Signaling and Communication in Plants

Jorge M. Vivanco
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# Secretions and Exudates in Biological Systems

Springer

# Signaling and Communication in Plants

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# Secretions and Exudates in Biological Systems

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# Preface

Secretions and emissions in biological systems play important signaling roles within the organism but also in its communication with the surrounding environment. This relatively recent knowledge is in stark contrast with the view of secretions that is available in most text books in different biological science disciplines. Not too long ago, secretions and emissions were considered biological waste products that were simply discharged out of plants through a chemical gradient with no function to the environment whatsoever. The realization of this void of information was the driving force behind the compilation of this volume. This volume brings together state-of-the-art information about the role of secretions and emissions in different organs and organisms ranging from flowers and roots of plants to human organs.

The plant chapters will relate information regarding the biochemistry of flower volatiles and root exudates, and their role in attracting pollinators and interacting with soil microbial communities, respectively. Furthermore, these chapters will illustrate information about the fine molecular and biochemical machinery that is involved in the biosynthesis and secretion of these compounds; which suggests that the organism actively coordinates the release of these chemical signals. The release of compounds by roots is further highlighted by the most economically important root–microbe association in agriculture. The rhizobium–legume root association forms an organ called the nodule that can fix the nitrogen from the air and entirely eliminates the need of nitrogen fertilization in legume plants such as soybean. Proteoid roots release proteases for improving nitrogen and phosphorus availability for their mineral nutrition. Roots of plants not only release compounds, but also entire cells and the detailed mechanisms and functions of this phenomenon will be highlighted. Plants emit fine bouquets of smell not only through their flowers but through several organs; the biosynthesis and function of volatile organic compounds (VOCs) in plants are also covered in this volume. Moreover, in order to manipulate their animal pollinators, plants provide them with nutritive exudates.

Microbial chapters will explain the biochemistry and ecology of quorum sensing and how microbial communities aggregate in different environments through the

continuous release and sensing of compounds that regulate the "quorum" in the community. A related chapter will touch upon highly coadapted association between plants and soil microbes that can aid in bioenergy applications by degrading lignocellulosic materials.

Other chapters will explain the biology of secretions by algae and humans, among other organisms. All in all, this volume will be a welcome addition to the literature as no other book covers aspects related to biological secretion in such a holistic and integrative manner.

Fort Collins, CO, USA — Jorge Vivanco
Bonn, Germany — František Baluška

# Contents

# Plant Root Secretions and Their Interactions with Neighbors

**Clelia De-la-Peña, Dayakar V. Badri, and Víctor M. Loyola-Vargas**

**Abstract** The rhizosphere biology at the molecular level has advanced dramatically since last decade. The continuous supply of carbon compounds from plant roots engages complex interactions among rhizosphere organisms including interactions between microbes and plants and between plants with other plants being these of the same or different species. Root exudation is part of the rhizodeposition process, which is a major source of soil organic carbon released by plant roots which clearly represents a significant carbon cost to the plant. Root exudates also play a role in soil nutrient availability by altering soil chemistry and soil biological processes. Different studies have highlighted that the rhizosphere soil surrounded by plant roots is more abundant in microbes than the nonrhizosphere soils. Therefore, the major responses in the interaction between plants and microbes must happen in that limited zone. Plants respond to the presence of microbes by releasing a mixture of phytochemicals, volatiles, and high-molecular-weight compounds. Soil microbes, on the other hand, modulate the secretion of root exudates to positively regulate plant growth and disease resistance. Several negative interactions are mediated by root exudates including antimicrobial, biofilm inhibitors, and quorum-sensing mimics to prevent soil-borne pathogens. There is a need to understand these rhizospheric multitrophic interactions in the realistic field conditions to improve the plant growth at species and community level. In addition, studies should be conducted in the field

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conditions to understand the rhizospheric complex interactions in monocultures and polycultures. This will help to understand the dynamics of interactions and their outcome in influencing the plant's success when they are in monocultures and in polycultures. The combination of techniques and the continuous development of new techniques in the field of rhizosphere biology coupled with systems approach will allow us partly to elucidate these complex interactions under field conditions.

## 1 Introduction

Until recently, the difficulty of working underground has kept the rhizosphere in a scientific state of "out of sight, out of mind." However, our understanding of the rhizosphere biology at the molecular level has advanced dramatically since last decade, thanks in large part to the completion of the Arabidopsis genome and the experimental tools and resources that have resulted from this key event. Rhizosphere processes are driven mainly by photosynthetically fixed carbon which is either directly transferred to symbionts or released as root exudates and is considered as a major factor in regulating soil microbiota. Soil microbiota regulates carbon storage via mineralization and immobilization of soil organic carbon (Paterson et al. 1997) and in terrestrial ecosystems the mineralization is not only due to the activity of microorganisms. The continuous supply of carbon compounds from plant roots engages complex interactions among rhizosphere organisms including interactions between microbes and plants, among microbes, between microfauna and microbes, between animals and plants, among animals, and among plants. Rhizosphere ecological interactions are broadly classified into two types: indirect ecological interactions and direct ecological interactions. Interactions between organisms that involve physical contact are considered direct ecological interactions and indirect ecological interactions include any mechanism of interaction between organisms mediated by a number of steps, where one organism affects another one without direct contact (Strauss 1991). These types of indirect interactions that occur in the rhizosphere can be grouped by considering the nature of the interacting organisms as plant–plant, plant–microbe, microbe– microbe, microbe–fauna, plant–fauna, etc., which are mediated by their secretions or mediator species. A large body of literature exists about rhizosphere interactions (Badri and Vivanco 2009; Badri et al. 2009; Bais et al. 2004, 2006, 2008; Bertin et al. 2003; Lambers et al. 2009; Prithiviraj et al. 2007). In this chapter, we focus on the current knowledge of the indirect ecological interactions mediated by their secretions.

