Chapter 6
The Complex Molecular Signaling Network in Microbe–Plant Interaction

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Contents

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Abstract Soil bacteria living around plants exert neutral, beneficial, or detrimental effects on plant growth and development. These effects are the result of signal exchange in which there is a mutual recognition of diffusible molecules produced by the plant and microbe partners. Understanding the molecular signaling network involved in microbe–plant interaction is a promising opportunity to improve crop productivity and agriculture sustainability. Many approaches have been used to decipher these molecular signals, and the results show that plants and microorganisms respond by inducing the expression of, and releasing, a mixture of molecules that includes flavonoids, phytohormones, pattern recognition receptors, nodulins, lectins, enzymes, lipo-chitooligosaccharides, exopolysaccharides, amino acids, fatty acids, vitamins, and volatiles.

This chapter reviews current knowledge of the diverse signaling pathways that are turned on when plants interact with beneficial microbes, with emphasis on bacteria belonging to the genera *Rhizobium*, *Azospirillum*, and *Pseudomonas*.

Beneficial Rhizospheric Microbes

Mutualistic association between microbes and plants brings benefits to the interacting partners. Some mutualistic microbes (plant–arbuscular mycorrhizal fungi interactions have been excluded from this chapter) are rhizospheric bacteria known as plant growth promoting rhizobacteria (PGPR) (Glick 1995) because they exert a positive influence on plant growth. Over the last decade, several PGPR have been isolated and used as bio-fertilizers, giving insight into good agronomical practices (Morel et al. 2012). Their contribution to plant growth promotion (PGP) can be exerted through direct and/or indirect mechanisms. Bacteria that use direct PGP mechanisms secrete metabolites such as hormones and polysaccharides, among other molecules, that influence root and shoot development. Indirect PGP effects include the secretion of bacterial metabolites with deleterious properties against the growth of phytopathogens (Lopez-Bucio et al. 2007). These bacteria are collectively called biocontrol agents.

The best-known microbe–plant mutualistic interaction is the diazotrophic microbial association with plants. Diazotrophs are free-living or symbiotic microbes that fix and reduce atmospheric nitrogen to ammonia. This process, called biological nitrogen fixation (BNF), is catalyzed by the bacterial enzyme nitrogenase (Masson-Boivin et al. 2009; Bhattacharjee et al. 2008). Examples of bacterial diazotrophs are *Azotobacter* (free-living diazotroph), *Azospirillum* (associative symbiont), *Azoarcus* and *Gluconacetobacter diazotrophicus* (endophytic non-nodular symbionts), and rhizobia (endophytic nodular symbionts). PGPR also produce phytohormones (Cassán et al. 2009), iron-sequestering siderophores (Yadegari et al. 2010), phosphate-solubilizing molecules (Wani et al. 2007), and/or 1-aminocyclopropane-1-carboxylate deaminase (Remans et al. 2007), among others. Examples of non-diazotrophic PGPR are *Pseudomonas* and *Bacillus* (Parmar and Dufresne 2011).

There is an exchange of signaling molecules between both interacting partner cells in mutualistic PGPR–plant interactions, leading to changes in gene expression.

This chapter reviews the progress in molecular signaling research involving beneficial microbe–plant interactions reported in recent years.

Rhizobia–Legume Symbiotic Association

The rhizobia–legume association is the best-known endosymbiotic microbe–plant interaction and, together with plant–mycorrhizal fungi interactions, is recognized for its importance in sustaining agricultural ecosystems and productivity. Rhizobia consist of several genera of the subclass Alpha- and Betaproteobacteria that are well known for their ability to form mutualistic associations, especially (but not exclusively) with leguminous plants (*Fabaceae*). Rhizobia induce the formation of root nodules where BNF occurs (Bapaume and Reinhardt 2012). The rhizobia–legume association is specific (each rhizobium establishes a symbiosis with only a limited set of host plants and vice versa). Plants mutually compatible with the same species of *Rhizobium* are called "cross-inoculation groups" (Morel et al. 2012).

Root colonization by rhizobia is accompanied with important changes in root architecture and gene expression in root and shoot, which lead to the nitrogen-fixing phenotype. During the process of BNF, rhizobia provide reduced nitrogen to the plant in exchange for carbohydrates and a micro-aerobic environment for the effective functioning of the oxygen-sensitive nitrogenase. Establishment of the symbiosis requires the reciprocal recognition of partners and the production of various signaling molecules that are required to regulate nodule initiation and differentiation and nitrogen fixation. Briefly, nitrogen fixation is preceded by root morphological changes that include highly coordinated events. Most legumes constitutively release root-diffusible attractant signal molecules (flavonoids), which trigger rhizobial production of specific lipo-chitooligosaccharides known as nodulation factors (Nod Factors or NFs) (Hassan and Mathesius 2012) (see section "Extracellular Polysaccharides"). NFs are among the most important molecules in the microbe–plant dialog, mediating rhizobia recognition by the plant root and nodule organogenesis (Masson-Boivin et al. 2009). NF recognition is accompanied by curling of root hairs, where bacteria are entrapped, and formation of plant-derived infection threads (IT) that carry the rhizobia into the dividing cells of the inner cortex, the nodule primordium (Fournier et al. 2008). Then, rhizobia are released into the nodule primordium where they differentiate into bacteroids, the symbiotic rhizobial form that expresses the nitrogen-fixing enzyme, nitrogenase (Oldroyd et al. 2011).

Rhizobia–legume symbiosis is regulated by transcriptional reprogramming of host cells that ensures the functioning of the nodule. Many reprogrammed genes are membrane proteins with important roles in signaling, intracellular accommodation, and nutrient transport (Bapaume and Reinhardt 2012; see section "PGPR and Plant Root Attachment"). In addition to BNF, most rhizobia have been found to produce auxins. The roles of auxins in rhizobia–legume interactions are related to plant growth and nodule organogenesis (Lambrecht et al. 2000; see section "Phytohormones Production").

Azospirillum–*Plant Association*

Bacteria belonging to the genus *Azospirillum* are free-living, nitrogen-fixing, surface-colonizing, and, sometimes, endophytic diazotroph Alphaproteobacteria (family Rhodospirillaceae). *Azospirillum* spp. establish associations that are beneficial to plants, but with no apparent preference for specific plants, and can be successfully applied to plants that have never been colonized before by azospirilla (Bianco and Defez 2011; Guerrero-Molina et al. 2011; Reis et al. 2011). Currently, there is a limited market for commercial bio-fertilizers for non-legume crops based on *Azospirillum* spp., but they have been shown to be efficient PGPR (Figueiredo et al. 2010).

Azospirillum is a nitrogen-fixing microbe, but given that azospirilla promote plant growth even in nitrogen-rich conditions, PGP by *Azospirillum* might be attributed to other mechanisms rather than BNF (Okon and Kapulnik 1986), such as deamination of the ethylene precursor 1-aminocyclopropane-1-carboxylate and siderophore (Tortora et al. 2011), auxin, or nitric oxide production (Baudoin et al. 2010; Spaepen et al. 2007). Among these PGP properties, auxin production is thought to be the main mode of action of *Azospirillum brasilense*. This assumption was corroborated in experiments using genetically modified azospirilla that showed enhanced auxin production (Baudoin et al. 2010; Spaepen et al. 2007, 2008). Many other workers have also reported that plant hormone production by *Azospirillum* spp. is the main mechanism that explains the PGP effect (Reis et al. 2011; Bashan et al. 2004; Lambrecht et al. 2000; Okon and Labandera-Gonzalez 1994). Auxin production by azospirilla promotes root development and proliferation, leading to enhanced nutrient uptake (Lambrecht et al. 2000) and increased root exudation of molecules to the rhizosphere. Molecules exuded by the root act as chemoeffectors that attract azospirilla to the rhizosphere (chemotaxis), thereby increasing the chance of root–bacterial interactions. This was, and still is, the mechanism that in fact explains how azospirilla promote plant growth (Hayat et al. 2010).

