

Chapter 11

Chemical Signalling in the Arbuscular Mycorrhizal Symbiosis: Biotechnological Applications

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11.1 Introduction

Plants are living organisms that continuously communicate with other organisms, including microorganisms, present in their environment. Unlike animals, plants are sessile organisms that largely rely on chemicals as signalling molecules to perceive and respond to environmental changes. For instance, plants use molecules to recognize potential pathogens and defend themselves as well as to establish mutualistic beneficial associations with certain microorganisms in the rhizosphere. These beneficial associations belowground can affect plant growth and development, change nutrient dynamics, susceptibility to disease, tolerance to heavy metals and can help plants in the degradation of xenobiotics (Morgan et al. 2005). As a result, these plant–microorganism interactions have considerable potential for biotechnological exploitation.

The rhizosphere is the narrow soil zone surrounding plant roots and constitutes a very dynamic environment. It harbours many different microorganisms and is highly influenced by the root and the root exudates (Bais et al. 2006; Badri et al. 2009). Plants produce and exude through the roots a large variety of chemicals including sugars, amino acids, fatty acids, enzymes, plant growth regulators and secondary metabolites into the rhizosphere, some of which are used to communicate with their environment (Siegler 1998; Bertin et al. 2003; Bais et al. 2006). Moreover, the release of root exudates together with decaying plant material provides carbon sources for the heterotrophic soil biota. On the other hand, microbial activity in the rhizosphere affects rooting patterns and the supply of available nutrients to plants, thereby modifying the quantity and quality of root exudates (Barea et al. 2005). Of special interest for rhizosphere communication are the so-called secondary metabolites from root exudates, which received this name

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because of their presumed secondary importance in plant growth and survival (Siegler 1998). These metabolites include compounds from different biosynthetic origins that are of ecological significance because they act as signals in multiple pathogenic and mutualistic plant–microorganism interactions, including the arbuscular mycorrhizal (AM) symbiosis (Bertin et al. 2003; Bais et al. 2006).

11.2 The Arbuscular Mycorrhizal Symbiosis

The AM symbiosis is one of the best known beneficial plant–microorganism associations that take place in the rhizosphere (Barea et al. 2005; Smith and Read 2008; Bonfante and Genre 2010). This interaction is the most widely distributed symbiosis in the world (Parniske 2008; Smith and Read 2008). The AM symbiosis is based on the mutualistic association between certain soil fungi of the monophyletic phylum Glomeromycota and the vast majority of land plants, including most agricultural and horticultural crop species. This association dates back to more than 400 million years and it has been postulated to be a key step in the evolution of terrestrial plants (Smith and Read 2008). AM fungi are obligate biotrophs and depend entirely on the plant to obtain carbon and complete their life cycle. They colonize the root cortex of the host plant and form specialized tree-shaped subcellular structures called arbuscules, which are involved in nutrient exchange between the two symbiotic partners. The AM symbiosis gives rise to the formation of extensive hyphal networks in the soil that facilitate the acquisition of nutrients beyond the area of nutrient depletion, thereby assisting the plant in the acquisition of mineral nutrients (mainly phosphorous) and water (Smith and Read 2008). Therefore, the AM symbiosis positively affects plant growth and enhances the recycling of nutrients (Morgan et al. 2005; Parniske 2008; Bonfante and Genre 2010). Moreover, the AM symbiosis has non-nutritional effects such as the improvement of soil quality by stabilizing soil aggregates and preventing erosion (Gianinazzi et al. 2010). It also provides tolerance against different types of abiotic stresses such as drought, salinity or heavy metals (Smith and Read 2008; Ruiz-Lozano et al. 2012) and enhances the ability of the host plant to cope with biotic stresses (Pozo and Azcón-Aguilar 2007; Parniske 2008; Jung et al. 2012). Altogether, these benefits highlight the “true helper” character of this symbiosis and envisage the potential use of the AM symbiosis as biofertilizers and bioprotection agents for the sustainable management of agricultural ecosystems (Gianinazzi et al. 2010).

11.3 Signalling During the AM Symbiosis

AM symbiosis is a complex and very dynamic interaction which requires a high degree of coordination between the two partners. Symbiosis establishment implies a signal exchange between both partners that leads to mutual recognition and development of symbiotic structures (Siegler 1998; Bonfante and Genre 2010). Noteworthy, this molecular dialogue must be very precise in order to avoid opportunities for malevolent organisms (Bouwmeester et al. 2007). The plant–AM fungus communication occurs beyond the early stages of the interaction as it is necessary for the proper maintenance and functioning of the symbiosis.

