



REVIEW ARTICLE

Regulation of the plant defence response in arbuscular mycorrhizal symbiosis

José Manuel García-Garrido¹ and Juan A. Ocampo

Departamento de Microbiología del Suelo y Sistemas Simbióticos. Estación Experimental del Zaidín, CSIC Profesor Albareda, 1, 18008 Granada, Spain

Received 30 July 2001; Accepted 3 January 2002

Abstract

The response of plants to arbuscular mycorrhizal fungi involves a temporal and spatial activation of different defence mechanisms. The activation and regulation of these defences have been proposed to play a role in the maintenance of the mutualistic status of the association, however, how these defences affect the functioning and development of arbuscular mycorrhiza remains unclear. A number of regulatory mechanisms of plant defence response have been described during the establishment of the arbuscular mycorrhizal symbiosis, including elicitor degradation, modulation of second messenger concentration, nutritional and hormonal plant defence regulation, and activation of regulatory symbiotic gene expression. The functional characterization of these regulatory mechanisms on arbuscular mycorrhiza, including cross-talk between them, will be the aim and objective of future work on this topic.

Key words: Arbuscular mycorrhiza, defence regulation, plant defence.

Introduction

The endosymbioses formed between plants and microorganisms play an important role in agriculture and natural ecosystems. The most widespread endosymbiotic interactions are formed between plant and fungi, including parasitic interactions, which result in diseases such as mildew and rust, and the mutualistic symbiosis established between plant roots and arbuscular mycorrhizal fungi (AMF).

AMF (zygomycotina) are typically associated with the vast majority of terrestrial plants, preferably with angiosperms. The successful establishment of this mutualistic association constitutes a strategy to improve the nutritional status of both partners. The fungi receive fixed

carbon compounds from the host plant, while the plant benefits from the association by the increased nutrient uptake (mainly phosphorus), enhanced tolerance to abiotic stress, and resistance to pests (Smith and Read, 1997).

AMF are considered to be biotrophic micro-organisms. Because of their obligate symbiotic nature, they cannot be cultured axenically. Generally, AMF show little or no specificity and the factors that determine whether mycorrhiza are formed or not appear to depend on the genotype of the host plant (Koide and Schreiner, 1992). Evidence for this is provided by the existence of non-host plant species (review by Giovannetti and Sbrana, 1998) and *Myc*⁻ mutant plants unable to form AM symbiosis (Gollotte *et al.*, 1993).

Penetration into the root and intercellular growth of the AM fungi involves a complex sequence of biochemical and cytological events and intracellular modifications (Bonfante-Fasolo and Perotto, 1992; Bonfante, 2001), which imply that the fungus must clearly be recognized by the host plant. The mechanisms controlling AM development are largely unknown. However, evidence suggests that, as in plant–pathogen interactions, the induction/suppression of mechanisms associated with plant defence play a key role in AM fungal colonization and compatibility with its host. The progress made in this area is reviewed here, focusing on the function and regulation of the host's defence mechanisms in the context of this mutualistic interaction.

Defence responses in plants during the development of arbuscular symbiosis

Plant defence responses during the early stages of plant–AM fungus interactions: the induction/suppression effect

The rapid recognition of a potential invader is a prerequisite for the initiation of an effective defence

¹ To whom correspondence should be addressed. Fax: +34 58 129600. E-mail: JoseManuel.Garcia@eez.csic.es

response by the plant. This is achieved through the recognition of specific signal molecules also known as elicitors. Elicitors can be secreted from the microbe (exogenous elicitors) or generated as a result of physical and/or chemical cleavage of the plant cell wall (endogenous elicitors). After perception of an elicitor, a number of biochemical changes contribute to the early response in host cells. These processes include changes in the ion permeability of the plasma membrane, the activation of plasma membrane-bound enzymes, the activation of kinases, phosphatases, phospholipases, and the production of signal molecules, including active oxygen species. The result of these processes is the transcriptional activation of defence-related genes (review by Somssich and Hahlbrock, 1998).

Some events similar to those found in plant–pathogen interactions have been also found in the plant interaction with AMF. These events include signal perception, signal transduction and defence gene activation. Salzer and Boller suggest that ectomycorrhizal and AM fungi secrete similar chitin elicitors, which could induce a defence response (Salzer and Boller, 2000). For instance, the elicitor derived from an extract of extraradical mycelium of *Glomus intraradices* was able to induce phytoalexin synthesis in soybean cotyledons (Lambais, 2000). Interestingly, recent results show that *G. intraradices* induces the expression of a chalcone synthase, the first enzyme in the metabolism of flavonoid compound, such as phytoalexin, in *Medicago truncatula* (Bonanomi *et al.*, 2001). This gene is induced in the roots of *M. truncatula* upon the first fungal contact (Bonanomi *et al.*, 2001). Furthermore, a hypersensitive-like response, commonly observed when the plant is confronted with a pathogen, could be observed in compatible AM associations. An oxidative burst could be detected at sites where hyphal tips of *G. intraradices* attempted to penetrate a cortical root cell of *M. truncatula* (Salzer *et al.*, 1999). Moreover, necrosis and cell death have been observed at sites of *Gigaspora margarita* infection of *Medicago sativa* roots (Douds *et al.*, 1998). Also, other reports demonstrate that AMF can elicit a defence response in the plant. The most evident effects were observed in incompatible associations between AM fungi and non-host plants or *Myc*⁻ mutant plants. In the case of non-host plants, the molecular bases for incompatibility with AM fungi remain unclear, but in some associations the resistance reaction seems to involve a hypersensitive-like response (Allen *et al.*, 1989). A stronger defence response, characterized by the deposition of callose, PR-1 protein, and phenolics has been observed when pea *Myc*⁻ mutant plants were challenged with an AMF (Gollotte *et al.*, 1993).

Not only a hypersensitive response but also some elements of signal transduction pathways activated after pathogen recognition by the plant have been observed transiently during the early stages of AM formation. In

mycorrhizal tobacco plants, transient increases of catalase and peroxidase activity were observed to coincide with appressoria formation and fungal penetration into the root (Blilou *et al.*, 2000a). Interestingly, similar results have been shown in onion and bean roots inoculated with AMF (Spanu and Bonfante-Fasolo, 1988; Lambais, 2000). The transient increase in catalase and peroxidase activity observed in tobacco mycorrhizal roots also coincided with the accumulation of salicylic acid (SA) (Blilou *et al.*, 2000a). SA is a signal molecule involved in the signal transduction pathway activated in plant–pathogen reactions (Malamy *et al.*, 1990; Métraux *et al.*, 1990). A transient accumulation of SA during the early stages of infection also has been observed in the interaction between rice and *Glomus mosseae* (Blilou *et al.*, 2000b). In this case, the accumulation of SA was also correlated to an increase in the expression of genes encoding lipid transfer protein (LTP) and phenylalanine ammonia-lyase (PAL) (Blilou *et al.*, 2000b). This provides evidence that induction of *Pal* and *Ltp* is part of the defence pathway (Blilou *et al.*, 2000b).