## 2 Root-Secreted Components

For the last decade the field of rhizosphere biology has discovered the importance of root exudates in mediating complex rhizospheric interactions (Bais et al. 2004; Broeckling et al. 2008; Walker et al. 2003; Weir et al. 2004). Root exudation is part of the rhizodeposition process, which is a major source of soil organic carbon

released by plant roots (Hütsch et al. 2002; Nguyen 2003). The quantity and quality of the compounds secreted by the roots depends on the plant species, the physiological stage of the plant, presence or absence of neighbors, plant nutritional status, mechanical impedance, sorption characteristics of the soil, and the microbial activity in the rhizosphere. Typically root exudation clearly represents a significant carbon cost to the plant (Marschner 1995) and with young seedlings typically exuding about 30–40% of their fixed carbon as root exudates (Lynch and Whipps 1990). Root exudates encompass ions (i.e., $H^+$), inorganic acids, oxygen, and water but mainly consist of carbon-based containing compounds (Bais et al. 2006; Uren 2000). These carbon-containing compounds can often be separated into two classes of compounds: low-molecular-weight compounds, which include amino acids, organic acids, sugars, phenolics, secondary metabolites, and volatile compounds such as terpenoids, and high-molecular-weight compounds, which include mucilage and proteins. The classes of compounds secreted by roots are listed in Table 1.

**Table 1** Classes of compounds released in plant root exudates

| Class of compounds | Single components[a] |
|---|---|
| Carbohydrates | Arabinose, glucose, galactose, fructose, sucrose, pentose, rhamnose, raffinose, ribose, xylose and mannitol, alanine, and threonine |
| Amino acids | All 20 proteinogenic amino acids, L-hydroxyproline, homoserine, mugineic acid, and aminobutyric acid |
| Organic acids | Acetic acid, succinic acid, L-aspartic acid, malic acid, L-glutamic acid, salicylic acid, shikimic acid, isocitric acid, chorismic acid, sinapic acid, caffeic acid, *p*-hydroxybenzoic acid, gallic acid, tartaric acid, ferulic acid, protocatacheuic acid, and *p*-coumaric acid |
| Flavonols | Naringenin, kaempferol, quercitin, myricetin, naringin, rutin, genistein, strigolactone, and their substitutes with sugars |
| Lignins | Catechol, benzoic acid, nicotinic acid, phloroglucinol, cinnamic acid, gallic acid, ferulic acid, syringic acid, sinapoyl aldehyde, chlorogenic acid, coumaric acid, vanillin, sinapyl alcohol, quinic acid, and pyroglutamic acid |
| Coumarins | Umbelliferone |
| Aurones | Benzyl aurones synapates and sinapoyl choline |
| Glucosinolates | Cyclobrassinone, desuphoguconapin, deslphoprogoitrin, desulphonapoleiferin, and desulphoglucoalyssin |
| Anthocyanins | Cyanidin, delphinidin, pelargonidin, and their substitutes with sugar molecules |
| Indole compounds | Indole-3-acetic acid, brassitin, sinalexin, brassilexin, methyl indole carboxylate, and camalexin glucoside |
| Fatty acids | Linoleic acid, oleic acid, palmitic acid, and stearic acid |
| Sterols | Campestrol, sitosterol, and stigmasterol |
| Allomones | Jugulone, sorgoleone, 5,7,4′-trihydroxy-3′, 5′-dimethoxyflavone, DIMBOA, and DIBOA |
| Proteins and enzymes | PR proteins, lectins, proteases, acid phosphatases, peroxidases, hydrolases, and lipase |
| Volatile organic compounds (VOCs) | Carbon dioxide, ethanol, methanol, acetone, acetaldehyde, β-phellanderene, 1,8-cineol, and longifolene |

[a]List of compounds presented in this table are mostly reported from model plant Arabidopsis (see Narasimhan et al. 2003) and this list is not complete. This table was adopted and modified from Badri and Vivanco (2009)

## 3 Plant–Plant Interactions by Root-Secreted Phytochemicals

Plants are sessile and therefore cannot move in response to biotic or abiotic attack. However, they respond to these attacks by releasing a mixture of chemical compounds. Communication between plants has not been studied in detail. The best known example is the communication mediated by volatile compound methyl salicylate (Shulaev et al. 1997). Plant hormones, such as ethylene and jasmonic acid, play an indispensable role in mediating plant–plant communication, and plant communication with other organisms (Lou et al. 2005; Ruther and Kleier 2005). Similarly, below-ground plant communication is orchestrated by roots through secreting phytochemicals and emitting volatiles. The most widely studied below-ground chemical mediated plant–plant interference is called allelopathy by which plants gain an advantage over their neighbors by using interfering chemicals called as allamones. Plant produced allamones vary considerably in structure, mode of action, and their effect on plants. Different compounds in root exudates affect metabolite production, respiration, photosynthesis, membrane transport, and inhibition of root and shoot growth in susceptible plants (Einhelling 1995; Weir et al. 2004). For example, a potent allamone juglone produced by black walnut (*Juglans nigra*) plants act as an electron donor and acceptor in photosynthesis and respiration reactions, affecting these processes in susceptible plants (Jose and Gillespie 1998). Recently, a flavonoid called catechin was identified in the root exudates of *Centaurea maculosa*, an invasive spotted knapweed exhibits a strong inhibitory effect on a number of plant species (Bais et al. 2003; Weir et al. 2003), and considered as a potent factor for its successful invasion in a nonnative range. Root exudates are also playing a big role in establishing associations between parasitic plants and their hosts. There are several examples that demonstrate the chemical cross talk to establish the parasitic association, including *Striga* spp. and *Orobanche* spp. (Palmer et al. 2004). Very recently, a root-secreted allelochemical identified as gallic acid from the roots of the noxious weed *Phragmites australis* which is considered a potent factor for its successful invasion in marsh and wetland communities by displacing the native species was identified (Rudrappa et al. 2007).