Azospirilla are considered "helper" bacteria that promote rhizobia–plant interactions (Morel et al. 2012). Co-inoculation with azospirilla stimulates nodulation (early nodulation and more nodules), nodule function, and plant growth and development when compared with inoculation with rhizobia alone (Bianco and Defez 2011; Remans et al. 2008). The evidence supports a mix of molecules secreted to the rhizosphere being involved in improving rhizobia–legume association. Auxin production by azospirilla, during co-inoculation, stimulates morphological and physiological changes in the root system, increasing the number of potential sites for rhizobial infection, thus leading to a much higher number of nodules (Bianco and Defez 2011). Some direct evidence also suggests that during co-inoculation, *Azospirillum* spp. induce the synthesis of chemoattractant flavonoids by roots of chickpea, common bean, and alfalfa (Star et al. 2012; Dardanelli et al. 2008; Burdman et al. 1996; Volpin et al. 1996).

Other PGPR–Plant Interactions

There is a long list of microbes that establish beneficial interactions with plants, but some endophytes and *Pseudomonas* head the list.

Endophytes

Endophytes are bacteria that infect and colonize the plant apoplast, evading or suppressing the host plant defense system. Many facultative endophytic bacteria can also survive in the rhizosphere, where they can enter their host plant via the roots (Badri et al. 2009). PGPR are bacteria that live in soil near the root, colonize the root surface, reside in root tissue, or live inside plant cells in specialized structures, promoting plant growth; thus, most endophytes might be considered PGPR. Given the semantic overlap and the difference between PGPR and endophytes, many researchers have adopted two simple terms: intracellular PGPR (iPGPR), for bacteria residing inside plant cells, and extracellular PGPR (ePGPR) for those bacteria living outside plant cells, root surface, or rhizosphere (Gray and Smith 2005). However, the definition of endophytes is still controversial. Many authors claim that ePGPR are simply epiphytes and iPGPR are just endophytes (Ikeda et al. 2010).

In endophyte–plant interactions, bacteria are not restricted to a specific compartment within the plant but can be found in roots, stems, and leaves. Like rhizobia, most endophytes commonly used as inoculants are diazotrophs that improve plant growth. Examples of endophyte–plant interactions are *Burkholderia* and sugarcane, *Herbaspirillum* and a broad range of host plants, and *Azospirillum* and rice (Govindarajan et al. 2008). It has been shown that crop yield increase after endophyte inoculation is mainly due to BNF. Details about endophytes for non-legumes can be read in Bhattacharjee et al. (2008).

Pseudomonas

The genus *Pseudomonas* includes the most diverse and ecologically significant group of bacteria, belonging to the class Gammaproteobacteria. They are ubiquitously distributed in terrestrial and marine environments and have been found associated with animals and plants (Kiil et al. 2008). Their genetic diversity is a reflection of their ecological diversity (Silby et al. 2009). Many *Pseudomonas* spp. have been extensively studied as PGPR. There is evidence that some *Pseudomonas* spp. produce siderophores (Rosas et al. 2006), phenolic compounds (Combes-Meynet et al. 2011), lytic enzymes (Egamberdieva et al. 2010), and phytohormones (Pallai et al. 2012; Khalid et al. 2011; Khakipour et al. 2008); solubilize phosphate (Azziz et al. 2012); act as biocontrol agents of phytopathogenic microbes (Quagliotto et al. 2009); and induce systemic resistance (Bakker et al. 2007), thus

promoting plant growth. Moreover, some rhizospheric *Pseudomonas* spp. interact synergistically with other PGPR, assisting PGPR–plant colonization and suppressing plant pathogens (Parmar and Dufresne 2011). Many studies support the action of *Pseudomonas* spp. as "helper" bacteria during the establishment of the rhizobia–legume interaction, evidenced by the promotion of plant growth during co-inoculation (Morel et al. 2012; Malik and Sindhu 2011). This helper effect might be explained by the production of phytohormones (Malik and Sindhu 2011; Egamberdieva et al. 2010), a qualitative change in plant-secreted flavonoids (Parmar and Dadarwal 1999), or the solubilization of non-available nutrients (mainly refixation of exogenously applied phosphorus), among other actions (Medeot et al. 2010).

Delftia

Recently, a new genus has emerged as a PGPR. Bacteria belonging to the genus *Delftia* have been described as novel PGP microbes (diazotrophic and biocontrol agents against various plant pathogens). They fix atmospheric nitrogen, produce the auxin indole-3-acetic acid and siderophores, promote alfalfa and clover growth under nitrogen-rich conditions, and assist as a "helper" bacterium during rhizobia–legume interaction, probably due to auxin production (Ubalde et al. 2012; Morel et al. 2011; Han et al. 2005).

Early Signaling Events: The Role of Root Exudates

The root system of plants imports water and nutrients from the soil solution but also releases low- and high-molecular-weight compounds to the rhizosphere. Root exudates are composed of a broad range of root-secreted molecules that act as a complex chemical cocktail that mediates interactions occurring in the rhizosphere and shapes soil microbial communities (Okumoto and Pilot 2011). Their chemical composition is influenced by environmental conditions, plant genotype, and the multipartite interactions occurring in the rhizosphere, among other factors.

Carbon-based compounds are the main constituent of this complex cocktail, but ions, oxygen, and inorganic acids are also important components with relevant roles during rhizospheric interactions (Badri and Vivanco 2009). Exuded molecules include low-molecular-weight compounds, such as sugars and phenolics, and high-molecular-weight compounds such as polysaccharides and proteins, which often compose a larger proportion of the total mass of the exudate (Cai et al. 2012). Even though these chemicals are root-secreted, many rhizobacteria also secrete metabolites that contribute to the pool of molecules that mediate rhizospheric interactions (Badri et al. 2009). Table 6.1 summarizes examples of these bacterial-secreted compounds and their general role in plants. The sections below describe current knowledge of different plant and bacterial metabolites involved in microbe–plant interactions.

Table 6.1 Some bacterial-secreted compounds and their role in plant physiology and architecture

Chemical group	Bacterial metabolite	Plant response	Reference
Phytohormones	Salicylic acid, jasmonic acid, and ethylene	Immune plant defense activation through SAR[a] (mainly) and ISR[b]	Bent (2010), Bakker et al. (2007), Ping and Boland (2004)
		Inhibition of legume response to NF and rhizobia	Oldroyd and Downie (2008), Ramos Solano et al. (2009), Ding et al. (2008), Sun et al. (2006)
	Cytokinins, auxins, and gibberellins	Phyto-stimulation. Morphogenesis	Morel et al. (2011), Cassán et al. (2009), Ferguson and Beveridge (2009), Boiero et al. (2007), Remans et al. (2007), (2008), Lopez-Bucio et al. (2007), Spaepen et al. (2007)
	Auxins	Pathogenesis (i.e., gall induction, necrotic lesions)	Ding et al. (2008), Chalupowicz et al. (2006), Robert-Seilaniantz et al. (2007), Lambrecht et al. (2000)
N-acyl-L-homoserine lactones (AHLs) and QS[c]-related signals	AHLs	Modulation of root system architecture	Ortiz-Castro et al. (2008a), von Rad et al. (2008)
		Induction of ISR	Schuhegger et al. (2006)
	AHL-degrading lactonases	Interference with QS signals required for virulence in phytopathogens	Friesen et al. (2011)
Volatile organic compounds	Acetoin, butanediol, 1-octen-3-ol, and butyrolactone	Modulation of root system architecture	Gutierrez-Luna et al. (2010), Lopez-Bucio et al. (2007)
		ISR	Ryu et al. (2005), Ping and Boland (2004)
Phenolic compounds	Flavonoids, phenolic acids	nod-gene inducers	Mandal et al. (2010), Parmar and Dadarwal (1999)
		Antimicrobial agents, ISR	Combes-Meynet et al. (2011), Parmar and Dufresne (2011)
Lipopolysaccharides (LPS) and extracellular-related factors	Siderophores, LPS	ISR	Ping and Boland (2004)