Most of the defence-related genes expressed during the early stages of AM fungal penetration are also activated by pathogen infection, treatment with elicitors, or by SA. In this context, the induction of defence gene expression could be considered to be a result of fungal elicitor recognition and signal transduction pathway activation. The weak and transient character of the plant defence response could be a consequence of the low capacity of the fungus to trigger such a response and/or to induce a plant mechanism which suppresses an already activated defence response at several levels to allow for fungal growth within the plant tissue. The possible implications these mechanisms could have in determining the compatibility of AM symbiosis will be discussed below.

Defence responses in plant cells containing arbuscules

In the early stages of root colonization by AMF, the plant defence response is characterized by a weak and transient activation, but at later stages, for example, the arbuscule formation, this activation appears to become stronger, although only in those cells which contain fungal structures. For instance, the use of specific probes in studies of *in situ* expression revealed that some mRNAs of genes associated with the plant defence response specifically accumulated in plant cells containing arbuscules. Likewise, members of different classes of plant defence genes, including genes encoding hydroxyproline-rich glycoproteins (HRGP) (Balestrini *et al.*, 1997; Blee and Anderson, 2000), phenylpropanoid metabolism enzymes (Volpin *et al.*, 1994, 1995; Harrison and Dixon, 1993, 1994), enzymes involved in the metabolism of reactive oxygen species (Blee and Anderson, 2000) and plant hydrolase (Lambais and Mehdy, 1993, 1998; Blee and

Anderson, 1996; David *et al.*, 1998; Salzer *et al.*, 2000) have been detected in plant cells containing arbuscules. In the case of HRP, an increase in the quantity of mRNA encoding HRP was observed in the root cells of maize and bean that contained arbuscules (Balestrini *et al.*, 1997; Blee and Anderson, 2000).

Phenylpropanoid compounds such as flavonoids and isoflavonoids are involved in diverse aspects of plant growth and development, but also in interactions with micro-organisms (reviewed by Dixon and Paiva, 1995). Genes encoding enzymes that catalyse core reactions of the metabolism of phenylpropanoid have been expressed in cells with arbuscules. For instance, *in situ* localization of phenylalanine ammonia lyase and chalcone synthase transcripts were observed in cells containing arbuscules (Harrison and Dixon, 1994). However, the expression of other genes encoding enzymes in the flavonoid/isoflavonoids pathway, such as chalcone isomerase or isoflavone reductase was not significantly affected in mycorrhizal roots (Harrison and Dixon, 1994). In *Medicago* species, transient increases in different flavonoid/isoflavonoid compounds were found to depend on the plant and fungal genotypes involved in the interaction (Volpin *et al.*, 1994, 1995; Harrison and Dixon, 1993, 1994). Altogether, the data available suggest an activation of phenylpropanoid metabolism in mycorrhizal roots, characterized by the weak, localized (preferably in cells containing arbuscules) and unco-ordinated induction of genes together with the accumulation of phytoalexin products, some of which are found at high levels (Morandi, 1989). Although no evidence exists which supports a specific role for flavonoids/isoflavonoids in the AM symbiosis, there are several results suggesting that flavonoids can stimulate AM symbiosis in a similar way to the rhizobial symbiosis (Xie *et al.*, 1995; Harrison, 1999).

Messengers RNAs of genes involved in the catabolism of reactive oxygen species, such as catalase and peroxidase have been localized in bean and wheat root cells containing arbuscules, respectively (Blee and Anderson, 2000). Blee and Anderson suggest that catalase and peroxidase play a role in the catabolism of hydrogen peroxide and/or in cross-linking reactions between proteins and polysaccharides in the interface between the arbuscule and the plant cell plasma membrane.

Corroborating the biochemical data, differential gene expression of acidic and basic forms of chitinase and β -1,3-glucanase has been observed during mycorrhiza formation in different plant–fungal combinations (Lambais and Mehdy, 1993, 1998; Blee and Anderson, 1996; David *et al.*, 1998; Salzer *et al.*, 2000). *In situ* localization of bean acidic endochitinase and β -1,3-glucanase transcripts in mycorrhizal roots showed that mRNAs accumulated predominantly in the vascular cylinder (Lambais and Mehdy, 1998). Nevertheless, the accumulation of basic forms of chitinase and

β -1,3-glucanase transcripts have been observed in the intercellular region between cortical cells containing arbuscules (Blee and Anderson, 1996; Lambais and Mehdy, 1998), suggesting that these enzymes might be involved in the control of intraradical fungal growth. Interestingly, the accumulation of β -1,3-glucanase mRNA in cells containing arbuscules was modulated by phosphorus (P) concentration. The level of mRNA accumulation increased as concentration of phosphorus decreased. In comparison, the amount of basic chitinase transcripts did not change with mycorrhization or P concentration (Lambais and Mehdy, 1998). The enzymatic activity and mRNA accumulation patterns observed, suggest that chitinases and β -1,3-glucanases could form part of the defence response by the plant to the invading fungus. Some recent data showed a specific induction of a class III chitinase gene family in mature *M. truncatula* mycorrhizae (Salzer *et al.*, 2000). The authors suggest a role for the induced chitinase genes in suppression of plant defence reactions in the later stages of the AM development (Salzer *et al.*, 2000). However, the role and regulation of these defence-related proteins during intraradical fungal growth is not clear.

Other genes implicated in the processes of senescence and stress have shown expression in cells containing arbuscules. That is the case of potato encoding a glutathione-S-transferase (Prp1), the transcripts of which accumulate only in certain cells containing arbuscules (Franken *et al.*, 2000). This gene product could be involved in the degradation of arbuscules (Franken *et al.*, 2000).

The role of defence genes expressed in the cells containing arbuscules could be the control of hyphal spread and arbuscule formation in the root. Nevertheless, the exact function of such gene products during mycorrhizas is unclear and should be clarified because the same activity due to a gene product might have a different consequence in a different context (pathogenesis or mutualism).