11.3.1 *The Pre-symbiotic Stage*

The chemical signalling between the two symbiotic partners takes place even in the absence of direct physical contact and involves molecules that are produced by either the host plant or the AM fungus. Among them, strigolactones and Myc factors play an essential role.

11.3.1.1 Plant-Derived Signals: Strigolactones

Plant–AM fungus communication starts in the rhizosphere with the production and exudation of signalling molecules by the host plant that are recognized by AM fungi. These cues stimulate hyphal growth and favour the establishment of the first physical contact between the partners (Fig. 11.1). Spores of AM fungi can germinate spontaneously and undergo an initial asymbiotic stage of hyphal germ tube growth, which is limited by the amount of carbon storage in the spore. However, if a partner is in the vicinity, the hyphal germ tube grows and ramifies intensively through the soil towards the host root (López-Ráez et al. 2012). Although other compounds such as flavonoids and hydroxy fatty acids have been reported as hyphal growth stimulators (Scervino et al. 2005; Nagahashi and Douds 2011), strigolactones have been shown to be crucial for a successful root colonization by the AM fungi (Akiyama et al. 2005; Bouwmeester et al. 2007; López-Ráez et al. 2012). Experimental evidence of strigolactone relevance in the AM symbiosis establishment has been provided by the reduction in mycorrhizal colonization of mutant plants affected in strigolactone biosynthesis (Gómez-Roldán et al. 2008, Vogel et al. 2010; Kohlen et al. 2012). In agreement with their role as signalling molecules, strigolactones are short-lived in the rhizosphere (Akiyama et al. 2010). When perceived by the AM fungus, the hyphal germ tube grows and branches, increasing the possibility of contact with the host root (Bouwmeester et al. 2007; López-Ráez et al. 2012). Although they were initially identified as molecular cues in the rhizosphere, strigolactones are multifunctional molecules that have been

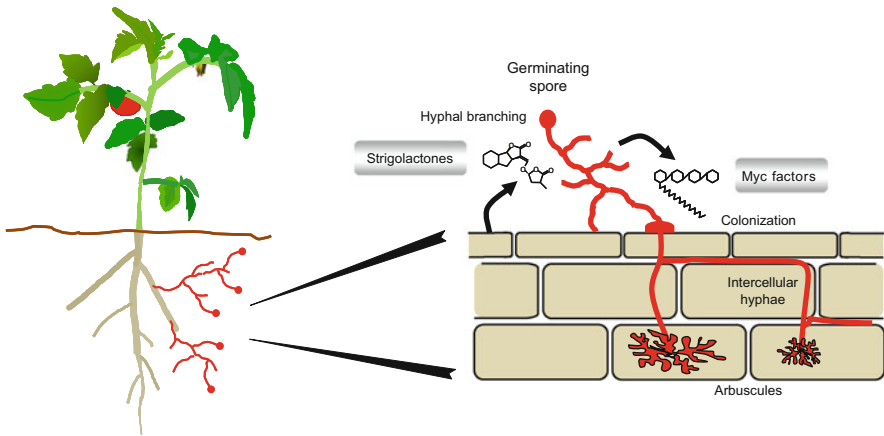


Fig. 11.1 Arbuscular mycorrhizal (AM) fungi–host plant symbiosis model. The process of AM symbiosis establishment begins with the production and exudation of strigolactones into the rhizosphere by the plant roots under deficient nutrient conditions (mainly phosphate shortage). Strigolactones induce hyphal branching of AM fungi-germinating spores, thus facilitating the contact with the host root. Parallel, AM fungus produces and releases the so-called Myc factors, which act on the plant roots inducing molecular responses required for a successful colonization. After contact, fungal hyphae grow intracellularly to the inner cortex, where the characteristic arbuscule is formed for nutrient exchange