Besides these negative interactions, root exudates can also have positive effects in plant–plant interactions. However, these positive interactions are less frequently reported. The best studied interaction is the root exudates that induce herbivore resistance in neighboring plants. For example, when *Hordeum vulgare* (barley) plants were treated with *Elytrigia repens* (couch-grass) root exudates or the phytotoxic compound identified from *E. repens* exudates called carboline, *H. vulgare* were chosen less by aphids than the control (Glinwood et al. 2003). Besides, having direct effect on herbivore behavior, root exudates have an indirect effect by inducing defense responses in neighboring plants resulting in reduced herbivore populations indirectly by attracting predators and parasites of the offending herbivore (Du et al. 1998; Guerrieri et al. 2002).

Root exudates also play a role in soil nutrient availability by altering soil chemistry and soil biological processes (Hopkins et al. 1998). Certain compounds

such as phytosiderophores, mugineic acid, and malate improve iron availability (Dakora and Phillips 2002; Fan et al. 2001). Roots secrete a range of chemicals including the secretion of organic acids and acid phosphatases and the production of proteoid roots to survive in P-deficient soils (Ascencio 1997; Raghothama 1999). For example, several plants including *Lupinus alba, Brassica napus*, and *Medicago sativa* increase the release of organic acids in P-deficient soils (Hoffland et al. 1992; Johnson et al. 1994; Lipton et al. 1987).

### *3.1 Plant–Plant Interactions Mediated by Root-Emitting Volatiles*

Many interactions between organisms are based on the emission and perception of volatiles. These volatiles act as communication signals for chemoattractant or repellent for species-specific interactions or mediators for cell-to-cell recognition. These volatiles do not only function as signals in the above-ground interactions, but below-ground volatile interactions are similarly complex. The majority of volatile organic compounds (VOCs) tend to be lipophilic, small in molecular mass (less than 300 Da), and have a high vapor pressure (0.01 kPa or higher at 20°C). Most of the volatile compounds belong to the following three chemical groups: terpenoids, phenylpropanoids, or fatty acid derivatives. Unlike, the root-secreted phytochemicals, volatiles can travel long distances in the atmosphere and also in the soil by permeating through air-filled pores. The efficiency of volatile penetration in the soil depends on the type of mineral, texture, and particle architecture (Aochi and Farmer 2005). Also, different VOCs exchange rates indicate that soils have the potential to act as VOC sinks rather than VOC sources (Asensio et al. 2007). Volatiles emitted in the underground enable plants to influence directly or indirectly the community of soil-dwelling organisms and combat competitive plant species (Nardi et al. 2000). Several studies demonstrated that the emission of terpenoids by plant roots and particularly obvious in forest soils (Hayward et al. 2001; Lin et al. 2007; Rohloff 2002). Furthermore, a blend of unidentified root volatiles of *Echinacea angustifolia* showed allelopathic effect on different plant species such as *Lactuca sativa, Panicum virgatum*, and *Sporobolus heterolepis* (Viles and Reese 1996).

## 4 Plant–Microbe Interactions Mediated by Root-Secreting Phytochemicals

The rhizosphere soil surrounded by plant roots is more abundant in microbes than the nonrhizosphere soils (Bending 2003; Lynch 1987; Rouatt and Katznelson 1960; Rouatt et al. 1960). However, more recently the term “rhizosphere” has broadened to include both the volume of soil influenced by the root and the root tissues

colonized by microorganisms (Pinton et al. 2001). Microorganisms in the rhizosphere react to the many metabolites secreted by plant roots. The microorganisms and their products also interact with plant roots or root-secreting compounds in a variety of positive, negative, and neutral ways. The positive interactions include classic symbioses, association with biocontrol agents, epiphytes, and mycorrhizal fungi. The negative interactions include association with parasitic plants, pathogenic bacteria, fungi, and invertebrate herbivores. Colonization and dominance of specific microbe species in the rhizosphere is very critical for pathogenic soil microbes and also important in the application of beneficial microorganisms for plant protective purposes. Although a general increase in microbes in the rhizosphere is always noted, the community structure and functional consequences of this increase are poorly understood.

The well-known classical example for positive plant–microbe interaction is the interaction between legume roots and *Rhizobia* bacteria, which are capable of forming dinitrogen-fixing nodules in the roots of legumes. However, in this chapter we intended to focus only on the nonlegumes–microbes interactions because legumes–microbe interactions are discussed as a separate chapter in this book. Similarly to *Rhizobia*, arbuscular mycorrhizal fungi (AMF) and plant roots form associations in more than 80% of terrestrial plants. Mycorrhizal fungus and bacterial rhizobial associations are thought to derive from a common-ancestral plant–microbe interaction likely of fungal origin and it was demonstrated that the activity of some host proteins regulates both mycorrhizal and rhizobial associations (Lévy et al. 2004). Root exudates play a role in the recognition of mycorrhizal fungi with the compatible host plant (Nagahashi and Douds 1999; Tamasloukht et al. 2003). Although root exudates have long been suspected to play a communicative role in mycorrhizal associations, the identification of specific molecule interactions from AMF and host still remains elusive. Recently, a sesquiterpene called strigolactone 5-deoxystrigol was identified in the root exudates of *Lotus japonicus* which is responsible for inducing AMF hyphal branching in germinating spores (Akiyama et al. 2005). In the presence of AMF symbiosis, plants trade carbon with phosphate from their fungal partners (Harrison 2005; Karandashov and Bucher 2005; Paszkowski 2006). Molecular data and fossil studies suggest that AMF have facilitated the adaptation and evolution of primitive plant species to life on land demonstrating more than 400 million years of coevolution which shows that plants and AMF are highly interdependent (Remy et al. 1994; Simon et al. 1993). Although less understood, similar processes are thought to control symbioses between nitrogen-fixing *Frankia* bacteria and their actinorhizal plant hosts (Wall 2000). Recently, a leucine-rich-repeat receptor kinase (SymRK) was identified as a common genetic basis for plant root endosymbioses with AMF, *Rhizobia*, and *Frankia* bacteria (Gherbi et al. 2008).