Mechanisms of defence regulation in AM symbiosis

Multiple signals and differential induction of gene expression mediate the complex interaction between AMF and plant cells. As described above, several investigations point to the existence of signals and the expression of genes related to a plant defence response in AM symbiosis. Nevertheless, the regulation and function of these signals and genes are still unknown. Some authors suggest that the plant defence response plays a role in its control over the development of the fungal symbionts (Lambais and Mehdy, 1993, 1996). However, the formation of AM in transgenic plants constitutively expressing several of the classical pathogenesis-related proteins were not affected (Vierheilig *et al.*, 1993, 1995).

Therefore, the effective function of these plant pathogenic proteins in AM symbiosis is debatable. The differential activation of defence genes reflect that some mechanism exists that regulates the suppression of the defence response to allow compatible AM fungi to form a mutualistic symbiotic interaction with the plant. The local or/and systemic nature of the regulation and the components of this mechanism of suppression or attenuation of the defence response are discussed below.

Plant and fungal hydrolases, a key to defence regulation in AM symbiosis

One possible mechanism to attenuate the plant defence response in the AM symbiosis might be the degradation of exogenous elicitor molecules produced by the AM fungi and/or the prevention of endogenous elicitor release from the plant cell wall. Theoretically, hydrolytic enzymes regulate both processes as plant hydrolases could hydrolyse fungal elicitor components while fungal enzymes could hydrolyse plant cell wall components. Data presented in the literature suggest that such a hypothetical model of regulation in AM symbiosis by hydrolases could exist. During AM development the expression of constitutive and mycorrhiza-specific chitinases, chitosanases and β -1,3-glucanase isoforms (potential hydrolases of fungal elicitors) are differentially regulated, thereby pointing toward a central role for these enzymes in AM formation (Dumas-Gaudot *et al.*, 1992; Dassi *et al.*, 1996; Pozo *et al.*, 1998; Salzer *et al.*, 2000). Furthermore, the induction of a plant defence response by an elicitor released from an extract of extraradical mycelium of *G. intraradices* has been observed (Lambais, 2000). In that work, differences in the induction of the plant response by extracts of intra and extraradical fungal components were observed, as the intercellular fluids of AM roots could not induce a response. As a result, the author suggests that a factor exists in plants, which is regulated by the level of P, which is responsible for the breakdown of the fungal elicitor molecules (Lambais, 2000). However, the factor/s responsible for the attenuation of the elicitor activity were not identified, although the data suggest that this effect was not related to chitinase activity in the root. By contrast, other authors suggest a similar mechanism for elicitor degradation and plant defence attenuation in AM symbiosis that does involve the differential regulation of chitinase enzymes by the host plant (Salzer *et al.*, 2000; Salzer and Boller, 2000). These authors proposed that constitutively expressed plant chitinase, in the early stages, and the micorrhiza-specific isoform in the later stages, were the enzymes responsible for elicitor degradation (Salzer and Boller, 2000).

The prevention of endogenous elicitor formation as a mechanism that mediated defence regulation should also be considered. If so, the production of endogenous

elicitors derived from the degradation of the cell wall does not seem to be an effective mechanism for the induction of the plant defence response. Evidence supporting this hypothesis is that mycorrhizal fungi produce very little plant cell wall degrading enzymes (García-Romera *et al.*, 1991; García-Garrido *et al.*, 1992). Furthermore, the cell wall degrading enzymes produced in mycorrhizal roots show similar electrophoretic and biochemical characteristics as plant enzymes produced in non-mycorrhizal roots (García-Romera *et al.*, 1997; García-Garrido *et al.*, 1996, 2000). This suggests that the role of fungal enzymes is only selectively and specifically to break plant cell wall components for fungal penetration, but not to participate in an unspecific and unco-ordinated plant cell wall degradation process, which could produce endogenous elicitors.

Nevertheless, the intracellular colonization by AM mycorrhizal fungi creates a new interface compartment composed of membranes from both partners separated by apoplastic material containing molecules common to the plant primary wall, such as cellulose, pectin, xyloglucan, and HRGP which are not assembled into a fully structured wall (Bonfante, 2001). The lytic activity of plant and/or fungal enzymes over these molecules could then putatively generate oligo-fragments which could act as elicitors for a localized defensive response at the arbuscular level.

Symbiotic genes: common steps in root endosymbioses

Studies of AM symbiosis have revealed similarities between the processes of AM fungal infection and rhizobial colonization and nodule formation in legume plants. Several reviews describe the most relevant similarities at the molecular, cytological and genetic level of both symbioses (Gianinazzi-Pearson and Dénarié, 1997; Hirsch and Kapulnik, 1998; Albrecht *et al.*, 1999; Parniske, 2000).

The large collection of nodulation and nitrogen fixation defective legume mutants allows the possibility of genetically identifying each step of the development of both symbioses. A recent review summarizes the current state of knowledge about the existing collection of *Myc*⁻ mutants, including information about the genetic loci affected by mutation and the stage of mycorrhizal colonization blocked (Marsh and Schultze, 2001).

Four loci (SYM8, SYM9, SYM19, and SYM30) are known to be involved in the early steps of both endosymbiotic interactions in pea plants. Cytological studies have shown that mutations in all of these four genes block the penetration of the AM fungus at the level of appressoria formation (*Myc*⁻ mutants) (Balaji *et al.*, 1994; Gianinazzi-Pearson *et al.*, 1991; Gollotte *et al.*, 1993). In the interaction with *Rhizobium*, these mutants are unable to form infection threads.

Nodulation-defective/*Myc*⁻ mutants have been reported in other legumes, such as *M. sativa* (Bradbury *et al.*, 1991), *Phaseolus vulgaris* (Shirtliffe and Vessey, 1996), *M. truncatula* (Catoira *et al.*, 2000), and *Lotus japonicus* (Wegel *et al.*, 1998). Most of these mutants are characterized by a blockage of fungal infection at the rhizodermis level. Three loci (DMI1, DMI2 and DMI3) were associated with the symbiotic mutants of *M. truncatula* defective for rhizobia and AM fungi infection (Catoira *et al.*, 2000). These mutants showed no induction of early nodulin genes and were affected in Nod factor-induced root hair branching (Catoira *et al.*, 2000). In *L. japonicus*, mutants in loci SYM2, SYM3 and SYM4 were affected in the early interaction with arbuscular fungi (Wegel *et al.*, 1998). One of these loci, SYM4, has been proposed to be necessary for the initiation or co-ordination of a host programme for the accommodation of the microsymbiont (Bonfante *et al.*, 2000).