[a] SAR systemic acquired resistance
[b] ISR induced systemic resistance
[c] QS quorum sensing

Phytohormones Production

Phytohormones are chemical messengers produced by plants and microorganisms, which coordinate plant cellular activities at low concentrations (Ferguson and Beveridge 2009). Common phytohormones belong to five major classes: auxins, cytokinins, gibberellins, abscisic acid, and ethylene. Other known phytohormones are brassinosteroids, salicylic acid, jasmonates, polyamines, nitric oxide, strigolactones, etc. (Pieterse et al. 2009). The following microbes are known phytohormone producers: *Pseudomonas* (Khakipour et al. 2008), *Azospirillum* (Khalid et al. 2011), rhizobia (Etesami et al. 2009), *Bacillus* (Lim and Kim 2009), and *Delftia* (Morel et al. 2011). Microbial secreted hormones, mainly cytokinins (CKs) and auxins, act as signaling molecules that coordinate changes in plant cell division and differentiation, affecting root and shoot architecture and functioning (Boiero et al. 2007; Lopez-Bucio et al. 2007; Ryu et al. 2005). In this section, we review information concerning phytohormones (auxins and CKs) that positively correlate with PGP during microbe–plant interaction (Tables 6.2 and 6.3).

The information supports the view that a mix of phytohormones, rather than a single effector, acts to control plant cellular processes at multiple levels (Yoshimitsu et al. 2011), including major effects on plant growth and the induction of plant immune defenses. During the microbe–plant interaction, bacterial-produced phyto-hormones, mainly auxins and CKs, also have phyto-stimulation effects (Robert-Seilaniantz et al. 2007). Most of the information that supports this affirmation was gathered working in the areas of rhizobia–legume and azospirilla–wheat interactions.

CKs are purine derivatives produced in root tips and developing seeds and are transported via the xylem from roots to shoots (Ortiz-Castro et al. 2009). Some effects of CKs in plants are the induction of root and shoot cell division, cell growth and dedifferentiation, apical dominance, lateral bud growth, leaf expansion, and delayed senescence. Zeatin is the most common CK, but other cytokinin-like substances are known: isopentenyladenine, isopentenyladenosine, zeatin riboside, and dihydrozeatin riboside (Davies 2010). CKs are probably the most studied phytohormones involved in nodule organogenesis (Ariel et al. 2012; Op den Camp et al. 2011; Oldroyd and Downie 2008; Murray et al. 2007; Tirichine et al. 2007). They have been proposed as secondary signal molecules that perceive NF at the root epidermis. In response to NF application at roots, a local increase in CK levels is detected, which induces nodule primordial development in the cortex cells, thus influencing bacterial infection (Heckmann et al. 2011; Ding et al. 2008; Murray et al. 2007; Oldroyd 2007; Tirichine et al. 2007). For instance, Murray et al. (2007) and Tirichine et al. (2007) showed that plant CK signaling pathway activation by rhizobial cells is necessary (and sufficient) to activate nodule formation in *L. japonicum*. CK production by plant-associated bacteria, other than rhizobia, has also been well documented. Some examples of CK-producing bacteria are *Bacillus megaterium* and *Azospirillum* (Ortiz-Castro et al. 2008b).

Many plant pathogenic bacteria also secrete CK analogs or activate plant CK production to form gall structures, leading to delayed senescence activity and suppression of plant basal defense mechanisms (Chalupowicz et al. 2006).

Table 6.2 Effects of bacterial auxins on microbe–plant interaction: root architecture and/or physiology

PGPR–plant system	Strategy used during the study	Effect	Possible mechanism of action	Reference
Sinorhizobium meliloti–Medicago truncatula	Proteomics of roots. Effect of inoculation and exogenous application of auxin (without inoculation)	Similar accumulation level in inoculated and auxin-treated plants	Auxin is a positive regulator of nodulation initiation	Van Noorden et al. (2007)
Azospirillum brasilense–Triticum sp. (wheat)	Plant growth. Inoculation with overproducing IAA[a]—*A. brasilense*	Increased shoot biomass, thinner roots, and no significant effect on root biomass (a month after inoculation)	Transient positive effect of bacterial IAA on root development	Baudoin et al. (2010)
Bacillus subtilis and *B. licheniformis–Capsicum* sp. (red pepper) and *Solanum lycopersicum* (tomato)	Plant growth and seed germination. *Bacillus* co-inoculation and *Bacillus* purified auxins exogenous application	Increased root, stem, and leaf growth and seed germination	Bacterial auxins are major factors responsible for plant growth promotion	Lim and Kim (2009)
Pseudomonas aeruginosa and *A. brasilense–wheat* and rice	Plant growth and yield. Inoculation under field conditions	Increased number of tiller, straw and grain yield	Auxin-producing PGPR positively affect plant growth	Khalid et al. (2011)
B. japonicum and *A. brasilense–Glycine max* (soybean)	Plant growth and seed germination. Inoculation and co-inoculation	Promotion of seed germination and PGP of soybean seedlings in co-inoculated plants	PGPR excretion of IAA (auxin) promotes young seedlings	Cassán et al. (2009)
Rhizobium galegae and *Pseudomonas* spp.–*Galega orientalis* (Galega)	Plant growth and nodulation. Inoculation and co-inoculation experiments	Increased shoot and root dry matter, number of nodules, and nitrogen content of co-inoculated plants	*Pseudomonas* spp. produce auxin and cellulase as mechanism to enhance symbiotic performance of rhizobia	Egamberdieva et al. (2010)
Mesorhizobium sp. and *Pseudomonas* spp.–*Cicer arietinum* (chickpea)	Plant growth and nodulation parameters. Inoculation and co-inoculation experiments	Increased shoot and root dry matter, number, and biomass of nodules in co-inoculated plants	Enhanced nodulation in chickpea by auxin secretion	Malik and Sindhu (2011)

[a] IAA indol-3-acetic acid

Table 6.3 Effects of cytokinins (CKs) on microbe–plant interaction: root architecture and/or physiology

PGPR–plant system	Strategy	Effect	Possible mechanism	Reference
Mesorhizobium loti–Lotus japonicum	Nodule organogenesis. Inoculation and CK application (without inoculation) of plant *hit1*[a] mutants	Hit1 roots are insensitive to exogenously applied CK and rhizobia inoculation	A CK receptor (LHK1) is required for the activation of *Nin*[b] and nodule organogenesis	Murray et al. (2007)
M. loti–L. japonicum	Nodule organogenesis in a L. japonicum snf2 mutant[c]	Spontaneous development of root nodules in absence of rhizobia or rhizobial signal molecules	CK signaling is required for nodule initiation and is a key element in dedifferentiation of cortical cells	Tirichine et al. (2007)
M. loti–L. japonicum	Plant growth and nodule organogenesis. Inoculation and exogenous CK application to plant mutants for CK response	Formation of discrete and easily visible nodule primordial. Expression of nodulin genes	Root cortical cell activation by CK depends on LHK1	Heckmann et al. (2011)
B. subtilis (CK-producing strain)–Lactuca sativa (lettuce)	Plant growth and hormone determination of shoot and root. Inoculation under drought stress	Increase of CKs in shoot. Weight increase in shoots and roots	Inoculation with CK-producing bacteria may have a beneficial result under moderate drought	Arkhipova et al. (2007)
B. megaterium–Arabidopsis thaliana	Plant growth. Inoculation of plants with mutations in three CK receptors (single, double, and triple mutants)	Reduced PGP in single and double mutant. Non-PGP in triple mutant	PGP can be mediated by different CK receptor homologs	Ortiz-Castro et al. (2008b)
B. japonicum and A. brasilense–G. max	Plant growth and seed germination. Inoculation and co-inoculation	Promotion of seed germination, PGP of soybean seedlings	PGPR excrete zeatin (CK) at sufficient concentration to produce PGP in young seed tissues	Cassán et al. (2009)

[a] *Hit1* genetic suppressor of the hyperinfected phenotype; abundant infection threads formation but failed cortical cell division
[b] *Nin* nodule inception regulator
[c] *snf2* an allele of a lotus histidine kinase LHK1 gene; spontaneous nodule formation

The production of CKs enhances pathogenicity and modulates the physiology of host plants (Choi et al. 2011). In contrast, plant-derived CKs may be involved in plant resistance to pathogen infection (Choi et al. 2011). However, the molecular mechanisms of CK action in disease resistance against a wide spectrum of pathogens and the reason for the opposite effects of CKs on plant responses against pathogens are still unclear.