classified as a new class of plant hormones regulating above- and belowground plant architecture (Bouwmeester et al. 2007; Gómez-Roldán et al. 2008; Umehara et al. 2008; Kapulnik et al. 2011a; Ruyter-Spira et al. 2011; López-Ráez et al. 2012). Strigolactones are present in the root exudates of a wide range of plants, and it has been shown that each plant produces a blend of different strigolactones, which suggest their broad spectrum action and importance in nature (Xie et al. 2010). They are derived from the carotenoids through sequential oxidative cleavage by *carotenoid cleavage dioxygenases* (CCD7 and CCD8) (Matusova et al. 2005; López-Ráez et al. 2008), thus belonging to the apocarotenoid class as the phytohormone abscisic acid (ABA) (Ohmiya 2009). All so far isolated and characterized strigolactones show a similar chemical structure (Fig. 11.2a), with a structural core consisting of a tricyclic lactone (the ABC-rings) connected via an enol ether bridge to a butyrolactone group (the D-ring) (Yoneyama et al. 2009; Zwanenburg et al. 2009). However, they can present different substituents on the AB-rings that make them different. It has been suggested that the biological activity of strigolactones resides in the enol ether bridge, which can be rapidly cleaved in aqueous and/or alkaline environments, indicating their short-lived and signalling character (Yoneyama et al. 2009; Zwanenburg et al. 2009; Akiyama et al. 2010). It was shown that phosphate and nitrogen deficiencies have a significant stimulatory effect on the production and exudation of strigolactones by plants (Yoneyama et al. 2007; López-Ráez et al. 2008), which will recruit AM fungi. Similarly, it has been recently shown that under salt stress conditions, plants also increase

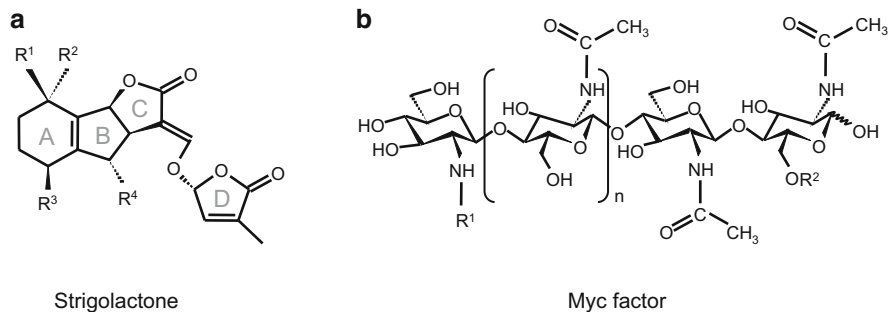


Fig. 11.2 Chemical structure of signalling molecules involved in AM symbiosis establishment. (a) General structure of host-derived strigolactones. (b) General Myc factor (lipochitooligosaccharide) structure

strigolactone production to promote AM symbiosis establishment (Aroca et al. 2013). According to the role of AM fungi, this increase in strigolactones is considered a cry for help under nutrient limiting and other stress conditions. Novel biological functions for strigolactones are being discovered continuously, indicating the relevance of these compounds in plant physiology and communication with other organisms from the rhizosphere (Kohlen et al. 2012).

Strigolactones present in the rhizosphere are perceived by the fungus through a so far uncharacterized receptor, thus inducing the so-called pre-symbiotic stage (Fig. 11.1). This stage is characterized by a profuse hyphal branching of the germinating spores, increasing the probability of contact with the root and that of establishing symbiosis (Akiyama et al. 2005; Besserer et al. 2006). Besides their role as hyphal branching factors, strigolactones stimulate spore germination in certain AM fungi (Besserer et al. 2006). Even, it has been suggested that they might act as the chemoattractant that directs the growth of the AM hyphae to the roots (Sbrana and Giovannetti 2005). Interestingly, strigolactones trigger a response only in AM fungi, but not in other beneficial fungal species such as *Trichoderma* and *Piriformospora indica* or in soilborne pathogens (Steinkellner et al. 2007), further supporting that they are rather specific signalling molecules.

