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REVIEW

Fungal and plant gene expression in arbuscular mycorrhizal symbiosis

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Abstract Arbuscular mycorrhizas (AMs) are a unique example of symbiosis between two eukaryotes, soil fungi and plants. This association induces important physiological changes in each partner that lead to reciprocal benefits, mainly in nutrient supply. The symbiosis results from modifications in plant and fungal cell organization caused by specific changes in gene expression. Recently, much effort has gone into studying these gene expression patterns to identify a wider spectrum of genes involved. We aim in this review to describe AM symbiosis in terms of current knowledge on plant and fungal gene expression profiles.

Keywords Arbuscular mycorrhizas · Cellular modification · Plant and fungal gene expression · Symbiosis · Functional genomics

Introduction

Gene expression studies during the establishment of arbuscular mycorrhiza (AM) symbiosis have targeted specific genes, especially those known to be involved in nutrient uptake and in other plant–microbe interactions. In addition, plant mutants have been key tools for the genetic dissection of AM development and have been instrumental in the identification of plant genes controlling specific steps (Kistner and Parniske 2002; Parniske 2004; Lévy et al. 2004; Kistner et al. 2005). The molecular similarity in symbiosis between legumes/Rhizobia and legumes/AM fungi has been a leading concept in the last decade (Gianinazzi-Pearson and Denarié 1997). The existence of plant mutants having nod$^-$ and myc$^-$ phenotypes (Duc et al. 1989; Hirsch and Kapulnik 1998; Albrecht et al. 1998; Marsh and Schultze 2001; Stougaard 2001; Morandi et al. 2005) has suggested a common genetic program for the two symbioses. Consequently, molecular probes developed for nodules have been used to investigate gene expression in AM hosts. The transcriptome—the mRNA pool of a cell at any one moment—can be analyzed using methods such as expressed sequence tag (EST) sequencing and macro/microarray analysis. They have revealed major changes in gene expression that accompany the establishment of AM and have identified a wide spectrum of genes involved (Journet et al. 2002; Liu et al. 2003, 2004; Wulf et al. 2003; Grunwald et al. 2004; Kuster et al. 2004; Manthey et al. 2004; Hohnjec et al. 2005; Güimil et al. 2005). In addition, differentially accumulating transcripts from AM developmental phases have been identified using various screening methods and plant/fungus systems (Martin-Laurent et al. 1997; Burleigh and Harrison 1997; Murphy et al. 1997; Krajinski et al. 1998; van Buuren et al. 1999; Weidmann et al. 2004; Maldonado-Mendoza et al. 2005). The introduction of advanced technologies (in vitro culture systems and methods based on polymerase chain reaction) has offered the opportunity to conduct a molecular investigation of the fungal partner too, thus overcoming the problem of the limited availability of biological material due to the obligate biotrophic status of the mycosymbiont. Gene expression profiles of AM fungi have also been explored by EST approaches based on the construction of

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cDNA libraries exclusively from fungal tissues (Lanfranco et al. 2000; Sawaki and Saito 2001; Stommel et al. 2001; Lammers et al. 2001; Jun et al. 2002).

After spore germination, establishment of the symbiosis includes hyphal branching, appressorium development after contacting the root, colonization of the root cortex, formation of intracellular arbuscules, and concomitantly, production of an extraradical mycelium from which spores are eventually formed (Smith and Read 1997; Fig. 1). At the molecular level, signals are exchanged between the partners, leading to stage-specific patterns of gene expression. The corresponding gene products could be responsible for the morphological and physiological changes necessary for integration of the two partners into one association. A number of recent reviews have discussed specific aspects of the AM symbiosis, i.e., signaling, nutrition, and plant–fungus interaction (Harrison 2005; Karandashov and Bucher 2005; Hause and Fester 2005; Balestrini and Bonfante 2005; Genre and Bonfante 2005). The aim of this review is to discuss the AM symbiosis on the basis of current knowledge on plant and fungal gene expression profiles (Table 1).

The fungal asymbiotic stage

The fungal asymbiotic phase is restricted to spore germination and production of a limited amount of mycelium, which also occurs in the absence of the host plant. This phase has been the object of transcriptome analyses with the construction of cDNA libraries from activated spores of *Gigaspora rosea* (Stommel et al. 2001) and germinated spores of *Gigaspora margarita* (Lanfranco et al. 2000; Podila and Lanfranco 2004) and of *Glomus intraradices*