In addition to CKs, auxins also influence plant growth. Auxins are compounds with aromatic ring and carboxylic acid groups. The increasing amount of data about bacterial strains with the ability to increase the pool of auxins available to plants leads to the assumption that their production is one of the major direct factors that promote root and plant growth (Khalid et al. 2011; Ali et al. 2009). Auxins act on root architecture increasing the number of lateral roots and root hair elongation. They are also responsible for apical dominance acting as a signaling molecule in root and shoot growth (Ferguson and Beveridge 2009). As a result of increased root bulk, the plant may scavenge a larger area for nutrient and water uptake, and the root has a larger number of potential niches for beneficial or pathogenic microbial infection.

Tryptophan is an amino acid commonly found in root exudates, and it is the main precursor of auxin biosynthesis (Etesami et al. 2009). Indole-3-acetic acid (IAA) is the main auxin in plants, controlling cell enlargement and division, tissue differentiation, and responses to light and gravity. Many PGPR, such as *Azospirillum*, *Pseudomonas*, *Delftia*, and *Rhizobium* species, induce root proliferation through IAA production (Morel et al. 2011; Spaepen et al. 2007, 2008; Kapulnik et al. 1985). However, various phytopathogens also have the ability to produce IAA and/or alter its levels in plants, facilitating host infection and virulence and causing uncontrolled growth in plant tissues (mainly tumor and gall induction) (Chalupowicz et al. 2006; Robert-Seilaniantz et al. 2007). *Agrobacterium*, *Pseudomonas*, and *Erwinia* produce IAA as part of their pathogenic behavior (Lambrecht et al. 2000). Other indolic compounds with auxin activity are indole-3-butyric acid, indole-3-pyruvic acid, indoleacetamide and indole-2-carboxylic acid (Lim and Kim 2009).

Gibberellins and brassinosteroids also play an important role during nodule formation (Oldroyd and Downie 2008). It has been shown that brassinosteroids act together with auxins on many developmental plant processes (Yoshimitsu et al. 2011). Strigolactones are plant hormones that contribute to apical dominance (Ferguson and Beveridge 2009). They are exuded by roots in extremely low concentrations (Steinkellner et al. 2007). They act as chemical signals for root colonization by symbiotic arbuscular mycorrhizal fungi and inhibit shoot branching. Even though there are no reports of microbial production of strigolactones, it has been suggested that this class of phytohormones has biological signaling functions in the rhizosphere (Tsuchiya and McCourt 2009; Steinkellner et al. 2007).

Other Secondary Metabolites

Plants produce an extremely diverse array of low molecular mass compounds, often called secondary metabolites, which include, among others, alkaloids, essential oils

or essences, steroids, terpenoids, and phenolic compounds. Some secondary metabolites are commonly found in plants, but others are specific to only a few related plant species and/or are produced in particular conditions (Pichersky and Gershenzon 2002). Most of them are signaling molecules, and even if their roles in signaling are unknown, some are strictly necessary, like flavonoids. Here, we summarize some of the highlights of plant secondary metabolites involved in plant–microorganism interaction, other than phytohormones, which have been covered in section "Phytohormones Production."

Volatile Organic Compounds (VOCs)

VOCs are molecules that have high vapor pressure and vaporize to the atmosphere under normal conditions (Ortiz-Castro et al. 2009). The first report of a plant VOC was the plant hormone ethylene in the year 1910 (recognized as cell-to-cell signal transmission in 1934 by Gane) (Bleecker and Kende 2000). Since then, it has been accepted that plants produce and release a variety of diffusible compounds, including low molecular weight compounds, such as terpenoids, modified fatty acids, benzenoids, and other scented substances (Ortiz-Castro et al. 2009; Ping and Boland 2004). Improved techniques for the collection and analysis of volatiles, such as gas chromatography-electroantennographic detection, have allowed the detection of new plant VOCs (Pichersky and Gershenzon 2002). VOCs act as plant growth regulating substances that affect other organisms, acting, for example, as attractants and/or repellents. Recently, some authors demonstrated that some PGPR can produce VOCs as signals that stimulate the growth of plants (Gutierrez-Luna et al. 2010). Examples of PGPR-producing VOCs are *B. megaterium* (acetoin and butanediol producer) (Lopez-Bucio et al. 2007) and *Bacillus* spp. (1-octen-3-ol and butyrolactone producer) (Gutierrez-Luna et al. 2010). Many VOCs have been detected in rhizospheric soil, but their role in microbe–plant interactions is still uncertain. It has been suggested that VOCs may have antibiotic functions acting in the control of plant pathogens, induce different phytohormonal signaling networks (Ortiz-Castro et al. 2009), or activate induced systemic resistance (ISR) via a salicylic acid- and jasmonic acid-independent pathway (Ping and Boland 2004). For example, the VOCs 2,3-butanediol and acetoin, released by *Bacillus* spp., trigger growth promotion of *Arabidopsis* seedlings and induce systemic resistance against *Erwinia carotovora* (Ryu et al. 2005). It was concluded that VOCs activate a CK-dependent pathway for PGP and an ethylene-dependent signaling pathway for ISR (Ping and Boland 2004).

Phenolic Compounds

Phenolic compounds are produced by plants and microbes as well, but they differ in chemical structure (Combes-Meynet et al. 2011; Mandal et al. 2010; Parmar and Dadarwal 1999). Increasing evidence suggests that root-secreted polyphenols initiate and modulate the dialog between roots and soil microbes (Badri and Vivanco 2009).

Table 6.4 Plant-secreted flavonoids induce *nod* genes

PGPR	Plant	Flavonoid(s)	Effect	Reference
Sinorhizobium meliloti	Alfalfa	Luteolin(3′,4′, 5,7-tetrahydroxyflavone)	*nod*-gene inducer	Peters et al. (1986)
S. meliloti	Alfalfa	4,4′-dihydroxy-2′-methoxychalcone, 4′,7-dihydroxyflavone, 4′-7-dihydroxyflavanone	Flavonoids, other than luteolin, are *nod*-gene inducers	Maxwell et al. (1989)
S. meliloti	Alfalfa	Chrysoeriol and luteolin	*nod*-gene induction	Hartwing et al. (1990)
Azospirillum brasilense (co-inoculation with *Rhizobium tropici* and *Rhizobium etli*)	Common bean	Daidzein, naringenin, genistein, and coumestrol	Increased root hair formation, nodule number, *nod*-gene induction	Burdman et al. (1996)
Rhizobium leguminosarum	Pea and lentil	Hesperetin and naringenin	*nod*-gene induction	Begum et al. (2001)
Bradyrhizobium japonicum	Soybean	Coumestrol	Increased number of nodules, high degree of biofilm formation. Weak *nod*-gene induction	Lee et al. (2012)