11.3.1.2 AM Fungi-Derived Signals: Myc Factors

As in the nodulation process, another ecologically important mutualistic association in the rhizosphere established between legumes and rhizobia (Sprent 2009), a fungal factor analogous to the rhizobial Nod factor, has been recently characterized (Maillet et al. 2011) (Fig. 11.1). This Myc factor is produced by the metabolically active AM fungus, although it is not clear yet whether its production is induced by strigolactones. This fungal signal was previously described as a diffusible molecule, which is perceived by the plant inducing molecular responses in the host root required for a successful colonization (Parniske 2008). The chemical nature of the

elusive Myc factor remained unknown for a long time. Maillet and co-workers recently showed that AM fungi produce and secrete a mixture of sulphated and non-sulphated simple lipochitooligosaccharides (LCOs) (Fig. 11.2b) that have structural similarities with rhizobial Nod factors (Maillet et al. 2011). Both consist of an *N*-acetylglucosamine backbone with various substitutions, which are the basis for their diversity. Since nodulation is more recent than AM symbiosis (it appeared about 60 million years ago), the authors suggest that the Nod factor-derived signalling pathway likely evolved from the AM association. Interestingly, Maillet and co-workers showed that Myc factors are not only symbiotic signals that stimulate the AM establishment, but they also act as plant growth regulators affecting root development (Maillet et al. 2011). Thus, AM fungi would trigger alterations in root architecture to increase the number of colonization sites. The discovery and characterization of these pre-symbiotic signals pave the way for the development of new environmentally friendly agricultural strategies, as we will discuss later in Sect. 11.5.

11.3.2 Communication During Symbiosis Establishment and Maintenance

After perception of the Myc factor by the host plant through a so far uncharacterized receptor, the plant cell actively prepares the intracellular environment for colonization by the AM fungus (Fig. 11.1). The plant responses comprise an extensive and specific reprogramming of root tissues for the establishment of the AM symbiosis under the control of the so-called common symbiosis (SYM) pathway, since it is common for a successful mycorrhization and nodulation in legumes (Parniske 2008; Bonfante and Genre 2010). The common SYM pathway seems to be primarily involved in controlling early events in AM establishment. As a consequence of sequential chemical and mechanical stimulation and reorganization, plant cells produce an AM-specific structure that is crucial for a successful fungal penetration known as the “prepenetration apparatus” (Genre et al. 2005). This transcellular path is structurally related to the pre-infection thread formed in legumes during nodulation (Parniske 2008). By this subcellular structure, the plant guides the fungal growth through root cells towards the cortex. Once in the inner root cortex, a perifungal membrane is assembled and it fuses with the plant plasma membrane to produce an invagination. Then, the intracellular hyphae branch repeatedly to form the characteristic arbuscules (Fig. 11.3) (Bonfante and Genre 2010).

Conversely to the pre-symbiotic stage, the information regarding the nature of the chemical cues involved in the molecular dialogue during AM establishment and maintenance is scarce. The central factor of this signalling pathway is calcium spiking. Thus, Myc factor perception triggers a rapid transient elevation of cytosolic calcium (Bonfante and Genre 2010). A receptor-like kinase has the potential to directly or indirectly perceive the fungal signals and transduce the signal to the

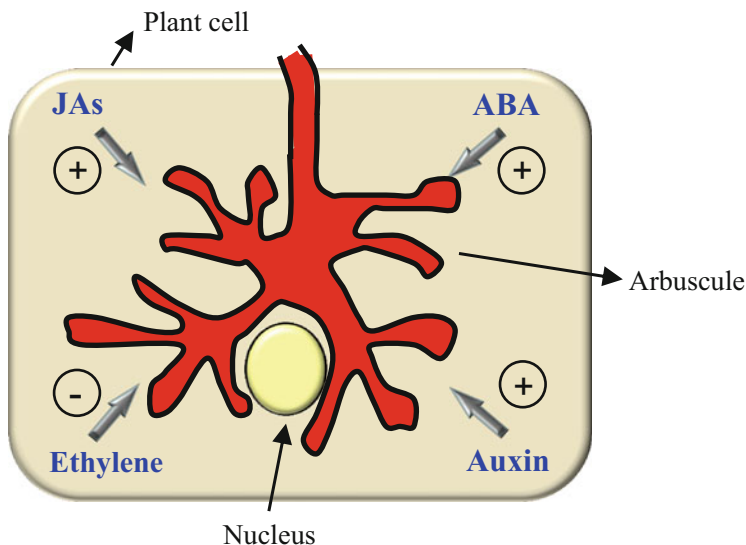


Fig. 11.3 Simplified model of the involvement of phytohormones in AM symbiosis. The picture gives a model of a root cortical cell containing an arbuscule, with the fungus in red. *Encircled plus* and *Encircled minus* indicates a positive and negative effect, respectively, of plant hormones on the establishment of AM symbiosis. *JAs* jasmonates, *ABA* abscisic acid