Soil microbes can also modulate the secretion of root exudates to positively regulate plant growth and disease resistance by indirect mechanisms. Plant growth promoting rhizobacteria (PGPR) have been found to positively influence plants through a wide variety of direct and indirect mechanisms (Gray and Smith 2005). Bacteria are likely to locate plant roots through cues extended from the root and the

carbohydrates and amino acids stimulate PGPR chemotaxis on root surfaces (Somers et al. 2004). A very recent report demonstrated that the rhizobacterial elicitor acetoin from *Bacillus subtilis* induces systemic resistance in Arabidopsis to reduce plant's susceptibility by pathogen attack (Rudrappa et al. 2010).

Several negative interactions are mediated by root exudates including antimicrobial, biofilm inhibitors, and quorum-sensing mimics to prevent soil-borne pathogens (Bais et al. 2006, 2008). Plants are known to use diverse chemical molecules for defense, although some groups of compounds (phenylpropanoids) are used for defensive function across taxa (Bouarab et al. 2002). Recent years of research have clarified the antimicrobial properties of root exudates. For example, rosamarinic acid from hairy root cultures of sweet basil (*Ocimum basilicum*), pigmented napthoquinones from *Lithospermum erythrorhizon* hairy root cultures, and aromatic phenolic compounds from the exudates of *Gladiolus* spp. have shown potent antimicrobial activity against an array of soil-borne pathogens (Bais et al. 2002; Brigham et al. 1999; Taddei et al. 2002).

Plant–microbe interactions in the rhizosphere are responsible for a number of intrinsic processes such as carbon sequestration, ecosystem functioning, and nutrient cycling (Singh et al. 2004). A great variety of biotic and abiotic factors shape soil and plants associated habitats, as well as modify the composition and activities of their microbial communities (Bever et al. 1997). Bacterial communities in root-associated habitats respond specifically with respect to density and composition of root exudates, eventually yielding plant species-specific microfloras which may also vary depending upon the plant developmental stage (Mahaffee and Kloepper 1997; Wieland et al. 2001; Yang and Crowley 2000). Recent evidence suggests that specific plant species are responsible for driving their own soil fungal community composition and diversity mediated by root-secreting compounds (Broeckling et al. 2008). In addition, a recent report demonstrated that a mutation in the ABC transporter (*At*PDR2) dramatically changes the composition of root-secreted phytochemicals which influenced the qualitative and quantitative changes in the Arabidopsis native soil microbiota by culturing more beneficial microbes compared to the wild type (Badri et al. 2009). Plant root exudates also affect the level of contamination found in soil and groundwater from various environmental pollutants by a process called "rhizoremediation." For example, *Pseudomonas putida* from the rhizosphere of corn and wheat helps to effectively decontaminate 3-methylbenzoate and 2,4-D, respectively (Kingsley et al. 1994; Ronchel and Ramos 2001). Several lines of evidences demonstrated the role of root exudates in metal remediation either directly by solubilizing the metals by root-secreting organic acids or indirectly with the help of soil microbes (Do Nascimento and Xing 2006).

## *4.1 Plant–Microbe Interactions Influenced by Root Volatiles*

The microbial community in the rhizosphere is limited by carbon availability but carbon-containing root volatiles especially monoterpenes contribute significantly to

the below-ground carbon cycle (Owen et al. 2007). Becard and Piche (1989) first demonstrated that the carbon dioxide is a crucial root volatile that stimulates hyphal growth of vesicular–arbuscular mycorrhizal (VAM) fungus. They also showed that elevated carbon dioxide promotes hyphal length of VAM and depresses the growth of non-VAM at low nitrogen availability. In addition, Scher et al. (1985) reported that *Pseudomonas fluorescens* was attracted by carbon dioxide. Besides carbon dioxide, there are other simple compounds such as acetaldehyde, acetone, acetic acid, ethanol, and methanol emitted by Arabidopsis roots (Steeghs et al. 2004). Root volatiles are important for defense response against microbial populations. For example, the root volatile β-phellanderene was effective against the root fungal pathogen *Fomes annosus* (Cobb et al. 1968), which was emitted from the roots of *Smyrnium olusatrum* (Bertoli et al. 2004) and *Rhodiola rosea* (Rohloff 2002).

Similarly, the monoterpene 1,8-cineol emitted from Arabidopsis roots in response to pathogen infection (Steeghs et al. 2004) had antimicrobial property against several microbes (Kalemba et al. 2002; Vilela et al. 2009). Besides the role of volatiles in plant defense, they also influence in symbiotic association either positively or negatively. For instance, the sesquiterpene longifolene from the roots of *Pinus sylvestris* inhibits the vegetative growth of mycorrhizal fungus *Boletus varigatus* and *Rhizopogon roseolus* (Melin and Krupa 1971).

## 5 Plant–Faunal Interactions

Protozoa and microbial feeding nematodes are known to be the most important grazers of the microflora in the terrestrial ecosystems (Ingham et al. 1985). Thus, grazing of the microflora by microbivores is considered as a critical mechanism to maintain the balance in the competition between microbes and plants. Despite the critical importance of interactions between roots (root exudates), microbes, and their predators for plant growth, knowledge of these interactions is still fragmentary and the mechanisms are poorly understood (Zwart et al. 1994). The outcome of the rhizosphere plant–faunal, plant–microbial, and faunal–microflora interactions may be either positive (e.g. mutualistic and associative) or negative (predatory and competitive) (Bonkowski et al. 2000). Much attention has been drawn only to the negative plant–faunal interactions (parasitic nematodes) (Curtis 2008). It should also be noted that root-feeding by nematodes may increase allocation of carbon below-ground and increase significantly the leaking of carbon from roots that stimulate rhizosphere microbial processes (Bardgett et al. 1998). Although most nematodes are free-living organisms that consume bacteria, there are some nematodes that are pathogenic for plants causing important economic losses each year (Barker and Koenning 1998). Some of the most harmful plant–parasitic nematodes include root-knot and cyst nematodes such as *Meloidogyne* spp., *Heterodera* spp., and *Globodera* spp. (Chitwood 2003).

