in *Sinorhizobium fredii* USDA257. *Mol Plant Microbe Interact* 16:617–625
- Lefebvre DD, Duff SM, Fife CA, Julien-Inalsingh C, Plaxton WC (1990) Response to phosphate deprivation in *Brassica nigra* suspension cells: enhancement of intracellular, cell surface, and secreted phosphatase activities compared to increases in Pi-absorption rate. *Plant Physiol* 93: 504–511
- Li M, Osaki M, Rao IM, Tadano T (1997) Secretion of phytase from the roots of several plant species under phosphorus-deficient conditions. *Plant Soil* 195:161–169
- Limpens E, Franken C, Smit P, Willemse J, Bisseling T, Geurts R (2003) LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. *Science* 302:630–633
- Lopez-Raez JA, Charnikhova T, Gomez-Roldan V, Matusova R, Kohlen W, De Vos R, Verstappen F, Puech-Pages V, Becard G, Mulder P, Bouwmeester H (2008) Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. *New Phytol* 178:863–874
- Loyola-Vargas VM, Broeckling CD, Badri D, Vivanco JM (2007) Effect of transporters on the secretion of phytochemicals by the roots of *Arabidopsis thaliana*. *Planta* 225:301–310
- Lucas Garcia JA, Barbas C, Probanza A, Barrientos ML, Gutierrez Manero FJ (2001) Low molecular weight organic acids and fatty acids in root exudates of two *Lupinus* cultivars at flowering and fruiting stages. *Phytochem Anal* 12:305–311
- Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J (2003) A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* 425:637–640
- Marie C, Deakin WJ, Viprey V, Kopcinska J, Golinowski W, Krishnan HB, Perret X, Broughton WJ (2003) Characterization of Nops, nodulation outer proteins, secreted via the type III secretion system of NGR234. *Mol Plant Microbe Interact* 16:743–751
- Maxwell CA, Hartwig UA, Joseph CM, Phillips DA (1989) A chalcone and two related flavonoids released from alfalfa roots induce nod genes of *Rhizobium meliloti*. *Plant Physiol* 91:842–847
- Mazzola M, Funnell DL, Raaijmakers JM (2004) Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species from resident soil populations. *Microb Ecol* 48:338–348
- Messens E, Geelen D, van Montagu M, Holsters M (1991) 7,4-Dihydroxyflavanone is the major *Azorhizobium* nod gene-inducing factor present in *Sesbania rostrata* seedling exudate. *Mol Plant-Microbe Interact* 4:262–267
- Micallef SA, Shiaris MP, Colon-Carmona A (2009) Influence of *Arabidopsis thaliana* accessions on rhizobacterial communities and natural variation in root exudates. *J Exp Bot* 60:1729–1742
- Miller RM, Reinhardt DR, Jastrow JD (1995) External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* 103:17–23
- Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. *Proc Natl Acad Sci USA* 104:19613–19618
- Mougel C, Offre P, Ranjard L, Corberand T, Gamalero E, Robin C, Lemanceau P (2006) Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytol* 170:165–175
- Mulligan JT, Long SR (1985) Induction of *Rhizobium meliloti* nodC expression by plant exudate requires nodD. *Proc Natl Acad Sci USA* 82:6609–6613
- Nakagawa T, Kaku H, Shimoda Y, Sugiyama A, Shimamura M, Takanashi K, Yazaki K, Aoki T, Shibuya N, Kouchi H (2011) From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume-*Rhizobium* symbiosis. *Plant Journal* 65:169–180
- Narasimhan K, Basheer C, Bajic VB, Swarup S (2003) Enhancement of plant-microbe interactions using a rhizosphere metabolomics-driven approach and its application in the removal of polychlorinated biphenyls. *Plant Physiol* 132:146–153

- Navazio L, Moscatiello R, Genre A, Novero M, Baldan B, Bonfante P, Mariani P (2007) A diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. *Plant Physiol* 144:673–681