cytoplasm by phosphorylating a so far unknown substrate (Bonfante and Genre 2010). Since all the downstream elements of the SYM pathway are located in the nucleus, it has been suggested that the signal should be rapidly transduced to the nucleus. Additional chemical cues are probably needed to induce the establishment of the symbiosis and to provide certain specificity (Parniske 2008; Bonfante and Genre 2010). Unfortunately, the mechanisms controlling colonization of the host root tissue and arbuscule development are also largely unknown, although a role for phosphorous and carbohydrates has been proposed (Javot et al. 2007; Balzergue et al. 2011; Helber et al. 2011). Despite the importance of strigolactones in the initiation of AM symbiosis, it is unclear whether they also play a role in subsequent steps of the symbiosis. In this sense, it has been recently shown that strigolactones are also regulators of root nodulation in legumes (Soto et al. 2010; Foo and Davies 2011). Unlike AM symbiosis, strigolactones here would not act as host detection cues, but they would be required for optimal nodule number. This finding suggests that strigolactones might be also involved in further stages of AM symbiosis establishment and/or maintenance. Indeed, it has been proposed that they would promote sustained intercellular root colonization (Kretzschmar et al. 2012). However, further research is needed to clarify this aspect.

11.3.2.1 Involvement of Phytohormones

Mycorrhizal colonization is a very dynamic process in which the plant must control fungal growth and development. In this process, phytohormones orchestrate the modifications that occur in the host plant (Hause et al. 2007; López-Ráez et al. 2011b). Among them, jasmonic acid and its derivatives, known as jasmonates (JAs), are believed to play a major role in the AM symbiosis (Isayenkov et al. 2005; Hause et al. 2007; Herrera-Medina et al. 2008; López-Ráez et al. 2010b). JAs have been proposed to be involved in the reorganization of cytoskeleton and in the alteration of sink status of mycorrhizal roots (Hause et al. 2007). In this sense, they could stimulate carbohydrate biosynthesis in the shoots and transport into the roots for nutrient exchange in the arbuscules. JAs might also increase fitness in mycorrhizal plants by interacting with other phytohormones such as cytokinins (Hause et al. 2007). Abscisic acid (ABA) was also recently shown to be a key component in the establishment of the symbiosis in tomato plants. This hormone is necessary to complete the arbuscule formation process, enhance its functionality and promote sustained colonization of the root (Herrera-Medina et al. 2007; Martín-Rodríguez et al. 2010). In addition, it has been recently suggested that ABA plays a role in the regulation of strigolactone biosynthesis (López-Ráez et al. 2010a). Thus, ABA would be involved both in the early stages of the plant–fungus interaction and in the maintenance of the symbiosis once established. A role for auxin signalling within the host root during the early stages of AM formation has also been described (Hanlon and Coenen 2011). Since auxins are involved in regulating plant architecture, they could be responsible for lateral root formation on the plant, favouring host plant–AM fungus contact and therefore symbiosis establishment. Ethylene has also arisen as an important regulator of the symbiosis. Several findings suggest that ethylene would act as a negative regulator of mycorrhizal intensity (Zsogon et al. 2008; Martín-Rodríguez et al. 2011; Mukherjee and Ane 2011). Interestingly, a cross talk between ethylene and ABA was also shown (Herrera-Medina et al. 2007). The authors showed that ABA deficiency induced ethylene production and proposed that one of the mechanisms by which ABA regulates fungal colonization might be through the negative modulation of ethylene biosynthesis. In summary, mycorrhizal symbiosis establishment and maintenance implies a fine regulation of phytohormone levels in the host plant, whose understanding is still in its infancy.