During coevolution with the host plant, parasitic nematodes have developed the capacity to recognize and respond to the chemical signals of particular host species.

Understanding the complexity of the chemical signal exchange and response during the early stage of host–parasite interactions is important to identify the critical steps in the parasite life cycle to disrupt the host–nematode recognition. Plant signals are indispensible for nematodes to locate hosts and feeding sites (Robinson et al. 1987). However, the identities of the plant signals involved in the key stages of the plant–nematode interactions are not yet clearly dissected. Chemical components of root exudates may deter one organism while attracting another and these compounds alter the nematode behavior by attracting the nematodes to the roots or result in repellence, motility inhibition, or even death (Robinson 2002; Wuyts et al. 2006). For instance, root exudates of cucumber and their fractions having both repellent and attractant activity to *M. incognita* were reported (Castro et al. 1989). Similarly, the root cap exudates that include enzymes, antibiotics, and other soluble chemicals and mucilage repelled both plant parasitic nematodes and free-living nematode *Caenorhabditis elegans* and resulted in reversible state of immobility in these nematodes (Hubbard et al. 2005; Wuyts et al. 2006; Zhao et al. 2000). This study indicates that the root tip delivered products has the potential to temporarily immobilize nematodes.

The best examples describing the role of plant signals in synchronizing host–parasite life cycle are the two species of potato cyst nematodes (*Globodera* spp.), as these nematodes are completely dependent on root exudates for hatching. Several hatching factors have been identified in crop plants to explore the potential of using these compounds for agrochemical use (Devine and Jones 2001; Timmermans et al. 2007). For example, *Solanum sisymbriifolium* is being used successfully as a trap crop for potato cyst nematodes in Europe, because the plant root exudates stimulate hatching of the second-stage juveniles but does not support their development to complete their life cycle (Timmermans et al. 2007). Mostly, the root exudates act as attractants for nematodes to move closer to individual host roots; these are considered "short distance attractants." However there are "long-distance attractants" that enable the nematodes to locate roots. So far, only carbon dioxide has been identified as a prime long-distance attractant to nematodes including *M. incognita* (Robinson 2002). Other additional short-distance attractants such as amino acids, sugars, and metabolites are also reported (Bird 1959; Perry 2001; Robinson 2002). In addition, plant roots also produce allelochemicals to defend other plant species or soil-borne pathogens, which have been shown to function as nematodes antagonists (Guerena 2006). For example, cucurbitacin A from cucumber plants repels nematodes and β-terthienyl from *Tagetes erecta* acts as repellent as well as nematotoxic (Castro et al. 1989). Other compounds such as cyclic hydroxamic acid from maize root exudates affect the behavior of *M. incognita, Pratylenchus zea*, and *Xiphinema americanum* (Friebe et al. 1998; Zasada et al. 2005). Root exudate compounds not only induce nematode hatching, attraction, and repellence, but also induce the exploratory behavior of nematodes including stylet thrusting and aggregation and increase in nematode mobility (Curtis 2007; Robinson 2002). In addition, root exudate compounds and phytohormones such as IAA induce the changes in the surface cuticle of nematodes (Curtis 2008). The surface changes in the cuticle might allow the nematodes (*M. incognita*) to adapt and

survive plant defense responses (Curtis 2007). Identifying the signaling and perception process executed by root exudate compounds occurring in plant–nematode interactions will reveal targets for chemical or genetic intervention.

Besides the role of root exudates compounds in attracting and repelling nematodes, nematodes also respond to the host by secreting specific proteins to complete their life cycle within the host. The way nematodes secrete proteins is mainly through their stylet, a hollow, protrusible spear at the anterior of the worm (Davis et al. 2000). The stylet secretions are studied widely for their role in host penetration, feeding site induction, and disease induction (Hussey 1989). The first analysis of the stylet-secreted proteins was realized in one-dimensional electrophoresis (Robertson et al. 1999; Veech et al. 1987). However, with the modern techniques of proteomics, knowledge of the nematode secretome has been extended (Bellafiore et al. 2008).

In the secretome of *M. incognita* were identified 486 proteins mainly required for invasion, immune suppression, and host cell reprogramming (Bellafiore et al. 2008). In another proteomic study on *M. incognita*, calreticulin a calcium-binding protein was identified as the most abundant stylet-secreted protein (Jaubert et al. 2002). Calreticulin has already been studied for its role in many host–parasite interactions (Nakhasi et al. 1998; Pritchard et al. 1999). In plants, calreticulin has been involved in cell-to-cell trafficking and pressure support (Baluska et al. 2001; Sivaguru et al. 2000). The fact that nematode protein homologues were found in plants indicates that nematodes could manipulate plant cell functions during the compatible interaction (Caillaud et al. 2008) to elude the defense plant response. Most of the nematode proteins secreted in the root–nematode interaction have been found in the first 18 h of infection being some of these glycoproteins (Veech et al. 1987) proteases and superoxide dismutases (Robertson et al. 1999).