- Neumann G, Martinoia E (2002) Cluster roots – an underground adaptation for survival in extreme environments. *Trends Plant Sci* 7:162–167
- Neumann G, Massonneau A, Langlade N, Dinkelaker B, Hengeler C, Römheld V, Martinoia E (2000) Physiological aspect of cluster root function and development in phosphorus-deficient White Lupin (*Lupinus albus* L.). *Ann Bot* 85:909–919
- Novak K, Chovanec P, Skrdleta V, Kropacova M, Lisa L, Nemcova M (2002) Effect of exogenous flavonoids on nodulation of pea (*Pisum sativum* L.). *J Exp Bot* 53:1735–1745
- O'Hara GW, Dilworth MJ, Boonkerd N, Parkpian P (1988) Iron-deficiency specifically limits nodule development in peanut inoculated with *Bradyrhizobium* sp. *New Phytol* 108:51–57
- Offre P, Pivato B, Siblot S, Gamalero E, Corberand T, Lemanceau P, Mougél C (2007) Identification of bacterial groups preferentially associated with mycorrhizal roots of *Medicago truncatula*. *Appl Environ Microbiol* 73:913–921
- Omote H, Hiasa M, Matsumoto T, Otsuka M, Moriyama Y (2006) The MATE proteins as fundamental transporters of metabolic and xenobiotic organic cations. *Trends Pharmacol Sci* 27:587–593
- Parke D, Rivelli M, Ornston LN (1985) Chemotaxis to aromatic and hydroaromatic acids: comparison of *Bradyrhizobium japonicum* and *Rhizobium trifolii*. *J Bacteriol* 163:417–422
- Parniske M (2005) Plant-fungal associations: cue for the branching connection. *Nature* 435:750–751
- Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6:763–775
- Peters NK, Frost JW, Long SR (1986) A plant flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. *Science* 233:977–980
- Phillips DA, Joseph CM, Maxwell CA (1992) Trigonelline and stachydrine released from Alfalfa seeds activate NodD2 protein in *Rhizobium meliloti*. *Plant Physiol* 99:1526–1531
- Priha O, Grayston SJ, Pennanen T, Smolander A (1999) Microbial activities related to C and N cycling and microbial community structure in the rhizospheres of *Pinus sylvestris*, *Picea abies* and *Betula pendula* seedlings in an organic and mineral soil. *FEMS Microbiol Ecol* 30:187–199
- Radutoiu S, Madsen LH, Madsen EB, Felle HH, Umehara Y, Gronlund M, Sato S, Nakamura Y, Tabata S, Sandal N, Stougaard J (2003) Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* 425:585–592
- Rani K, Zwanenburg B, Sugimoto Y, Yoneyama K, Bouwmeester HJ (2008) Biosynthetic considerations could assist the structure elucidation of host plant produced rhizosphere signaling compounds (strigolactones) for arbuscular mycorrhizal fungi and parasitic plants. *Plant Physiol Biochem* 46:617–626
- Rasmann S, Kollner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TC (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434:732–737
- Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. *Science* 289:1920–1921
- Redmond J, Batley M, Djordjevic M, Innes R, Kuempel P, Rolfe B (1986) Flavones induce expression of nodulation genes in *Rhizobium*. *Nature* 323:632–635
- Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc Natl Acad Sci USA* 91:11841–11843
- Rispail N, Hauck B, Bartholomew B, Watson AA, Nash RJ, Webb KJ (2010) Secondary metabolite profiling of the model legume *Lotus japonicus* during its symbiotic interaction with *Mesorhizobium loti*. *Symbiosis* 50:119–128
- Rivilla R, Sutton JM, Downie JA (1995) *Rhizobium leguminosarum* NodT is related to a family of outer-membrane transport proteins that includes TolC, PrtF, CyaE and AprF. *Gene* 161:27–31