11.3.2.2 Autoregulation of AM Symbiosis

A plant regulatory mechanism to control excessive root colonization has been proposed, a phenomenon known as autoregulation (García-Garrido et al. 2009; Staehelin et al. 2011). This mechanism shares some similarities with control of nodulation in legumes and allows the host plant to suppress further colonization once a certain level of mycorrhization has been reached, presumably to prevent

costs of the symbiosis exceeding the benefits. It is known that mycorrhizal plants with a well-established colonization produce fewer strigolactones than non-mycorrhizal ones (López-Ráez et al. 2011a). Therefore, it has been suggested that the reduction in strigolactone biosynthesis might be involved in the autoregulation process (García-Garrido et al. 2009; López-Ráez et al. 2011b). However, the mechanism by which AM symbiosis reduces strigolactone production is unknown. As aforementioned, strigolactone biosynthesis is negatively affected by phosphate levels (Yoneyama et al. 2007; López-Ráez et al. 2008). Moreover, phosphate acts systemically to repress AM colonization by affecting essential symbiotic genes, in particular genes coding enzymes of carotenoid and strigolactone biosynthesis, and symbiosis-associated phosphate transporters (Breuillin et al. 2010). Therefore, it is plausible that changes in phosphate levels within the plant are responsible of autoregulation in mycorrhizal plants (Breuillin et al. 2010).

More recently, a different mechanism has been proposed for autoregulation of mycorrhization. Staehelin and co-workers (2011) suggested that long-distance transport of chemical signals would be related to autoregulation. The authors proposed that fungal Myc factors induce expression or post-translation processing of root-derived short peptides belonging to the CLE peptide family, which likely function as ascending long-distance signals to the shoot. Extracellular CLE peptides show hormone-like activities and are involved in the regulation of several processes in plant development. In legumes, some of these short peptides have been shown to be involved in autoregulation of rhizobial colonization (Mortier et al. 2010). Somehow, these CLE peptides would induce the production of a descending signal (shoot-derived inhibitor) that inhibits mycorrhizal root colonization (Staehelin et al. 2011). In this scenario, it would be logical to think that this unknown shoot-derived signal inhibits strigolactone production in the roots. A third mechanism proposed to mediate autoregulation has been suggested. Microarray analysis revealed that mycorrhization in tomato induced the expression of key genes in the 9-LOX branch of the oxylipin pathway (López-Ráez et al. 2010b; León-Morcillo et al. 2012). Oxylipins are a diverse class of lipid metabolites that include biologically active molecules as the phytohormone JAs (Mosblech et al. 2009). The 9-LOX pathway gives rise to the biosynthesis of, among others, colnelenic and colneleic acids for which a role in defence against plant pathogens has been proposed (Mosblech et al. 2009). Therefore, these compounds might act as inhibitors to avoid an excessive colonization (López-Ráez et al. 2010b). In any case, none of the above-described hypothesis are exclusive, and most likely they act in concert. Further research is needed to elucidate how the phenomenon of autoregulation during AM symbiosis is regulated and what are the chemical molecules involved.

11.4 Strigolactones: Ecological Significance in the Rhizosphere

In addition to their role as signalling molecules in AM symbiosis, strigolactones have been recognized as a new class of plant hormones that inhibits shoot branching and hence controls above-ground architecture (Gómez-Roldán et al. 2008; Umehara et al. 2008). More recently, it was shown that they also affect root growth, root hair elongation and adventitious rooting (Kapulnik et al. 2011a; Ruyter-Spira et al. 2011; Kohlen et al. 2012; Rasmussen et al. 2012), which shows they are even more important components in the regulation of plant architecture than already postulated. Root system architecture (RSA) is of great importance for plants. In addition to a site of interaction with symbiotic microorganisms, roots are essential for different plant functions such as uptake of nutrients and water and anchorage in the substrate (Den Herder et al. 2010). RSA is altered under nutrient-deficient conditions such as phosphorous starvation, resulting in a shorter primary root, more and longer lateral roots (which are the preferred colonization sites for AM fungi) and a greater density of root hairs, thus expanding the exploratory capacity of the root system (Sánchez-Calderón et al. 2005; Rouached et al. 2010). These responses are regulated through a complex network of interconnected signalling pathways in which plant hormones, including strigolactones, play a key role (Kapulnik et al. 2011b; Ruyter-Spira et al. 2011). According to this positive effect on root development, strigolactone biosynthesis is promoted under limited Pi conditions (Yoneyama et al. 2007; López-Ráez et al. 2008). Consequently, it has been proposed that they play a pivotal role in plants as modulators of the coordinated development of roots and shoots and acting as a “cry for help” signal in the rhizosphere in response to Pi starvation (López-Ráez et al. 2011b; Ruyter-Spira et al. 2011).