Flavonoids are plant phenolic compounds recognized as important signaling molecules in microbe–plant interaction events. The main subclasses of flavonoids include chalcone, flavone, isoflavone, flavonol, flavanone, and isoflavonoid compounds (Cesco et al. 2012). The effects of flavonoids in the rhizosphere depend on their chemical composition and concentration. In the rhizosphere, they have a critical role in early stages of the rhizobia–legume symbiotic interaction and in plant defense. The best-known roles attributed to plant flavonoids are in chemoattraction of rhizobia to the legume root and as primary molecular signals for rhizobial *nod-gene* induction and NF production (Mandal et al. 2010; Badri et al. 2009; Oldroyd and Downie 2008; Steinkellner et al. 2007). A wide variety of plant flavonoids have been shown to induce NF production in different rhizobia–legume interactions (Table 6.4). In the presence of compatible rhizobial strains, the legume host increases the exudation of a particular set of flavonoids, e.g., in the presence of *Sinorhizobium* strains, alfalfa produces increased amounts of the flavonoid luteolin. Flavonoids protect dividing cells from oxidative damage due to their antioxidant properties and ability to modulate several enzymes (Ariel et al. 2012; Cesco et al. 2012).

The genome-wide transcriptional response of *Bradyrhizobium japonicum* to genistein showed that 100 genes were induced, including all *nod* box-associated

genes and flagellar and transport process genes, suggesting that genistein has a much broader function than *nod*-gene induction (Lang et al. 2008). Flavonoids (naringenin and hesperetin) are also factors that influence rhizobial competitiveness in soils, as showed in several biovars of *Rhizobium leguminosarum* (Maj et al. 2010). Flavonoids also participate in plant host specificity for few rhizobial species. Plants secrete a characteristic group of inducing and non-inducing flavonoids that are recognized by rhizobial outer membrane protein, NodD (the LysR-type transcriptional regulator that mediates the expression of *nod* genes and a key determinant of host specificity). Both inducing and non-inducing flavonoids bind NodD and mediate conformational changes at *nod*-gene promoters, but only a few set of flavonoids are capable of promoting *nod* genes. The production of non-inducing flavonoids may be a mechanism by which legumes prevent overnodulation (Peck et al. 2006). The rhizospheric microbial community may also alter the amount and composition of *nod*-inducing signals secreted by the plant. Many reports showed that the inoculation of leguminous plants with *Azospirillum* induces the secretion of a particular set of *nod*-inducing flavonoids that facilitate the establishment of the rhizobia–plant interaction, even under stress conditions (Burdman et al. 1996; Volpin et al. 1996; Dardanelli et al. 2008).

Flavonoids shape rhizosphere microbial community structure because they may be used as potential carbon sources or may act as toxic substances for microbes that do not possess flavonoid biodegradation pathways (Shaw et al. 2006). They may also accelerate the biodegradation of xenobiotics, since the chemical structures of many flavonoids and xenobiotics are similar (Cesco et al. 2012; Shaw and Burns 2003), and flavonoids may have allelopathic effect on other plants (Cesco et al. 2012).

The role of phenolic compounds as signaling compounds in pathogenic microbe–plant interactions is undeniable. Usually, phenolic compounds released from seeds and roots act against soilborne pathogens and have high antifungal, antibacterial, and antiviral activities (Mandal et al. 2010). For example, *Pseudomonas* produces 2,4-diacetylphloroglucinol (DAPG), a phenolic compound with antibiotic properties, and a signal molecule that induces systemic resistance in plants and stimulates root exudation and branching (Combes-Meynet et al. 2011). The secretion of catechin by *Combretum albiflorum* interferes with the production of virulence factors by *P. aeruginosa* (Vandeputte et al. 2010).

Quorum Sensing Responses

Quorum sensing (QS) is a phenomenon where microbes communicate and coordinate activities by the accumulation of signal molecules at sufficient concentration (Adonizio et al. 2008). Both pathogenic and symbiotic bacteria require QS to interact successfully with their hosts (Badri et al. 2009). In Gram-negative bacteria, QS is typically mediated by *N*-acyl-L-homoserine lactones (AHLs). AHLs are freely diffused through the bacterial membrane and distributed within the rhizosphere where they regulate the behavior of rhizospheric bacteria. Increasing evidence

indicates that higher plants may produce metabolites that mimic AHLs, interfering with rhizospheric QS behavior (Gao et al. 2003). For example, *Medicago sativa* produces multiple signal molecules, including L-canavanine, capable of interfering with QS gene expression in *S. meliloti* (Keshavan et al. 2005). Canavanine is an arginine analog commonly found in seed and root exudates of a variety of legumes. Cai et al. (2009) found that canavanine is toxic to many soil bacteria but not to some rhizobia and suggest that host legumes may exude canavanine to optimize the bacterial population and select beneficial rhizobia in their rhizospheres. The role of these plant AHL-like compounds is still unclear (Ortiz-Castro et al. 2009), but some authors report direct effects on plant development in a similar way to auxins, by modulating root system architecture (more lateral roots and root hairs) (Ortiz-Castro et al. 2008a; von Rad et al. 2008). Plant AHL-like compounds are also involved in protection against pathogens. Vandeputte et al. (2010) reported the secretion by *Combretum albiflorum* of the flavonoid catechin that interferes in the QS signaling of *Pseudomonas aeruginosa* PAO1, as the first line of defense against this pathogen. Some PGPR can also protect plants by disrupting the QS signals required for pathogen–pathogen communication, interfering with the expression of virulence genes. For example, *Bacillus*, *Arthrobacter*, and *Klebsiella* produce AHL-degrading lactonases which inactivate AHLs (Friesen et al. 2011). Moreover, QS in the rhizosphere can also be disrupted by abiotic factors such as alkaline pH (Reis et al. 2011). Other PGPR secrete AHLs that induce plant systemic resistance to pathogens. For example, AHL molecules produced by *Serratia liquefaciens* MG1 and *P. putida* IsoF induce ISR in tomato plants against *Alternaria alternata* via a salicylic acid and ethylene-dependent pathway (Schuhegger et al. 2006). It is important to highlight the relevance of disrupting bacterial QS signaling as a strategy to fight against phytopathogens. This field is still unexplored.

Extracellular Polysaccharides

Bacterial extracellular polysaccharides (exopolysaccharides, EPSs; lipopolysaccharides, LPSs; capsular polysaccharides, CPSs; and cyclic β-glucans) are usually accumulated on cell surfaces and/or secreted into the cell surroundings (Gray and Smith 2005). They have multiple roles, such as protection against stress (Qurashi and Sabri 2012; Upadhyay et al. 2011), attachment to surfaces (Tsuneda et al. 2003), plant invasion (Fraysse et al. 2003; Troch and Vanderleyden 1996), and inhibition of the plant defense response in plant–microbe interactions (Kyungseok et al. 2008). PGPR also produce EPS and other surface polysaccharides as essential components that promote interaction with plants (Upadhyay et al. 2011).

Rhizobial surface polysaccharides are highly important during the early steps of microbe–legume interaction. They are essential for the formation of infection thread (IT), for nodule development, and for adaptation and survival of rhizobia under different environmental conditions (Fischer et al. 2003). In rhizobia, surface

polysaccharides form a hydrated matrix that contributes to protection against abiotic factors and plant products secreted as a defense response during the infection process. Moreover, CPSs may have an active signaling role during beneficial infections (Parada et al. 2006; Becker et al. 2005).