- Rossen L, Johnston AW, Downie JA (1984) DNA sequence of the *Rhizobium leguminosarum* nodulation genes nodAB and C required for root hair curling. *Nucleic Acids Res* 12:9497–9508
- Rossen L, Shearman CA, Johnston AWB, Downie JA (1985) The nodD gene of *Rhizobium leguminosarum* is autoregulatory and in the presence of plant exudate induces the nodA, B, C genes. *EMBO J* 4:3369–3373
- Scheidemann P, Wetzel A (1997) Identification and characterization of flavonoids in the root exudate of *Robinia pseudoacacia*. *Trees* 11:316–321
- Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363:67–69
- Smil V (1999) Nitrogen in crop production. *Global Biogeochem Cycles* 13:647–662
- Smit G, Puvanesarajah V, Carlson RW, Barbour WM, Stacey G (1992) *Bradyrhizobium japonicum* nodD1 can be specifically induced by soybean flavonoids that do not induce the nodYABCSUIJ operon. *J Biol Chem* 267:310–318
- Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, San Diego
- Spaink HP, Wijffes AH, Lugtenberg BJ (1995) *Rhizobium* NodI and NodJ proteins play a role in the efficiency of secretion of lipochitin oligosaccharides. *J Bacteriol* 177:6276–6281
- Staunton S, Leprince F (1996) Effect of pH and some organic anions on the solubility of soil phosphate: implications for P bioavailability. *Eur J Soil Sci* 47:231–239
- Stergiopoulos I, De Wit PJ (2009) Fungal effector proteins. *Annu Rev Phytopathol* 47:233–263
- Sugiyama A, Shitan N, Yazaki K (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
- Sugiyama A, Shitan N, Yazaki K (2008) Signaling from soybean roots to *rhizobium*: An ATP-binding cassette-type transporter mediates genistein secretion. *Plant Signal Behav* 3:38–40
- Suzuki H, Sasaki R, Ogata Y, Nakamura Y, Sakurai N, Kitajima M, Takayama H, Kanaya S, Aoki K, Shibata D, Saito K (2008) Metabolic profiling of flavonoids in *Lotus japonicus* using liquid chromatography Fourier transform ion cyclotron resonance mass spectrometry. *Phytochemistry* 69:99–111
- Takagi S (1976) Naturally occurring iron-chelating compounds in oat and rice root-washings. *Soil Sci Plant Nutr* 22:423–433
- Tawarayama K, Watanabe S, Yoshida E, Wagatsuma T (1995) Effect of onion (*Allium cepa*) root exudates on the hyphal growth of *Gigaspora margarita*. *Mycorrhiza* 6:57–59
- Tawarayama K, Hashimoto K, Wagatsuma T (1998) Effect of root exudate fractions from P-deficient and P-sufficient onion plants on root colonisation by the arbuscular mycorrhizal fungus *Gigaspora margarita*. *Mycorrhiza* 8:67–70
- Teplitski M, Robinson JB, Bauer WD (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol Plant Microbe Interact* 13:637–648
- Thavarajah D, Thavarajah P, Sarker A, Vandenberg A (2009) Lentils (*Lens culinaris* Medikus Subspecies culinaris): a whole food for increased iron and zinc intake. *J Agric Food Chem* 57: 5413–5419
- Ueda H, Sugimoto Y (2010) Vestitol as a chemical barrier against intrusion of parasitic plant *Striga hermonthica* into *Lotus japonicus* roots. *Biosci Biotechnol Biochem* 74:1662–1667
- Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyoizuka J, Yamaguchi S (2008) Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455:195–200
- Uren NC (2007) Types, amounts and possible functions of compounds released into the rhizosphere of soil-grown plants. In: Pinton R, Varanini Z, Nannipiero P (eds) *The rhizosphere: biochemistry and organic substances at the soil-plant interface*. CRC, New York, pp 1–22
- Verma D, Hong Z (1996) Biogenesis of the peribacteroid membrane in root nodules. *Trends Microbiol* 4:364–368
- Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu U, Lee Y, Martinoia E, Murphy A, Rea PA, Samuels L, Schulz B, Spalding EJ, Yazaki K, Theodoulou FL

- (2008) Plant ABC proteins – a unified nomenclature and updated inventory. *Trends Plant Sci* 13:151–159
- Vieira RF, Cardoso EJBN, Vieira C, Cassini STA (1998) Foliar application of molybdenum in common beans. I. Nitrogenase and reductase activities in a soil of high fertility. *J Plant Nutr* 21:169–180
- Viprey V, Greco AD, Golinowski W, Broughton WJ, Perret X (1998) Symbiotic implications of type III protein secretion machinery in *Rhizobium*. *Mol Microbiol* 28:1381–1389
- Wan J, Zhang XC, Neece D, Ramonell KM, Clough S, Kim SY, Stacey MG, Stacey G (2008) A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. *Plant Cell* 20:471–481
- Wang J, Raman H, Zhou M, Ryan PR, Delhaize E, Hebb DM, Coombes N, Mendham N (2007) High-resolution mapping of the Alp locus and identification of a candidate gene HvMATE controlling aluminium tolerance in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 115: 265–276
- Weeraratna CS (1980) Studies on the molybdenum application to soybean. *Beitr Trop Landwirtschaft Veterinarmed* 18:131–134
- Weisskopf L, Abou-Mansour E, Fromin N, Tomasi N, Santelia D, Edelkott I, Neumann G, Aragno M, Tabacchi R, Martinoia E (2006a) White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. *Plant Cell Environ* 29:919–927
- Weisskopf L, Tomasi N, Santelia D, Martinoia E, Langlade NB, Tabacchi R, Abou-Mansour E (2006b) Isoflavonoid exudation from white lupin roots is influenced by phosphate supply, root type and cluster-root stage. *New Phytol* 171:657–668
- Werner D (2007) Molecular biology and ecology of the *rhizobia*-legume symbiosis. In: Pinton R, Varanini Z, Nannipiero P (eds) *The rhizosphere: biochemistry and organic substances at the soil-plant interface*. CRC, New York, pp 237–266
- Werner D, Muller P (2002) Communication and efficiency in the symbiotic signal exchange. In: Heldmaier G, Werner D (eds) *Environmental signal processing and adaptation*. Springer, Heidelberg
- Yang CH, Crowley DE (2000) Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Appl Environ Microbiol* 66:345–351
- Yanni YG (1992) Performance of chickpea, lentil and lupin nodulated with indigenous or inoculated rhizobia micropartners under nitrogen, boron, cobalt and molybdenum fertilization schedules. *World J Microbiol Biotechnol* 8:607–613
- Yazaki K, Sugiyama A, Morita M, Shitan N (2008) Secondary transport as an efficient membrane transport mechanism for plant secondary metabolites. *Phytochem Rev* 7:513–524
- Yazaki K, Shitan N, Sugiyama A, Takanashi K (2009) Cell and molecular biology of ATP-binding cassette proteins in plants. *Int Rev Cell Mol Biol* 276:263–299
- Yoneyama K, Xie X, Sekimoto H, Takeuchi Y, Ogasawara S, Akiyama K, Hayashi H, Yoneyama K (2008) Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. *New Phytol* 179:484–494
- Yost CK, Rochepeau P, Hynes MF (1998) *Rhizobium leguminosarum* contains a group of genes that appear to code for methyl-accepting chemotaxis proteins. *Microbiology* 144:1945–1956
- Zaat SAJ, Schripsema J, Wijffelman CA, Brussel AAN, Lugtenberg BJJ (1989) Analysis of the major inducers of the *Rhizobium nodA* promoter from *Vicia sativa* root exudate and their activity with different nodD genes. *Plant Mol Biol* 13:175–188.
- Zhang J, Subramanian S, Zhang Y, Yu O (2007) Flavone synthases from *Medicago truncatula* are flavanone-2-hydroxylases and are important for nodulation. *Plant Physiol* 144:741–751

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