11.4.1 *Strigolactones Are Germination Stimulants of Root Parasitic Plants*

Long before the discovery of their function as plant hormones and signalling molecules for mycorrhization, strigolactones were described as germination stimulants for the seeds of root parasitic plants of the family Orobanchaceae, including *Striga*, *Orobanche* and *Phelipanche* genera (Cook et al. 1972; Bouwmeester et al. 2003; López-Ráez et al. 2012). Strigolactones are produced and exuded into the rhizosphere by plants in very low amounts, being able to stimulate the germination of these parasitic plants in nano- and pico-molar concentrations. These parasitic weeds are some of the most damaging agricultural pests, causing large crop losses worldwide in cultivars such as rice, maize, tomato and legumes (Joel et al. 2007; Parker 2009). They attach to the roots and acquire nutrients and water from their host through a specialized organ called haustorium

(Bouwmeester et al. 2003). The life cycle of these parasitic weeds involves germination in response to strigolactones, radicle growth towards the host root, attachment and penetration. After emergence from the soil, the parasitic plants flower and produce enormous amount of seeds that are scattered in the soil, increasing the seed bank (Bouwmeester et al. 2003; López-Ráez et al. 2009). Parasitic weeds are difficult to control because most of their life cycle occurs underground, making the diagnosis of infection difficult and possible only when irreversible damage has already been caused to the crop. Therefore new control strategies focused on the initial steps in the host–parasite interaction, and especially those targeting seed germination induced by strigolactones are required (López-Ráez et al. 2009).

11.5 Biotechnological Applications of Signalling Communication in the AM Symbiosis: The Green Technology

Biotechnological tools are defined as those processes of biological interest that use chemistry of living organisms to develop new and more effective ways of producing traditional products while maintaining the natural environment. Even though the mechanistic bases of chemical signalling and regulation of plant responses during the AM symbiosis are not fully elucidated, it is worthy to eavesdrop this signalling process and use it for crop protection and yield increase. Thus, the AM symbiosis can greatly contribute to crop productivity and ecosystem sustainability (Gianinazzi et al. 2010). They not only improve plant growth through increased uptake of water and nutrients but also have “non-nutritional” effects such as stabilizing soil aggregates, preventing erosion as well as alleviating abiotic and biotic stress (Parniske 2008; Smith and Read 2008). Since the identity and chemical structure of the key molecules involved in AM symbiosis establishment below-ground is now being elucidated, manipulating their production to promote AM symbiosis, and its benefits, seems of great potential for a “green agriculture.”

11.5.1 AM Symbiosis as Bioprotective Agent Against Root Parasitic Plants

The fact that strigolactones play a dual role in the rhizosphere as signalling molecules for AM fungi and root parasitic plants makes them a suitable candidate to develop environmentally friendly control methods against these parasitic weeds. Remarkably, it was shown that AM fungal inoculation of maize and sorghum led to a reduction in *Striga hermonthica* infection in the field (Lendzemo et al. 2005). It was proposed that this reduced infection was caused, at least partially, by a reduction in the production of strigolactones in mycorrhizal plants (Lendzemo

et al. 2007). Similarly, AM colonization in pea and tomato induced less germination of seeds of *Orobanch*e and *Phelipanche* species compared to non-colonized plants (Fernández-Aparicio et al. 2010; López-Ráez et al. 2011a). Moreover, in the case of tomato, it was demonstrated that such reduction was caused by a decrease in the production of strigolactones (López-Ráez et al. 2011a). This decrease was associated to a fully established symbiosis, likely related to the phenomenon mycorrhizal autoregulation.

These observations in different plant systems suggest that the reduction in strigolactone production induced by AM symbiosis is conserved across the plant kingdom. Therefore, since AM fungi colonize roots of most agricultural and horticultural species and are widely distributed around the globe, AM symbiosis could be used as a biocontrol strategy for economically important crops that suffer from these root parasitic weeds. In addition, these crops would also take advantage of all the other well-known benefits of the symbiosis, such as the positive effect on plant fitness and higher tolerance/resistance against biotic and abiotic stresses. Addressing the possible specificity of different strigolactones in plant–parasitic plant and plant–AM fungus interactions is a major research challenge to further develop biotechnological strategies that favour one against another. In this line, by generating tomato transgenic plants blocked at the CCD8 enzyme (biosynthesis of strigolactones), we have recently shown that a mild reduction in strigolactone exudation is sufficient to reduce parasitic infection about 90 % without severely compromising AM symbiosis (Kohlen et al. 2012).