Cytological studies of *Myc*⁻ pea mutants challenged with AM fungi have revealed that the plant reacts against the fungus by apposition of material in the cell wall of epidermal and hypodermal cells adjacent to the invading hyphae (Gollotte *et al.*, 1993; Gianinazzi-Pearson *et al.*, 1996). Furthermore, during the early stages of root interactions with *G. mosseae* and *R. leguminosarum* bv. *viciae* the accumulation of transcripts of defence-related genes was higher in roots of the *Myc*⁻ pea mutant than in the wild-type (Ruíz-Lozano *et al.*, 1999). Interestingly, in one of these mutants (SYM30), the resistance against AM fungi and rhizobia correlates to enhanced levels of endogenous SA in its roots (Blilou *et al.*, 1999). It is tempting to speculate that a defence mechanism associated with cell wall apposition and PR gene expression, mediated by SA accumulation is responsible for the incompatible interactions between this *Myc*⁻/*Nod*⁻ mutant and endosymbiotic micro-organisms.

It is clear that the products of the *Sym* genes have a fundamental role in the establishment of the mycorrhizal and rhizobial symbioses, and that a single mutation could result in incompatibility and resistance. The finding that some of the genes important for both symbioses are necessary for Nod factor perception (Albrecht *et al.*, 1998; Catoira *et al.*, 2000) suggests that a signal similar to the Nod factor could also be important in the AM association. Although this hypothesis has been proposed by other authors (Albrecht *et al.*, 1998, 1999; Blilou *et al.*, 1999; Catoira *et al.*, 2000; Vierheilig and Piché, 2002), the signals by which AMF could activate the symbiotic programme in the root remain unknown.

Some hypothetical roles could be attributed to the genes mutated in *Nod*⁻/*Myc*⁻ plants. First, any of the genes could participate directly in the Nod factor and/or *Myc* factor perception. The mutation of these genes could lead to the non-recognition of a mutualistic micro-organism and, subsequently, to the activation of the

plant defence. In this case the elicitation of the plant defence by *Rhizobium* and AMF elicitors could cause a resistance response in the plant. In this regard, evidence exists that *Rhizobium* Nod factor is involved in the inhibition of the SA-mediated defence response in alfalfa roots (Martínez-Abarca *et al.*, 1998).

The function of the products of *Sym* genes in the signal transduction pathway after signal perception should also be considered (Catoira *et al.*, 2000). The disruption by mutation of some of the steps of the signal transduction cascade implies that the plant does not perceive a symbiotic signal, and thus the resistance response take place. It is also possible that the *Sym* genes could play a role in restricting the plant defence response induced by endosymbiotic micro-organisms (Gianinazzi-Pearson, 1996; Blilou *et al.*, 1999). In this regard, two possible mechanisms have been proposed to describe how symbiotic genes could regulate the plant defence response. Firstly, these genes could be involved in the production of a negative regulator of plant defence response. Secondly, the products of the symbiotic genes might induce the production of specific suppressors of the defence response by the endosymbiotic micro-organism (Gianinazzi-Pearson, 1996).

Further work and comparative analysis of the plant defence response at the cytological, physiological and biochemical levels in plants mutated in different symbiotic genes are necessary to place these genes within the pathway leading to nodulation and mycorrhization. In this way, it is exciting to investigate if AMF and rhizobia share a similar mechanism of signal perception, and how these micro-organisms that differ vastly in their host specificity could activate similar genes and plant response in such a wide range of plant species. In this regard, the use of pea *Nod*⁻/*Myc*⁻ mutant plants in experiments of calcium quantification in pea root hairs has provided evidence that calcium spiking may be a common step to both mycorrhizal and nodulation signalling (Walker *et al.*, 2000).

Regulation of plant defence response in AM symbiosis by alterations in the signal transduction pathway

Another possible mechanism to attenuate the plant defence response could be by blocking components of the signal transduction pathway that activate this response. Among these components, salicylic acid and reactive oxygen species (ROS) have been implicated as second messengers in AM associations.

Although levels of H₂O₂ and other ROS have not been measured in AM roots, indirect evidence exists that suggests that the levels of H₂O₂ in mycorrhizal associations are increased (Salzer *et al.*, 1999). Alterations in the pattern of anti-oxidative enzymes, such as catalase and peroxidase in mycorrhizal roots may indicate that

oxidative compounds are produced during the colonization process (Blilou *et al.*, 2000a; Lambais, 2000). The increase in catalase and peroxidase activity could be due to their function as antioxidants for any active oxygen molecules generated during the initial stages of fungal penetration. Since H₂O₂ and other reactive oxygen species are involved in signal transduction cascades in plant–pathogen interactions, it is possible that degradation of H₂O₂ by catalase in AM could be a possible mechanism for avoiding the activation of defence response genes. In bean roots, catalase activity was regulated according to the infectivity of the AM fungi and the availability of P (Lambais, 2000). Another example of regulation of catalase and peroxidase has been shown in bean and wheat colonized by *G. intraradices* (Blee and Anderson, 2000). The accumulation of these enzymes in cells containing arbuscules may be due to a localized regulation of the defence mechanism.

Transient increases in catalase and peroxidase activities in AM tobacco roots occur at the same time as the transient enhancement of free SA (Blilou *et al.*, 2000a). In this way there may be a synergistic relationship between SA and H₂O₂ in some plant defence responses (van Camp *et al.*, 1998). The role and significance of SA in AM interactions remains unclear. Nevertheless, the findings that transient increases of SA are detected in tobacco and rice AM roots (Blilou *et al.*, 2000a, b) and that the exogenous application of SA to rice roots did not affect appressoria formation, but did cause a transitory delay of mycorrhization of root (Blilou *et al.*, 2000b) suggest that the regulation of defence response in plants against AM fungi may be through the SA pathway. In fact, during the early stages of infection, the level of mycorrhization was higher in NahG tobacco plants (unable to accumulate free SA) than in wild-type plants (JM García-Garrido and H Vierheilig, unpublished results). These findings suggest a link between SA accumulation and fungal infectivity in compatible mycorrhizal associations. Evidence supporting this hypothesis has been obtained with Nod⁻/Myc⁻ pea P2 plants, which are resistant to both rhizobia and AMF colonization. In these studies SA accumulation was observed both in the roots of wild-type and mutant plants when associated with rhizobia or AMF. However the amplitude of SA accumulation was higher in P2 plants and increased with time, an effect that was not observed in the roots of the wild-type plants (Blilou *et al.*, 1999).

Regulation of the plant defence response by hormonal and nutritional imbalances

Alterations in the flux of nutrients and in the levels of plant hormones could generate cell-signalling pathways that regulate the plant defence response during AM development. Evidence for this is provided by the fact that phosphate levels in the plant are negatively, and

carbohydrate levels are positively correlated with root colonization by AMF (Jasper *et al.*, 1979; Clarkson, 1985). The exact mechanisms and molecular basis that could mediate the inhibition of AM colonization by P remain unknown. One possibility is that defence gene expression is mediated by a signalling mechanism that senses the level of P in the root, resulting in an up-regulation of these genes in plants with high levels of P. This has been confirmed for catalase (Lambais, 2000), chitinase and glucanase genes (Lambais and Mehdy, 1998).