LPSs are anchored to the surface membrane by a lipidic moiety and inserted into the bacterial phospholipid monolayer, and the saccharidic part is oriented outside. Although LPS is a constitutive component of the bacterial membrane in Gram-negative bacteria, it is commonly found in very low concentrations in growth media, being released from cells in vesicles (Becker et al. 2005), and consequently it seems likely that LPSs may act as long-distance signaling molecules to target cells (Fraysse et al. 2003). They play various roles at different stages of the symbiotic process, act as inhibitors of plant defense responses, and/or help bacteria to adapt to the endosymbiotic environment. Experimental evidence demonstrates that root exudates, mainly plant-exuded flavonoids, induce changes in the PGPR-extracellular polysaccharide (EPS, LPS-O antigen, and CPS) composition, affecting the PGPR–plant interaction (Fischer et al. 2003; Fraysse et al. 2002, 2003; Reuhs et al. 1994; Dunn et al. 1992).

The importance of bacterial surface polysaccharides during the symbiotic process has been extensively demonstrated. *Azorhizobium caulinodans* mutants with LPS deficiency (Mathis et al. 2005) and LPS with reduced rhamnose content (Gao et al. 2001) established defective interactions with *Sesbania rostrata*, suggesting that both correct LPS amount and composition are required to sustain an effective rhizobia–legume interaction. In addition, LPS affects competitiveness and colonization as demonstrated by working with *Mesorhizobium loti* mutants defective in LPS and cyclic β-glucans (D'Antuono et al. 2005) and LPS mutants of *A. brasilense* in maize (Jofre et al. 2004), respectively.

EPSs are mostly species-specific heteropolysaccharides with an important role for an efficient symbiotic process. Bacterial mutants which fail to produce EPS induce nodules on the roots of the host plant but fail to invade these root nodules. Rhizobial EPSs are involved in the invasion process, IT formation, bacteroid and nodule development, and plant defense response and also confer protection to rhizobia when exposed to environmental stress (Bomfeti et al. 2011). EPSs are also involved in plant colonization and cell aggregation, as widely shown in *Azospirillum* species (Bahat-Samet et al. 2004; Jofre et al. 2004; Fischer et al. 2003; Burdman et al. 2000). The data showed that aggregation and root colonization properties of *Azospirillum* depend on the concentration and composition of EPS. The influence of EPS during aggregation on rhizospheric soil results in increased water and fertilizer availability to inoculated plants (Qurashi and Sabri 2012). Some PGPR-EPS can also bind cations, including Na^+, suggesting a role in mitigation of salinity stress by reducing the content of Na^+ available for plant uptake (Upadhyay et al. 2011). EPS produced by specific rhizobacteria can also elicit plant-induced resistance against biotic stress. For example, inoculation with the EPS-producing *Paenibacillus polymyxa* on peanut seeds significantly suppressed crown rot disease caused by *Aspergillus niger* (Haggag 2007), and the purified EPS from the PGPR *Burkholderia gladioli* induced resistance against *Colletotrichum orbiculare* on cucumber (Kyungseok et al. 2008).

Among extracellular polysaccharides, the rhizobial lipo-chitooligosaccharide known as nodulation factor (Nod factor or NF) is the most studied and probably the "movie star" of rhizobia–legume interaction. NFs have an oligomeric backbone of β-1,4-linked *N*-acetyl-ᴅ-glucosamine, N-acylated at the nonreducing terminal residue, and trigger the nodule developmental process. Depending on the rhizobial species, NFs have different chemical structures (variation in acyl chain, substitutions at the reducing and nonreducing terminal sugar, and additional decorations) (D'Haeze and Holsters 2002; Geurts and Bisseling 2002). Rhizobia perceive plant-secreted flavonoids by binding to NodD, a member of the LysR family of transcriptional regulators that triggers NF synthesis. NodD binds to conserved DNA sequences, known as *nod* boxes, found in the promoter regions of inducible *nod* genes. NF synthesis is commanded by the common *nodABC* genes which encode enzymes involved in the core structure, and many other *nod* genes are involved in decorations. Properties and functions of NFs are described throughout the body of the text of this chapter.

PGPR and Plant Root Attachment

Successful colonization and persistence in the rhizosphere are required for PGPR to exert their beneficial effect on plants. Many studies have shown that rhizobacteria are attracted to seed and root (chemotaxis) by plant-exuded molecules, the "rhizosphere effect" (Bais et al. 2006). Plant roots provide a carbon-rich environment and produce signals that are recognized by microbes which in turn produce others signals that initiate colonization. What are the most important traits in root–microbe interaction events? Motility, chemotaxis, and electrotaxis (the ability to use electric potentials produced at the root surface which act as attractants) enhance competitiveness during root colonization. Many microbe–plant interactions are mediated by the flagella which modulate attachment of the microbial cell to the root system. This process is well known in root colonization by azospirilla. Azospirilla undergo a biphasic attachment process, with an initial flagella-dependent adsorption phase, followed by an irreversible anchoring of the bacterium to the surface, and then the formation of bacterial aggregates embedded within the fibrillar material (Reis et al. 2011; Troch and Vanderleyden 1996).

A model described by Genre and Bonfante (2007) suggests alternative routes to biotrophy in interactions between plants and PGPR, endophytes, and pathogens, where precontact signaling contributes to the recognition of rhizobacteria as beneficial or pathogenic. A weak, nonspecific, and reversible first contact occurs mediated by lectins, bacterial surface proteins, CPS, and/or flagella (Rodriguez-Navarro et al. 2007). Then, a direct contact occurs characterized by a rapid translocation of the cytosolic and subcellular elements to the contact site (localized secretion). In beneficial interactions, this secretion leads directly to (1) epiphyte–bacterial aggregates on the plant surface or (2) a preemptive assembly of an intracellular apoplastic compartment to host the endophyte (Genre and Bonfante 2007). In this step,

extracellular polysaccharides are the main determinants, required for tight and irreversible binding of bacteria (Rodriguez-Navarro et al. 2007).

In the rhizobia–legume interaction, the endophytes access the root by the ITs, tubular structures derived from plant plasma membranes that act as "tourist guides" to the root cortex. The process of rhizobia accommodation into the nodule primordium may be explained by a sequence of events described by Held et al. (2010). The extracellular colonization of roots by rhizobia leads to the uptake of cells through an intracellular (through root hairs) or intercellular ("crack-entry") infection (Held et al. 2010). The latter is thought to be the ancestral mechanism of root infection and involves the formation of transcellular ITs within the root cortex (Downie 2010). The next section gives a brief but more detailed description of rhizobia–legume interaction events.

Proteins Involved in Rhizobia–Plant Interaction

Proteomics, the identification of a set of proteins under specific conditions, is a valuable tool to decipher part of the complex network involved in plant and microbe communication. Most works dealing with plant–microbe exchange of information through a proteomic approach have been performed on plant tissues after bacterial inoculation, bacteroids, or nodules. Additional information has also been achieved by transcriptomic and metabolomic analysis (Stacey et al. 2006).

It has been shown that rhizobia inoculation induces or increases the level of several proteins in soybean root hairs (calcium/calmodulin kinase, lipoxygenases, phospholipase D, ascorbate peroxidase, phosphoglucomutase, lectin), roots (enzymes involved in energy, carbohydrate, amino acid, and flavonoid metabolism), and bacteroids (proteins involved in carbon and nitrogen metabolism, stress response and detoxification, ABC transporters and receptors) (Mathesius 2009). In addition, large amount of information has been generated about the regulation of signal transduction involved in bacterial infection and nodule organogenesis and long-distance signaling to control nodule number (Oka-Kira and Kawaguchi 2006; Popp and Ott 2011). However, few experiments have analyzed proteins secreted in the rhizosphere or those that are associated with the bacterial outer membrane. These experiments involve plant growth in liquid media, protein concentration by lyophilization or precipitation, desalting, two-dimensional gel electrophoresis, and protein identification by mass spectrometry (Jayaraman et al. 2012). In addition, proteins secreted by bacteria or associated with their outer membrane have been found using a classical approach, by the analysis of culture medium after adding plant-secreted molecules, or a genomic approach through the study of mutants. Using different approaches, many proteins secreted to the rhizosphere and involved in plant–microbe communication have been identified.