11.5.2 AM Symbiosis as Biofertilizer

AM fungi are already being used as biofertilizers for enhancing plant growth and biomass production in agriculture, although their use is still rather low compared with conventional methods (Barea et al. 2005; Gianinazzi et al. 2010). Remarkably, the benefits of the association for the host plant depend on the plant and AM fungus combination. Therefore, selecting appropriate AM fungal isolates adapted to local conditions and functionally optimal for the plant of interest is essential. Understanding the mechanisms regulating colonization, symbiosis efficiency and competence is crucial to optimize mycorrhizal benefits. Elucidation of the molecules involved in the interaction of host plant–AM fungus will definitively contribute to a better implementation of the “mycorrhizal technology” agrosystems.

11.5.2.1 Use of Strigolactones to Promote AM Symbiosis

Strigolactones have been shown to be essential for AM symbiosis establishment through the induction of hyphal branching, thus increasing the possibility of contact with the host plant (Akiyama et al. 2005; Bouwmeester et al. 2007). Therefore, breeding for high strigolactone production cultivars appears as an attractive strategy

to improve mycorrhization in agronomical conditions. On the other hand, this improved mycorrhizal colonization could be achieved by the exogenous application of strigolactones or strigolactone analogues. However, due to their stimulatory role of germination of root parasitic plants in the rhizosphere, a higher plant infection by these parasitic weeds would also take place. Moreover, strigolactones as phytohormones are involved in multiple physiological functions, which could give rise to undesired side effects. Therefore, a better understanding in the biology of strigolactones is essential prior to its application to agriculture. As mentioned above, one single plant species produces a mixture of different strigolactones and in different quantities (Xie et al. 2010). In addition, structural differences between strigolactones on the AM fungal branching response and for parasitic plant seed germination have been reported (Yoneyama et al. 2009; Akiyama et al. 2010). Similarly, specificity in strigolactone transport *in* and *ex planta* has been recently reported (Kohlen et al. 2012). Kohlen and co-workers showed that certain strigolactones are mainly exuded into the rhizosphere, while others are preferentially loaded into the xylem and transported to the shoot. Thus, new strategies for breeding or strigolactone application should be focused on strigolactone specificity in order to favour mycorrhization without affecting other functions in the plant.

11.5.2.2 Application of Myc Factors

With regard to the Myc factors, they are the fungal signals that stimulate AM symbiosis establishment in leguminous and other mycotrophic plant species. Besides stimulating AM symbiosis, they act as plant growth regulators by stimulating root branching and development (Maillet et al. 2011). Elucidation of the chemical nature of these Myc factors (Maillet et al. 2011) makes Myc factors as the ideal candidates for their use as biofertilizers. Indeed, this “green technology” has currently been applied for the treatment of leguminous seeds with Nod factors and yielded increase in crops such as soybean, alfalfa and pea. The fact that more than 80 % of land plants are mycotrophic suggests that Myc factors might have a broader spectrum of activity than Nod factors. Besides their beneficial effect on plant growth through an improved mycorrhization, they would facilitate the development of the root system, with the consequent improvement in nutrient uptake and resistance to abiotic stresses. In the search of methods for efficient synthesis of Myc factors, its production by bacteria has been already developed (Maillet et al. 2011), allowing their production on a large scale. Still further research is needed on the biological activity and specificity of Myc factors and the optimal conditions for application before its implementation in agriculture.

11.6 Conclusions

Chemical fertilizers and pesticides are being used to promote plant growth and to prevent plant diseases. However, the environmental pollution caused by excessive use and misuse of agrochemicals has led to public concerns about the use of these chemicals in agriculture. Therefore, there is a need to find more environmentally friendly alternatives for fertilization and disease control. The key to achieve successful biological control is the knowledge on plant interactions in an ecological context. We emphasize here the importance of the chemical communication that occurs in the rhizosphere between plants and beneficial microorganisms, especially AM fungi, and its potential use as biofertilizers and biocontrol agents. Further research on the mechanisms regulating the production and release of these cues and about their specificities requirements will expand our knowledge on biological processes occurring underground. The information generated may result in the development of new green technologies for sustainable agriculture in the near future.

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