Apart from changes in the metabolism of P in mycorrhizal plants, changes in the carbohydrate metabolism also occur. Recently, it was observed that arbusculated cells are greater sucrose sinks than cortical cells without arbuscules (Vierheilig *et al.*, 2001). This might be due to the high energetic needs these cells require to support enhanced metabolism in combination with the nutrition requirements of the AM fungus. In arbusculated cells, the enhanced transcription of genes involved in the catabolism of sucrose was correlated to the enhanced transcription of defence genes (Blee and Anderson, 1998). These authors suggest that a sensor system based on the flux of carbohydrates regulates defence gene expression in plant cells containing arbuscules (Blee and Anderson, 2000). Furthermore, these authors postulated that an increase in the flux of sucrose, glucose, and fructose and the activation of genes associated with the catabolism of sucrose in the cells containing arbuscules constitute a mechanism responsible for the activation of defence genes (Blee and Anderson, 2000). Relationships between sugar regulation and defence gene expression and activation of the systemic resistance have also been described by other authors (Herbers *et al.*, 1996). However, the mechanism that mediates defence regulation by nutritional factors remain unknown. At least in the regulation by sugar, some results suggest that the ethylene signal transduction pathway could be implicated in this mechanism of defence regulation (Zhou *et al.*, 1998).

It is intriguing that plant hormone levels in mycorrhizae are altered, as the ethylene level in AM tobacco roots is low (Vierheilig *et al.*, 1994) and cytokinin levels are significantly increased in alfalfa and tobacco AM roots (van Rhijn *et al.*, 1997; Ginzberg *et al.*, 1998). Additionally, one study has shown that AMF can produce plant hormones (Barea and Azcón-Aguilar, 1982). The significance and role of plant hormones in AM symbiosis have been reviewed (Beyrle, 1995), but the exact role of plant hormones in the regulation of gene expression during the plant–AMF colonization is not clear. Cytokinins act as suppressive factors of chitinase activity (Shinshi *et al.*, 1987), and some authors suggest that elevated levels of cytokinins in mycorrhizal roots could suppress the induction of some PR-protein genes, specifically chitinase and glucanase genes (Spanu *et al.*,

1989; Shaul *et al.*, 2000). Nevertheless, the effective role of plant hormones during AM is not clear and it is difficult to determine if changes in hormone levels in AM symbiosis are directly implicated in the process of mycorrhization.

Conclusions and perspectives

The combination of molecular and genetic approaches in comparative studies of defence-related processes generated in AM- and pathogen-infected roots, have revealed that the defence response in plants colonized by AMF is temporally and spatially regulated. The initial, transient and weak defence response is continuous with a

prolonged and subtle activation of defence genes in cells containing arbuscules. The role and significance of the defence mechanism activated during the AM interaction remains unclear. Whether the alteration of defence gene expression has a particular or functional role in the establishment of the symbiosis, or if it is a consequence of non-specific activation, should be clarified. An investigation of the signalling mechanisms underlying the activation and cross-talk processes that participate in the regulation of the AM symbiosis (Fig. 1) may contribute to our understanding of the formation and functioning of this symbiosis, including the repercussions that these mechanisms could have on the induction of the plant resistance against pathogens observed in mycorrhizal plants.