Rhizobial proteins are secreted by general secretion (Sec) and two-arginine (Tat) systems of general use (NodO, adhesins, PlyA and PlyB polysaccharide lyases, ExoK and ExsH succinoglycan depolymerases, calsymin, cellulose, etc.) and by specialized secretion systems (Nops or nodulation outer proteins secreted by the

type III secretion system, Msi059 and Msi061 by the type IV secretion system, ribose-binding protein-like by the type V and VI secretion systems) (Downie 2010; Deakin and Broughton 2009; Tseng et al. 2009; Fauvart and Michiels 2008). Plant roots secrete compounds mainly by passive process mediated diffusion, ion channels, and vesicle transport. But excretion of high-molecular weight compounds by roots, including proteins, generally involves vesicular transport. Rhizobial cells secrete adhesins such as rhicadhesin that plays an important role in attachment to root hairs (Smit et al. 1992), hydrolytic proteins such as cellulase that erodes the noncrystalline cellulose in the root hair cell wall allowing rhizobial penetration (Robledo et al. 2008), and glycanases that cut emerging EPS produced by rhizobia and are required for biofilm formation (Russo et al. 2006). Many extracellular glycanases, involved in nodulation and EPS modification, have been identified and characterized in rhizobia: PlyA and PlyB of *R. leguminosarum* bv. *viciae* and ExoK and ExsH of *S. meliloti*. The secreted nodulation-signaling protein NodO was purified from the supernatant of cultures of *R. leguminosarum* bv. *viciae* supplemented with flavonoids (Sutton et al. 1994). NodO is a calcium-binding protein that forms cation-selective channels in membranes and may complement NF function by promoting the movement of cations across the root hair membrane (Downie 2010). *M. sativa* inoculation with *S. meliloti* caused an increase in the secretion of plant hydrolases (chitinases that use NFs as substrates, glycosidases, and peptidases), peroxidase precursors, pathogenesis-related proteins (thaumatin-like protein), lectins, bacterial superoxide dismutase, glycine betaine-binding ABC transporter, and a putative outer membrane lipoprotein transmembrane (De la Peña et al. 2008).

Rhizobia–Legume Interaction Events

Rhizobia–legume signaling strategies are mainly based on sugars such as the NFs, EPSs, lipopolysaccharides and capsular polysaccharides, as well as cyclic β-glucans. However, roots and microorganisms also produce diverse proteins that play a dynamic role in the process of signaling and recognition that occurs during their interaction. A picture of events implicated in legume–rhizobia interaction involving carbohydrates, flavonoids, phytohormones, and proteins may be summarized as follows (Fig. 6.1).

Plant roots release species-specific mixtures of molecules, such as phytohormones and flavonoids (that act as bacterial attractants), that initiate the symbiotic chemical dialog. Rhizobial cells recognize flavonoids by their binding to NodD, an extracellular membrane protein that works as an environmental sensor and master transcriptional activator of genes downstream of promoters known as *nod* boxes. In response to *nod*-gene activation, rhizobia produce and release the signaling molecule NF that is identified by plant root receptor-like kinases (NFR-LKs). Many NFR-LKs have been identified, e.g., LysM-type RLKs NFR5/NFR1 of *L. japonicus*, NFP/LYK3/LYK4 of *M. truncatula*, SYM10/SYM2 of *Pisum sativum*, and NFR5αβ/NFR1β of *G. max*. After the NFR-LK-ligand recognition,
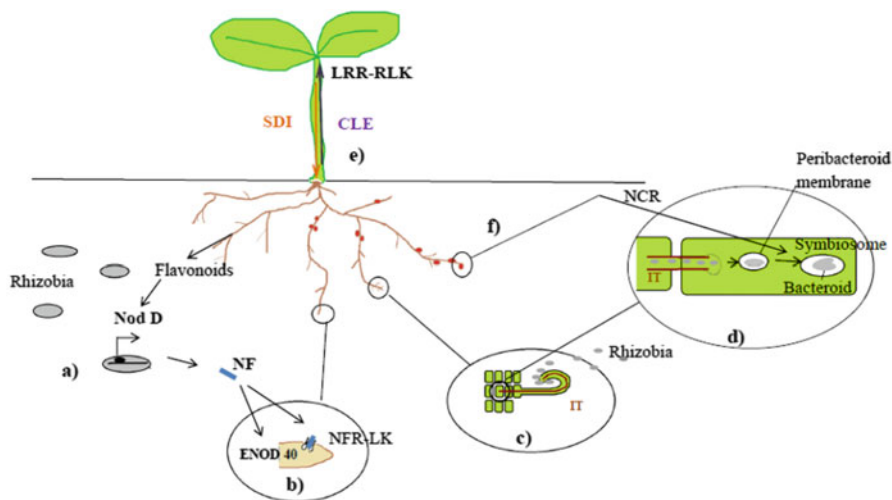
Fig. 6.1 Overview of rhizobia–legume interaction events. (**a**) Induction of *nod* genes by root-exuded flavonoids and NF production; (**b**) NF perception by NFR-LK elicits calcium signaling that leads to localized CK biosynthesis. CK induces the ENOD40 production and downstream signaling for activation of symbiotic response and nodule organogenesis; (**c**) deformation of root hair and formation of IT. Bacteria move through the IT; (**d**) rhizobia penetrate cortical cells via IT. They are released from unwalled IT into the host cell cytoplasm as membrane-delimited symbiosome into bacteroids; (**e**) CLE peptide synthesis in the nodule and recognition by shoot-specific receptor kinase (LRR-RLK). Production of shoot-derived inhibitor (*SDI*) that regulates nodule number (*AON*); (**f**) indeterminated nodule produces NCRs that induce bacteroid differentiation

many physiological events are turned on, such as root hair deformation and IT initiation, depolarization of the plasma membrane, rhizosphere alkalinization, Ca spiking by a calcium-dependent calmodulin kinase (CCaMK), cytoskeletal rearrangement, early nodulin gene expression, and finally nodule formation.

In addition to NF, some rhizobia secrete proteins involved in host specificity and symbiotic efficiency by a type III secretion system or T3SS. T3SS delivers virulence proteins called effectors directly into the host cells. Rhizobial effector proteins are known as Nops (nodulation outer proteins). Rhizobial NopL and NopP interfere with plant signaling pathways acting as positive effectors that enhance nodule formation. These and other Nops effectors might contribute to suppression of plant innate immune response or modulate cytoskeletal rearrangements in root cells during nodule formation. Thus, rhizobial effectors could facilitate bacterial release from IT, initiate symbiosis, and/or promote or maintain persistence of bacteroids (Saeki 2011; Deakin and Broughton 2009).

The invading bacteria move through the IT and are taken into the plant cell by a type of endocytosis in which they are surrounded by a plant-derived peribacteroid membrane. Nodule organogenesis, cell proliferation and dedifferentiation, and bacteroid differentiation are driven by plant hormones and systemic signaling peptides (ENOD40, CLE, NCR) (Ding et al. 2008; Batut et al. 2011). Ethylene, jasmonic

acid, and abscisic acid (ABA) regulate NF signaling and affect the nature of NF-induced calcium spiking, with ABA being capable of coordinating regulation of diverse development pathways associated with nodule formation (Ding et al. 2008).