Some of the secreted proteins have been determined to have important roles in parasitism (Davis et al. 2000; De Meutter et al. 1984; Popeijus et al. 2000). Enzymes such as β-1,4-endoglucanases, cellulases, pectate lyase, and polygalacturonase are likely to be used by nematodes in softening the cell wall in order to facilitate their movement through the root (Davis et al. 2000; Doyle and Lambert 2002; Goellner et al. 2000; Popeijus et al. 2000; Smant et al. 1998). Mawuenyega et al. (2003) found, by two-dimensional liquid chromatography (2DLC) coupled with electrospray ionization (ESI) tandem mass spectrometry (MS/MS), 110 secreted/targeted proteins and 242 transmembrane proteins. Also, it was found that many peptides of these proteins have *N*-terminal glycosylation and phosphorylation, which suggest the importance of posttranslational modification for recognition and infection. Depending on the modification that the protein has, it would have critical effects on cell regulatory and signaling processes (Mann and Jensen 2003). For instance, it was found in a human–filanial nematode parasite *Acanthocheilonema viteae* that the covalent attachment of phosphorylcholine to a major secreted protein named ES-62 is likely involved in the interference of the host immune system (Houston et al. 1997). This effect may also happen in plant nematodes that secrete numerous posttranslational modified proteins (Bellafiore et al. 2008; Caillaud et al. 2008; Mawuenyega et al. 2003).

Reports are also available in the model plant *A. thaliana* response to the nematodes. Huang et al. (2006) found that the root-knot nematode *M. incognita* secretes a peptide named 16D10 that interacts with the plant SCARECROW-like transcription factor; this peptide–protein interaction probably represents an early signaling event in the plant–nematode interaction. Some nematodes induce the well-known salicylic acid-related defense response during incompatible interactions. The fact that salicylic acid inhibits the parasitism of *H. schachtii* by inducing the expression of PRP genes in Arabidopsis roots (Wubben et al. 2008) suggests that pathogenesis-related proteins play a role in signaling and perception process in the host–nematode interactions.

## 6 Plant-Root-Secreting Proteins Involve in Neighbors Interaction

Studies on proteins involved in root–microorganism interaction have provided strong evidence about the importance of root-secreted proteins during the recognition between pathogenic and nonpathogenic interactions. One of the most studied common proteins found in root exudates are lectins (De-la-Peña et al. 2008; Wen et al. 2007). Lectins are a diverse group of carbohydrate binding proteins that are found in dual systems, functioning in defense with some pathogens, and in recognition of a compatible symbiosis (De Hoff et al. 2009; Sharon and Lis 2004). De Hoff et al. (2009) illustrated a hypothetical model of the perception of pathogenic and symbiotic bacteria where the lectin gradient is secreted from the root to permit the growth of symbiotic bacteria and agglutination of the pathogenic bacteria. However, those bacteria that elude the first line of the plant defense can be recognized by specific receptors in the root triggering a cascade of MAP kinase signaling leading to antipathogen response or prosymbiotic response depending on the microbe that is in contact with the root. Another set of proteins found highly secreted in the rhizosphere are the PRP. PRP, such as chitinases, osmotin, and thaumatin-like proteins, have been found in root exudates under pathogen contact as well as secreted constitutively (Basu et al. 2006; De-la-Peña et al. 2008; Nóbrega et al. 2005). Root-secreted proteins are not only important for defense, but also for attracting microbes to the roots, a process known as chemotaxis (Currier and Strobel 1977). Chemotaxis is one of the earliest essential events in the interaction between plants and bacteria (Hawes and Smith 1989; Manson 1990). Proteins in the rhizosphere are so important for chemotaxis that even a glycoprotein, named trefoil chemotactin, from *Lotus corniculatus* has been identified (Currier and Strobel 1977, 1981). After that, other secreted proteins, able to recognize bacterial surface carbohydrate structures, which help to adhere to root hairs of many plants, have been identified in Rhizobium (Ausmees et al. 2001).

In order to colonize the roots, bacteria usually congregate together by using of quorum-sensing (QS) signals. The most common type of QS signal in proteobacteria are the *N*-acyl-homoserine lactones (AHL) (von Bodman et al. 2003), which affect the expression of more than 600 genes in bacteria (Schuster et al. 2003). Biofilm-forming bacteria is a dense population that perform many biological responses as community, including production of extracellular polysaccharides, degradative enzymes, antibiotics, Hrp protein secretion, Ti plasmid transfer, and other functions (von Bodman et al. 2003). QS stimulates the production of extracellular enzymes which has been related to pathogenesis in *P. aeruginosa* PAO1 (Passador et al. 1993), *P. fluorescens* (Worm et al. 2000), and *Aeromonas hydrophila* (Swift et al. 1999). More recent evidence suggests that QS-related enzymes such as chitinases and proteases could be involved in nitrogen mineralization process instead of pathogenesis (DeAngelis et al. 2008). On the other hand, *M. truncatula* roots are able to detect low concentrations of bacterial QS signals from the pathogenic bacterium *P. aeruginosa* by change and accumulation of 154 proteins, from which 21 are related to defense and stress responses (Mathesius et al. 2003). Based on these information one can easily predict that considerable percentage of root-secreted proteins function in the rhizosphere still remains elusive.

Studies on the secretome of Gram-positive bacteria *B. subtilis* started 10 years ago (Hirose et al. 2000; Tjalsma et al. 2000) revealed nearly 300 possible proteins secreted into the soil, among them more than a half have not been yet identified (Antelmann et al. 2006). The secretome analysis of pathogenic bacteria is very important in revealing new virulence proteins (Desvaux and Hébraud 1978; Kaffarnik et al. 2009; Kazemi-Pour et al. 2004; Saarilahti et al. 1992; Watt and Wilke 2005). For instance, Kazemi-Pour et al. (2004) analyzing the secretome of *Erwinia chrysanthemi*, a well-known plant pathogenic bacterium, found proteins related to virulence, disease symptoms, and pathogenicity: Avr-like protein, elongation factor EF-Tu, flagellin, pectate lyases, and metalloproteases. Furthermore, *E. chrysanthemi* and *E. carotovora* secrete proteases, polygalacturonase, and proteins that degrade plant cell walls, such as pectin lyase and cellulose (Collmer and Keen 1986; Perombelon and Kelman 1980). Polyglacturonases are considered to be key enzymes involved in pathogenesis (Palomski and Saarilahti 1997). Another secretome study on *Xanthomonas campestris* revealed 97 proteins; some of these are involved in element acquisition, protein maintenance and folding, compound degradation, and proteins with unknown functions (Watt and Wilke 2005). It is worth noting that in both bacteria, *E. chrysanthemi* and *X. campestris*, there are some shared secreted proteins that could be linked with pathogenicity. *E. chrysanthemi* (Kazemi-Pour et al. 2004) secreted an elongation factor, a chaperonin GroEL, flagellin, and celluloses that also were found in the secretome of *X. campestris* (Watt and Wilke 2005). Furthermore, the secretion of these proteins has found to increase in the presence of plants and plant compounds (De-la-Peña et al. 2008; Kazemi-Pour et al. 2004).