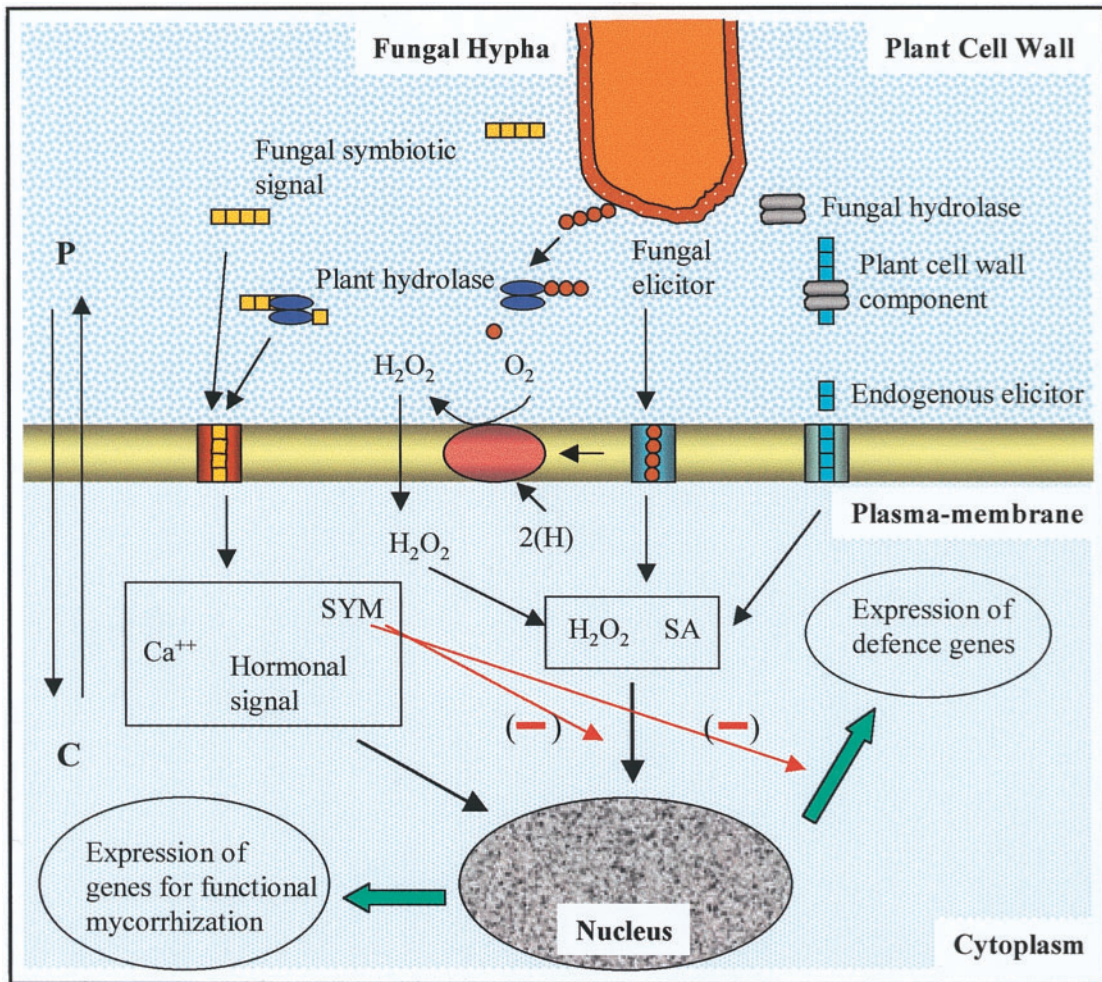


Fig. 1. Hypothetical model representing the regulatory mechanisms involved in the plant defence response during the establishment of the AM symbiosis. The binding of fungal elicitors to plant receptors triggers the activation of the signal transduction pathway leading to defence gene activation. Numerous events participate in the signal transduction pathway, including generation of reactive oxygen species and increases in SA. The action of fungal hydrolases on plant cell wall components could also generate endogenous elicitors that contribute to the generation of the signal transduction chain after elicitor perception. Constitutive and/or inducible plant hydrolases are the enzymes responsible for elicitor degradation and, consequently, plant defence attenuation. In parallel, the recognition of a putative fungal symbiotic signal by the plant cell leads to the activation of a specific symbiotic pathway.

Acknowledgements

The authors wish to thank Drs H Vierheilig, Université Laval, Québec, Canada and P van Dillewijn, EEZ, CSIC, Granada, Spain, for critical reading of the manuscript.

References

- Albrecht C, Geurts R, Bisseling T. 1999. Legume nodulation and mycorrhizae formation; two extremes in host specificity meet. *The EMBO Journal* **18**, 281–288.
- Albrecht C, Geurts R, Lapeyrie F, Bisseling T. 1998. Endomycorrhizae and rhizobial Nod factors both require SYM8 to induce the expression of the early nodulin genes *PsENOD5* and *PsENOD12A*. *The Plant Journal* **15**, 605–614.
- Allen MF, Allen EB, Friese CF. 1989. Responses of the non-mycotrophic plant *Salsola kali* to invasion by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **111**, 45–49.
- Balaji B, Ba AM, LaRue TA, Tepfer D, Piché Y. 1994. *Pisum sativum* mutants insensitive to nodulation are also insensitive to invasion *in vitro* by the mycorrhizal fungus, *Gigaspora margarita*. *Plant Science* **102**, 195–203.
- Balestrini R, José-Estanyol M, Puigdoménech P, Bonfante P. 1997. Hydroxyproline-rich glycoprotein mRNA accumulation in maize root cells colonized by an arbuscular mycorrhizal fungus as revealed by *in situ* hybridization. *Protoplasma* **198**, 36–42.
- Barea JM, Azcón-Aguilar C. 1982. Production of plant growth-regulating substances by vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Applied and Environmental Microbiology* **43**, 810–813.
- Beyrle H. 1995. The role of phytohormones in the function and biology of mycorrhizas. In: Varma A, Hock B, eds. *Mycorrhiza structure, function, molecular biology and biotechnology*. Berlin: Springer-Verlag, 365–390.
- Blee KA, Anderson AJ. 1996. Defense-related transcript accumulation in *Phaseolus vulgaris* L. colonized by the arbuscular mycorrhizal fungus *Glomus intraradices*, Schenk & Smith. *Plant Physiology* **110**, 675–688.
- Blee KA, Anderson AJ. 1998. Regulation of arbuscule formation by carbon in the plant. *The Plant Journal* **16**, 523–530.
- Blee KA, Anderson AJ. 2000. Defense responses in plants to arbuscular mycorrhizal fungi. In: Podila GK, Douds DD, eds. *Current advances in mycorrhizae research*. Minnesota, USA: The American Phytopathological Society, 27–44.
- Blilou I, Bueno P, Ocampo JA, García-Garrido JM. 2000a. Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae*. *Mycological Research* **104**, 722–725.
- Blilou I, Ocampo JA, García-Garrido JM. 1999. Resistance of pea roots to endomycorrhizal fungus or *Rhizobium* correlates with enhanced levels of endogenous salicylic acid. *Journal of Experimental Botany* **50**, 1663–1668.
- Blilou I, Ocampo JA, García-Garrido JM. 2000b. Induction of *Ltp* (Lipid transfer protein) and *Pal* (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*. *Journal of Experimental Botany* **51**, 1969–1977.
- Bonanomi A, Oetiker JH, Guggenheim R, Boller T, Wiemken A, Vögeli-Lange R. 2001. Arbuscular mycorrhizas in mini-mycorrhizotrons: first contact of *Medicago truncatula* roots with *Glomus intraradices* induces chalcone synthase. *New Phytologist* **150**, 573–582.
- Bonfante P. 2001. At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Esser K, Hock B, eds. *The Mycota IX*. Berlin, Heidelberg: Springer-Verlag, 45–61.
- Bonfante P, Genre A, Faccio A, Martini I, Schauser L, Stougaard J, Webb J, Parniske M. 2000. The *Lotus japonicus* *LjSym4* gene is required for the successful symbiotic infection of root epidermal cells. *Molecular Plant–Microbe Interactions* **13**, 1109–1120.
- Bonfante-Fasolo P, Perotto S. 1992. Plant and endomycorrhizal fungi: the cellular and molecular basis of their interaction. In: Verma D, ed. *Molecular signals in plant–microbe communications*. Boca Raton, FL: CRC Press, 445–470.
- Bradbury S, Peterson RL, Bowley SR. 1991. Interactions between three alfalfa nodulation genotypes and two *Glomus* species. *New Phytologist* **119**, 115–120.
- Catoira R, Galera C, de Billy F, Varma Penmetsa R, Journet E-P, Maillet F, Rosenberg C, Cook D, Gough C, Dénarié J. 2000. Four genes of *Medicago truncatula* controlling components of a Nod factor transduction pathway. *The Plant Cell* **12**, 1647–1665.
- Clarkson DT. 1985. Factors affecting mineral nutrient acquisition in plants. *Annual Review of Plant Physiology* **36**, 77–115.
- Dassi B, Dumas-Gaudot E, Asselin A, Richard C, Gianinazzi S. 1996. Chitinase and β -1,3-glucanase isoforms expressed in pea roots inoculated with arbuscular mycorrhizal or pathogenic fungi. *European Journal of Plant Pathology* **102**, 105–108.
- David R, Itzhaki H, Ginzberg I, Gafni Y, Galili G, Kapulnik Y. 1998. Suppression of tobacco basic chitinase gene expression in response to colonization by the arbuscular mycorrhizal fungus *Glomus intraradices*. *Molecular Plant–Microbe Interactions* **11**, 489–497.
- Dixon RA, Paiva NL. 1995. Stress-induced phenylpropanoid metabolism. *The Plant Cell* **7**, 1085–1097.
- Douds DD, Galvez L, Bécard G, Kapulnik Y. 1998. Regulation of arbuscular mycorrhizal development by plant host and fungus species in alfalfa. *New Phytologist* **138**, 27–35.
- Dumas-Gaudot E, Furlan V, Grenier J, Asselin A. 1992. New acidic chitinase isoform induced in tobacco roots by vesicular–arbuscular mycorrhizal fungi. *Mycorrhiza* **1**, 133–136.
- Franken P, Requena N, Bütehorn B, Krajinski F, Kuhn G, Laponin L, Mann P, Rhody D, Stommel M. 2000. Molecular analysis of the arbuscular mycorrhizas symbiosis. *Archives of Agronomy and Soil Science* **45**, 271–286.
- García-Garrido JM, García-Romera I, Ocampo JA. 1992. Cellulase production by the vesicular-arbuscular mycorrhizal fungus *G. mosseae*. *New Phytologist* **121**, 221–226.
- García-Garrido JM, García-Romera I, Parra-García MD, Ocampo JA. 1996. Purification of an arbuscular mycorrhizal endoglucanase from onion roots colonized by *Glomus mosseae*. *Soil Biology and Biochemistry* **25**, 1443–1449.
- García-Garrido JM, Tribak M, Rejón-Palomares A, Ocampo JA, García-Romera I. 2000. Hydrolytic enzymes and ability of arbuscular mycorrhizal fungi to colonize roots. *Journal of Experimental Botany* **51**, 1443–1448.
- García-Romera I, García-Garrido JM, Ocampo JA. 1991. Pectolytic enzymes in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *FEMS Microbiology Letters* **78**, 343–346.
- García-Romera I, García-Garrido JM, Rejón-Palomares A, Ocampo JA. 1997. Enzymatic mechanisms of penetration and development of arbuscular mycorrhizal fungi in plant. In: Pandl SG, ed. *Recent research developments in soil biology and biochemistry*. India: Research Signpost, 121–136.