CLE (CLAVATA3/endosperm surrounding region) are peptides that have been identified in a wide variety of plants. They are key molecules in the regulation of nodulation acting as a root-derived ascending signal to the shoot. This peptide is probably recognized as a ligand for a leucine-rich repeat (LRR) autoregulation receptor kinase that controls multiple aspects of shoot development, jasmonate signaling, and the production of a shoot-derived inhibitor (produced in leaves) that regulates root nodule number. These LRR receptor kinases (GmNARK, *Glycine max* nodule autoregulation receptor kinase of soybean; HAR1, hypernodulation and aberrant root of *Lotus japonicus*; SYM29, symbiosis of pea; and SUNN, super numeric nodules of alfalfa) are key regulators of the autoregulation of nodulation (AON) signaling pathway that controls a hypernodulated unproductive phenotype (Staehelin et al. 2011; Popp and Ott 2011; Miyazawa et al. 2010; Kinkema and Gresshoff 2008; Oka-Kira and Kawaguchi 2006). AON is the major pathway that controls nodulation events acting through the inhibition of nodule development in a long-distance signaling fashion between root and shoot. NF is also involved in the expression of several early nodulin (ENOD) genes (ENOD12 y ENOD40).

It has been suggested that CK is an epidermal cell synthesized secondary signal, which after translocation to cortex cells triggers the initiation of nodule primordial ahead of the upcoming IT (see section "Phytohormones Production"). CK induces the expression of the *enod40* gene serving as an amplification mechanism, thus triggering a localized hormone imbalance, a state that initiates cell divisions in the root cortex (Fang and Hirsch 1998). The *enod40* gene codes for two short conserved peptides, A and B, which strongly bind the cytosolic sucrose synthase (SuSy) enzyme-stimulating sucrose breakdown activity. The data support the view that Enod40 peptide may participate in phloem uploading, increasing the carbon sink strength in pre-dividing root cortical cells and in mature nodule tissues (Batut et al. 2011). CK induces the expression of the *Nin* transcriptional regulator within the root cortex through the activation of the LHK1 cytokinin receptor, subjected to HAR1-mediated autoregulation (Heckmann et al. 2011).

Some legumes such as *Medicago*, *Pisum*, *Vicia*, and *Trifolium* maintain active apical meristems that produce indeterminate nodules. This type of nodule undergoes an irreversible differentiation mediated by nodule-specific cysteine-rich (NCR) peptides. NCRs are produced by the host cells and targeted to bacteroids where they interfere with the rhizobial cell cycle affecting terminal bacterial differentiation. In addition, NCRs resemble antimicrobial peptides (Batut et al. 2011; Van de Velde et al. 2010). Findings suggest that after the root epidermal cell recognition of NF, several kinase receptors are activated, working as a signal transduction cascade responsible for the control and progression of IT, nodule organogenesis, and nitrogen fixation (activation of downstream common *nod* and *sym* genes). These kinase receptors are regulated by E3-ubiquitin ligases that act as dynamic modulators of cellular reprogramming during rhizobial infections (Popp and Ott 2011; Mathesius 2009). Hundreds of proteins from nodule, xylem, root, and shoot have been

implicated in rhizobia–legume interaction (Mathesius 2009), but insufficient work has been done on proteins secreted in the soil by roots and bacteria during microbe–plant interaction.

A large variety of regulatory molecules, including kinases, transcriptional factors, and other regulatory molecules, are involved in symbiotic nodule organogenesis, and recent reports showed that sRNAs, especially microRNAs (miRNAs), are also key regulatory factors of this process. Thus, miRNAs are emerging as riboregulators that control gene networks in plant cells through interactions with specific target mRNAs. Only a few nodulation-responsive miRNAs have been linked to nodule formation: among other miRNAs, miR169 and miR166 overexpression in *M. truncatula* led to lower densities of lateral roots and nodules, and they might be responsible for nodule meristematic zone regulation during nodule differentiation into nitrogen-fixing cells; soybean miR482 targets the resistance gene receptor kinase involved in the defense response, playing a role during nodule initiation; miR1511 and miR1512 target transcripts encoding signaling proteins, including a calmodulin-binding protein (Bazin et al. 2012; Khan et al. 2011; Voinnet 2008). In addition, there is strong evidence that there is a connection between miRNA regulation and hormone response. Some miRNAs facilitate hormone-induced responses, e.g., the miRNAs miR160, 167, and 393 that are implicated in the regulation of auxin signaling target transcripts to reduce lateral root production and are potentially involved in nodulation (Simon et al. 2009; Bazin et al. 2012).

Concluding Remarks

Compounds exuded by plants and microbes provide a cocktail of molecules (carbohydrates, phytohormones, flavonoids, amino acids, and proteins) that constitute the words of a chemical dialog between plants and microbes in the rhizosphere (Fig. 6.2). The massive variety of metabolites released by plants suggests that they provide a specific language for communication. Researchers are deciphering the content and significance of the cells' signaling and responses. Recent advances in analytical skills and biochemical and molecular approaches have provided new tools for evaluating the natural roles of these substances and for investigating the mechanisms underlying their regulation.

In brief, the picture of microbe–plant interaction events involves a huge number of molecules that span our imagination. Every year a new signaling molecule is found, and the overall scene is getting much bigger and more complex. The new information on proteins involved in two-component signal transduction systems that allow sensing and responding to different stimuli, transcriptional regulators, and plant-derived peptides is far from completing the picture of the microbe–plant interaction. In this chapter, only some recent and relevant earlier information related to molecules involved in microbe–plant interaction have been used to present a partial panorama.
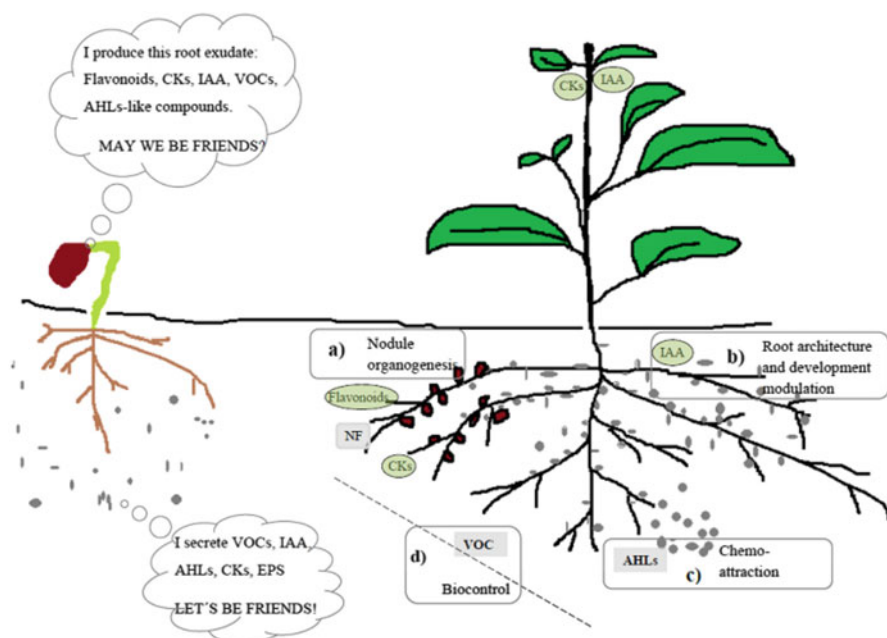
Fig. 6.2 Summary of early signaling events in microbe–plant interaction. *Left*: Some bacterial and root-exuded compounds involved in microbe–plant interaction. *Right*: Four mechanisms of PGP: (**a**) In legume–rhizobia symbiosis, root-secreted flavonoids trigger rhizobial production of NF and nodule formation (see Fig. 6.1); (**b**) bacteria-produced IAA modulate root architecture and development. In legume–rhizobia interaction, they act on nodule functioning; (**c**) bacterial AHLs and plant AHL-like compounds regulate the behavior of rhizobacteria, selecting beneficial ones and interfering with QS of non-beneficial ones. AHLs may also modulate root architecture in an auxin-like fashion; (**d**) VOCs may function as biocontrol compounds against phytopathogens

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