As far as root–fungus interactions are concerned, several secretomes have been annotated (Choi et al. 2010), being these from symbiotic to pathogenic, proteomics

have been applied to complement the genomics analysis of the fungal secretome (Phalip et al. 2005). Pathogenic fungus in comparison to symbiotic mycorrhizal fungi represents serious damage and loss to agriculture. Furthermore, some fungi such as *Trichoderma* spp. represent a group of fungi that have been used as plant disease control against a wide diversity of phytopathogenic fungi and even they have positive effect on plants as a plant growth enhancer (Harman and Björkman 1998; Harman et al. 2004; Yedidia et al. 2003). This mycoparasitic activity has been attributed to the secretion of complex mixture of hydrolytic enzymes such as chitinases, glucanases, and proteases able to degrade different cell wall systems (Benítez et al. 1978; Suárez et al. 2005; Szekeres et al. 2004). The secretome of *T. harzianum* revealed to vary both qualitatively and quantitatively on different ascomycetes, oomycetes, and basidiomycetes cell walls (Suárez et al. 2005).

In pathogenic fungi the most important molecules that promote the infection process are the extracellular effectors that produce the elicitation of plant defense responses (Birch et al. 2006; Colditz et al. 2004; Dean et al. 2005; Hahn 1996; Rose et al. 2002). These effectors have been found in the secretome of different oomycetes (Kamoun 2006). Among the secretomes studied on plant pathogenic fungi are those from *Fusarium graminearum*, and *Sclerotinia sclerotiorum* (Phalip et al. 2005; Yajima and Kav 2006). In *F. graminearum* secretome, in the presence of *Humulus lupulus* L. cell wall, were identified 84 proteins (Phalip et al. 2005) which 45% of them are actually involved in cell wall degradation and the most abundant proteins were cellulases, endoglucanases, proteases, and chitinases. In *S. sclerotiorum* were identified 18 secreted proteins from the liquid culture of this fungus (Yajima and Kav 2006) where L-arabinofuranosidase was one of the most abundant in the secretome but not in the mycelia. This protein is much known for its function in the virulence process of this fungus. The study of secreted proteins not only in pathogens alone, but also in the presence of the host, should be persuaded in order to know the principal signals involved between fungal and roots interaction. Some of these proteins could lead to the identification of new effector proteins produced by fungi and defense-related proteins produced by roots specific-secreted to a given fungus.

The way plants and pathogenic fungi cross talk is very dynamic and complex, and it is not known which one of the organisms emits the first signal. Fungus as much as plants turn on their genetic and biochemical systems to generate a series of signals to invade or defend (Nürnberger and Brunner 2002). In the case of fungus, once contact with the plant host root has been established, elicitors start to be produced and secreted by the fungus (Nürnberger and Brunner 2002). These elicitors are perceived by the plant and plant-specific proteins rapidly phosphorylated in response to fungus signals (Peck et al. 2001). Dietrich et al. (1990) and after Felix et al. (1991) found that protein kinase-mediated phosphorylation might be the first trigger immediately after the fungal is perceived by the plant cells. Posttranslational modifications by phosphorylation/dephosphorylation in the signal transduction cascade produced by pathogens have been studied in other plants as an early plant defense response (Grant and Mansfield 1999; Stone and Walker 1995). The possible existence of an extracellular phosphorylation network (Ndimba et al.

2003) has opened the possibility to investigate the plant–microbe interaction signaling in the rhizosphere mediated by kinases and phosphatases.

The principal way that roots combat the fungal invasion is through enzymes such as PRP (Colditz et al. 2004; Fagoaga et al. 2001), which some have been seen to be effective in repressing the growth of root pathogenic fungi (Nóbrega et al. 2005). For instance, the root protein profile of *M. truncatula* infected with the pathogen *Aphanomyces euteiches* (Colditz et al. 2004) showed an induction of proteins belonged to the family of class 10 of PRP (PR10). Because the fungal cell walls are build with chitin and β-glucans, proteins belonging to the family PR2 such as β-1,3-endoglucanases and family PR-3, -4, -5, and -11 such as several types of endo-chitinases are effective to inhibit the growth of fungi while depolymerizing poly-saccharides of mycelia walls and disturbing intracellular targets (Abad et al. 1996; Ferreira et al. 1984; Joosten and De Wit 1989; Li et al. 2000). PRPO have been studied extensively and they are found in both pathogenic and nonpathogenic interactions. This is the case of osmotin, a PR5 protein found in the root exudates of alfalfa inoculated with *Sinorhizobium meliloti* (De-la-Peña et al. 2008), which also participates in plasma membrane permeabilization, which is associated with patho-genic fungal spore lysis (Abad et al. 1996). This specificity is very important for the plant in order to avoid the killing of beneficial microbes or the free invasion of pathogenic organisms. How the plants provoke such specificity is a research that needs to be persuaded. Although mycorrhiza are beneficial fungi for plants, the induction of plant-defense-related genes still takes place at early stages of the interaction (Gianinazzi-Pearson et al. 1996; Harrison 1999, 2005). During the earlier stages of the development of the VAM symbiosis association between *Allium porrum* L. and *Glomus versiforme,* the root chitinase activity was almost twice as high as in uninfected roots (Spanu et al. 1989). However, once the symbiosis was fully established, the chitinase activity in mycorrhizal roots was even lower than in the control roots. The possible explanation for this observation is that at the earliest stages of the interaction with the fungus, roots respond with a defense response. However, once the symbiotic interaction is established, the fungus is able to suppress the plant stress reaction and grow inside the roots.