- Gianinazzi-Pearson V.** 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. *The Plant Cell* **8**, 1871–1883.
- Gianinazzi-Pearson V, Dénarié J.** 1997. Red carpet genetic programmes for root endosymbioses. *Trends in Plant Science* **2**, 371–372.
- Gianinazzi-Pearson V, Dumas-Gaudot E, Gollotte A, Tahiri-Alaoui A, Gianinazzi S.** 1996. Cellular and molecular defense-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytologist* **133**, 45–57.
- Gianinazzi-Pearson V, Gianinazzi S, Guillemin JP, Trouvelot A, Duc G.** 1991. Genetic and cellular analysis of resistance to vesicular arbuscular (VA) mycorrhizal fungi in pea mutants. In: Hennecke H, Verma DPS, eds. *Advances in molecular genetics of plant-microbe interactions*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 336–342.
- Giovannetti M, Sbrana C.** 1998. Meeting a non-host: the behaviour of AM fungi. *Mycorrhiza* **8**, 123–130.
- Ginzberg I, David R, Shaul O, Elad Y, Wininger S, Ben-Dor B, Badani H, Fang Y, van Rhijn P, Li Y, Hirsch AM, Kapulnik Y.** 1998. *Glomus intraradices* colonization regulates gene expression in tobacco plants. *Symbiosis* **24**, 145–157.
- Gollotte A, Gianinazzi-Pearson V, Giovannetti M, Sbrana C, Avio L, Gianinazzi S.** 1993. Cellular localization and cytochemical probing of resistance reactions to arbuscular mycorrhizal fungi in a 'locus a' mutant *Pisum sativum* L. *Planta* **191**, 112–122.
- Harrison M.** 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 361–389.
- Harrison M, Dixon R.** 1993. Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular arbuscular mycorrhizal associations in roots of *Medicago truncatula*. *Molecular Plant-Microbe Interactions* **6**, 643–659.
- Harrison M, Dixon R.** 1994. Spatial patterns of expression of flavonoid/isoflavonoid pathway genes during interactions between roots of *Medicago truncatula* and the mycorrhizal fungus *Glomus versiforme*. *The Plant Journal* **6**, 9–20.
- Herbers K, Meuwly P, Frommer WB, Métraux J-P, Sonnewald U.** 1996. Systemic acquired resistance mediated by the ectopic expression of invertase, possible hexose sensing in the secretory pathway. *The Plant Cell* **8**, 793–803.
- Hirsch AM, Kapulnik Y.** 1998. Signal transduction pathways in mycorrhizal associations: comparisons with the *Rhizobium*-legume symbiosis. *Fungal Genetics and Biology* **23**, 205–212.
- Jasper DA, Robson AD, Abbott LK.** 1979. Phosphorus and the formation of vesicular-arbuscular mycorrhizas. *Soil Biology and Biochemistry* **11**, 501–505.
- Koide RT, Schreiner RP.** 1992. Regulation of the vesicular arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology Plant Molecular Biology* **43**, 557–581.
- Lambais MR.** 2000. Regulation of plant defence-related genes in arbuscular mycorrhizae. In: Podila GK, Douds DD, eds. *Current advances in mycorrhizae research*. Minnesota, USA: The American Phytopathological Society, 45–59.
- Lambais MR, Mehdy MC.** 1993. Suppression of endochitinase, β -1,3-endoglucanase and chalcone isomerase expression in bean vesicular-arbuscular mycorrhizal roots under different soil phosphate conditions. *Molecular Plant-Microbe Interactions* **6**, 75–83.
- Lambais MR, Mehdy MC.** 1996. Soybean roots infected by *Glomus intraradices* strains differing in infectivity exhibit differential chitinase and β -1,3-endoglucanase expression. *New Phytologist* **134**, 531–538.
- Lambais MR, Mehdy MC.** 1998. Spatial distribution of chitinases and β -1,3-glucanase transcripts in bean arbuscular mycorrhizal roots under low and high soil phosphate conditions. *New Phytologist* **140**, 33–42.
- Malamy J, Carr JP, Klessig DF, Raskin I.** 1990. Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* **250**, 1002–1004.
- Marsh JF, Schultze M.** 2001. Analysis of arbuscular mycorrhizas using symbiosis-defective plant mutants. *New Phytologist* **150**, 525–532.
- Martínez-Abarca F, Herrera-Cervera JA, Bueno P, Sanjuan J, Bisseling T, Olivares J.** 1998. Involvement of salicylic acid in the establishment of the *Rhizobium meliloti*-alfalfa symbiosis. *Molecular Plant-Microbe Interactions* **11**, 153–155.
- Métraux JP, Signer H, Ryals J, Ward E, Wyss-Benz M, Gaudin J, Raschdorf K, Schmid E, Blum W, Inverardi B.** 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* **250**, 1004–1006.
- Morandi D.** 1989. Effect of xenobiotics on endomycorrhizal infection and isoflavonoid accumulation in soybean roots. *Plant Physiology and Biochemistry* **27**, 697–670.
- Parniske M.** 2000. Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? *Current Opinion in Plant Biology* **3**, 320–328.
- Pozo MJ, Azcón-Aguilar C, Dumas-Gaudot E, Barea JM.** 1998. Chitinase and chitinase activities in tomato roots during interactions with arbuscular mycorrhizal fungi or *Phytophthora parasitica*. *Journal of Experimental Botany* **49**, 1729–1739.
- Ruíz-Lozano JM, Roussel H, Gianinazzi S, Gianinazzi-Pearson V.** 1999. Defense genes are differentially induced by a mycorrhizal fungus and *Rhizobium* sp. in wild-type and symbiosis-defective pea genotypes. *Molecular Plant-Microbe Interactions* **12**, 976–984.
- Salzer P, Bonanomi A, Beyer K, Vögeli-Lange R, Aeschbacher RA, Lang J, Wiemken A, Kim D, Cook DR, Boller T.** 2000. Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation and pathogen infection. *Molecular Plant-Microbe Interactions* **13**, 763–777.
- Salzer P, Boller T.** 2000. Elicitor-induced reactions in mycorrhizae and their suppression. In: Podila GK, Douds DD, eds. *Current advances in mycorrhizae research*. Minnesota, USA: The American Phytopathological Society, 1–10.
- Salzer P, Corbière H, Boller T.** 1999. Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus mosseae*. *Planta* **208**, 319–325.
- Shaul O, David R, Sinvani G, Ginzberg I, Ganon D, Wininger S, Ben-Dor B, Badani H, Ovdad N, Kapulnik Y.** 2000. Plant defense responses during arbuscular mycorrhiza symbiosis. In: Podila GK, Douds DD, eds. *Current advances in mycorrhizae research*. Minnesota, USA: The American Phytopathological Society, 61–68.
- Shinshi, H, Mohnen D, Meins F.** 1987. Regulation of a plant pathogenesis-related enzyme: inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin. *Proceedings of the National Academy of Sciences, USA* **84**, 89–93.
- Shirliffe SJ, Vessey JK.** 1996. A nodulation (Nod⁺/Fix⁻) mutant of *Phaseolus vulgaris* L. has nodule-like structures lacking peripheral vascular bundles (Pvb⁻) and is resistant to mycorrhizal infection (Myc⁻). *Plant Science* **118**, 209–220.
- Smith S, Read DJ. (eds)** 1997. *Mycorrhizal symbiosis*. London: Academic Press.

- Somssich IE, Hahlbrock K.** 1998. Pathogen defence in plants: a paradigm of biological complexity. *Trends in Plant Science* **3**, 86–90.
- Spanu P, Boller T, Ludwig A, Wiemken A, Faccio A.** 1989. Chitinase in roots of mycorrhizal *Allium porrum*: regulation and localization. *Planta* **177**, 447–455.
- Spanu P, Bonfante-Fasolo P.** 1988. Cell wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. *New Phytologist* **109**, 119–124.
- van Camp W, Van Montagu M, Inzé D.** 1998. H₂O₂ and NO: redox signals in disease resistance. *Trends in Plant Science* **3**, 330–334.
- van Rhijn P, Fang Y, Galili S, Shaul O, Atzmon N, Winer S, Eshed Y, Lum M, Li Y, To V, Kapulnik Y, Hirsch AM.** 1997. Expression of early nodulin genes in alfalfa mycorrhizae indicates that signal transduction pathways used in forming arbuscular mycorrhizae and *Rhizobium*-induced nodules may be conserved. *Proceedings of the National Academy of Sciences, USA* **94**, 5467–5472.
- Vierheilig H, Alt M, Lange J, Gut-Rella M, Wiemken A, Boller T.** 1995. Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Applied and Environmental Microbiology* **61**, 3031–3034.
- Vierheilig H, Alt M, Mohr U, Boller T, Wiemken A.** 1994. Ethylene biosynthesis and activities of chitinase and β -1,3-glucanase in the roots of host and non-host plants of vesicular-arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. *Journal of Plant Physiology* **143**, 337–343.
- Vierheilig H, Alt M, Neuhaus JM, Boller T, Wiemken A.** 1993. Colonization of transgenic *Nicotiana glauca* plants, expressing different forms of *Nicotiana glauca* chitinase, by root pathogen *Rhizoctonia solani* and by the mycorrhizal symbiont *Glomus mosseae*. *Molecular Plant–Microbe Interactions* **6**, 261–264.
- Vierheilig H, Knoblauch M, Juergensen K, van Bel AJE, Grundler FMW, Piché Y.** 2001. Imaging arbuscular mycorrhizal structures in living of *Nicotiana tabacum* by light, epifluorescence and confocal laser scanning microscopy. *Canadian Journal of Botany* **79**, 231–237.
- Vierheilig H, Piché Y.** 2002. Signaling in arbuscular mycorrhiza: facts and hypotheses. In: Manthey J, Buslig B, eds. *Flavonoids in the living system*. New York: Plenum Press (in press).
- Volpin H, Elkind Y, Okon Y, Kapulnik Y.** 1994. A vesicular arbuscular mycorrhizal fungus (*Glomus intraradices*) induces a defense response in alfalfa roots. *Plant Physiology* **104**, 683–689.
- Volpin H, Phillips DA, Ocón Y, Kapulnik Y.** 1995. Suppression of an isoflavonoid phytoalexin defense response in mycorrhizal alfalfa roots. *Plant Physiology* **108**, 1449–1454.
- Walker SA, Viprey V, Downie A.** 2000. Dissection of nodulation signalling using pea mutants defective for calcium spiking induced by Nod factors and chitin oligomers. *Proceedings of the National Academy of Sciences, USA* **21**, 13413–13418.
- Wegel E, Schauser L, Sandal N, Stougaard J, Parniske M.** 1998. Mycorrhiza mutants of *Lotus japonicus* define genetically independent steps during symbiotic infection. *Molecular Plant–Microbe Interactions* **11**, 933–936.
- Xie ZP, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton WJ.** 1995. Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. *Plant Physiology* **108**, 1519–1525.
- Zhou L, Jang J, Jones TL, Sheen J.** 1998. Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant. *Plant Biology* **95**, 10294–10299.