## 7 Methods for Studying Rhizosphere Interactions

The rhizosphere is complex; quantitatively a single gram of soil has over 10,000 distinct microbes (Kent and Triplett 2002). The traditional culture-based techniques are inadequate to study the actual goings-on of the microbes of the interest because most of the rhizosphere organisms are unculturable (Kent and Triplett 2002). Novel approaches are needed to probe this complex environment and recently a broad range of techniques and strategies have been used to study the rhizosphere interactions. The increasing applications of molecular techniques will provide a basis for studying rhizosphere interactions at broad-scale (community level) to

fine-scale (species level) investigations. Previously, Biolog has been used to characterize the differences in microbial communities between contrasting habitat and soil types (Zak et al. 1994). The Biolog assay uses microtiter plates consisting of 96 wells containing separate sole carbon sources and a redox indicator dye, which produce patterns of potential carbon utilization for microbial communities. However, this method is completely dependent on the growth of microbial population in artificial media and also biased toward faster growing microbes (Paterson et al. 1997). To overcome this problem, phospholipid fatty acid analysis (PLFA) has been used to analyze the microbial population at community level and this method is totally culture-independent analyses and provides the broad number of bacterial taxes present in the samples (Zelles 1997). The combination of Biolog and PLFA techniques has shown differences in the microbial community composition of bulk and rhizosphere soils (Söderberg et al. 2004), but these two methods cannot identify certain microbial species at community level. Later, polymerase chain reaction (PCR) amplification of rDNA genes combined with fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment analyses (T-RFLP), and amplified rDNA restriction analysis (ARDRA) is proven to study the species composition of whole communities in detail (Nicol et al. 2003; Torsvik and Ovreás 2002). In addition, development of novel methods such as fluorescence in situ hybridization (FISH) and microautoradiography allows to determine the phylogenetic identification of uncultured bacteria in natural environments by using fluorescence-specific phylogenetic probes (targeting rRNA) and fluorescence microscopy to detect and quantify the active population utilizing a specific substrate (Gray et al. 2000). The recent development of metagenomics coupled with bioinformatics will allow studying the genomics analyses of uncultured microbes in the rhizosphere (Rondon et al. 2000), but it does not make any sense to gather data on every microbe present in the rhizosphere instead of the organisms of interest which are actively interacting in the rhizosphere. The discovery of an elegant technique called stable isotope probing (SIP) allows studying the organisms of interest which are actively interacting in the rhizosphere with the root exudates. In this technique, plants are exposed to $^{13}CO_2$, which has a heavier carbon atom than regular $CO_2$, metabolized by the plant, and deposited in the rhizosphere through rhizodeposition and utilized by the microbes present in the rhizosphere. The nucleic acids of the microbes utilizing the $^{13}CO_2$ will be heavier than the noninteracting microbes and analyzed using density gradient centrifugation (Kiely et al. 2006) and also yield the entire genome of all the participating microbes in the rhizosphere (Singh et al. 2004). In addition, the recent development of "omics" technologies coupled with bioinformatics studies is appropriate to study the rhizospheric soil microbe's interactions at community levels to species level. The "omics" techniques such as transcriptomics, proteomics, and metabolomics allow studying the microbial interactions in a given environment as a part of functional genomics. The recent development of next-generation sequencing methods will complement these "omics" techniques to study the rhizospheric microbial interactions in detail to detect and quantify the unculturable microbes that actively participate in the rhizosphere. Finally, the rhizosphere is a complex system and no

single method is sufficient to describe the complex nature of the rhizosphere. Therefore, there exists a need to develop systems approach to describe the complex nature of the rhizosphere. In addition, the multitude of interactions in the rhizosphere requires high-throughput techniques in order that they can be elucidated in a reasonable time frame.

## 8 Concluding Remarks and Future Perspectives

Competition between plants is high because plant growth in the natural environment frequently takes place in dense stands of established vegetation. The complex interactions that take place in the rhizosphere between plants and microbes and their regulation by soil faunal activity may be of fundamental importance for individual plant success but also at community level.

Although there is a large body of literature available to prove the significance of plant–microbes, plant–microfaunal, and microbial–microfaunal interactions to enhance plant growth, but still there is a lacuna on the knowledge of multitropic interactions occurring in the rhizosphere. In addition, most information about important processes in the rhizosphere comes from studies in controlled environments where roots are grown in simple uniform media and organisms of interest are applied. There is a need to understand these rhizospheric multitrophic interactions in the realistic field conditions to improve the plant growth at species and community level. In addition, studies should conduct in the field conditions to understand the rhizospheric complex interactions in monocultures and polycultures. This will help to understand the dynamics of interactions and their outcome in influencing the plant's success when they are in monocultures and in polycultures.

Obviously, studying these complex rhizospheric interactions by employing single method is impossible. However, the combination of techniques and the continuous development of new techniques in the field of rhizosphere biology coupled with systems approach will allow us partly to elucidate these complex interactions under field conditions. For many years, ecologists have viewed soil organisms and plants as relatively independent from each other. But to unravel these complex interactions, further research requires multidisciplinary system approach, which includes involving and exchange of knowledge between plant physiologists, soil scientists, microbiologists, and zoologists with the help of bioinformatics specialists. In addition, the differences in plant growth and plant community composition can only be understood in relation to indirect microbial–faunal, plant–microbial, faunal–plant, and microbial–microbial interactions in the rhizosphere.

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