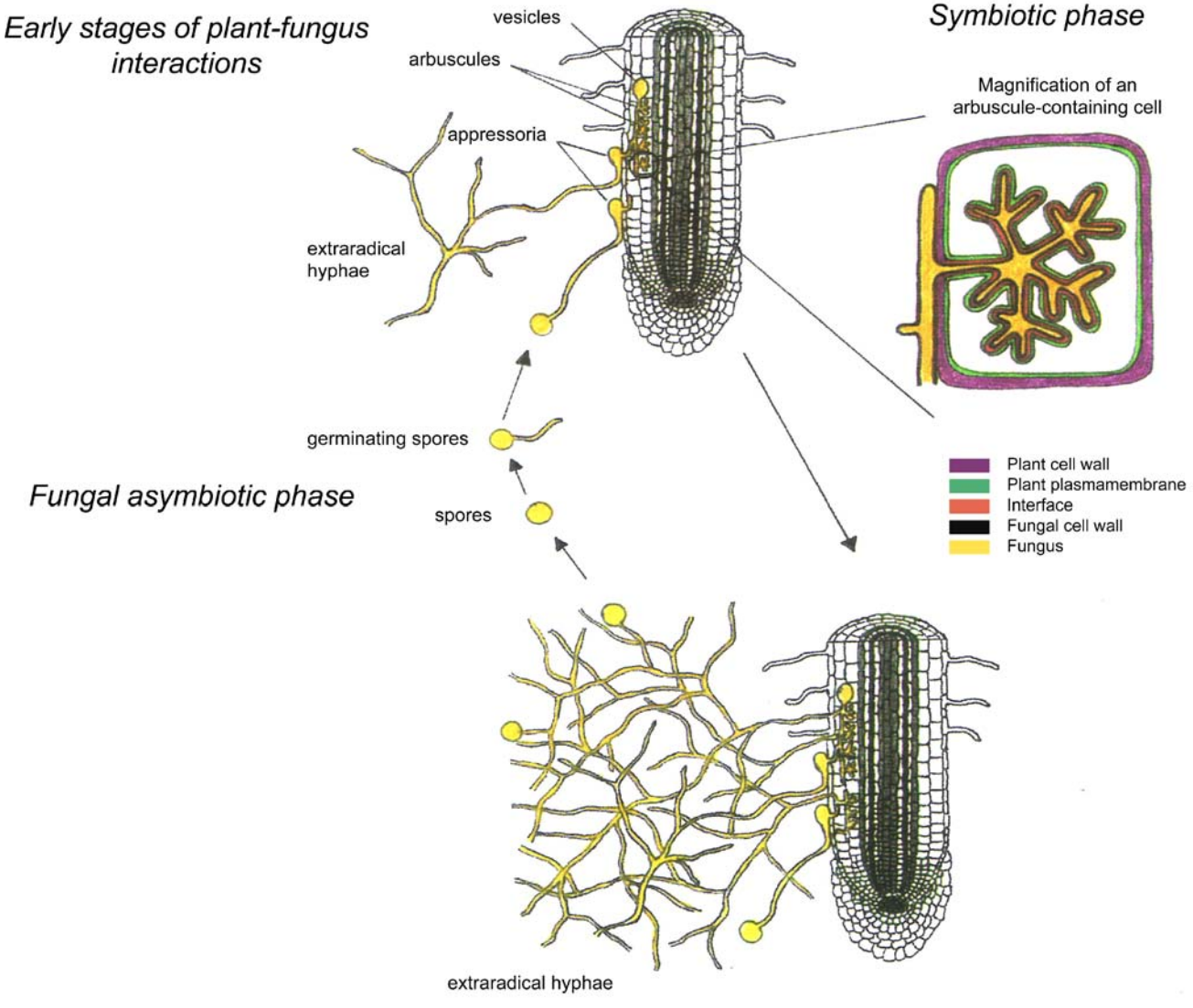


Fig. 1 Scheme of the different stages of root colonization by an arbuscular mycorrhizal fungus

(Lammers et al. 2001). These tools have allowed rapid identification of increased numbers of sequences expressed in AM fungi and are valuable for characterizing specific genes involved in carbohydrate metabolism (Lammers et al. 2001; Bago et al. 2003) and in stress responses (Lanfranco et al. 2002, 2005).

A crucial point for understanding the biology of AM fungi is to explain the basis of their obligate biotrophism, the molecular mechanisms of which are unknown. In particular, the reason why mycelium growing in vitro shows growth arrest 2–3 weeks after spore germination is unknown. Cytological studies have shown that nuclear division, which occurs in nuclei within germination hyphae, is necessary for asymbiotic hyphal growth (Bianciotto et al. 1995; Requena et al. 2000). Requena et al. (2000) characterized the *GmTOR2* gene, a homolog to the cell cycle checkpoint gene *TOR2* from *Saccharomyces cerevisiae*, in *Glomus mosseae* and hypothesized that *GmTOR2* may control cell cycle arrest in the absence of the plant as a response to nutrient starvation.

As mentioned above, an AM fungus relies almost entirely on the host plant for its carbon supply. Investigations on C metabolism in AM fungi have proved useful to offer some explanation for their obligate biotrophism. The asymbiotic phase is inefficient in the uptake of hexoses, and hyphal growth is likely to be supported by the mobilization of triacylglycerides and glycogen, the main carbon storage compounds of spores (Beilby and Kidby 1980; Bonfante et al. 1994; Gaspar et al. 1994). Expression of lipid breakdown genes was found from small-scale random sequencing of an EST collection of germinating spores (Bago et al. 2002). Moreover, genes of the glyoxylate cycle, which converts lipids into carbohydrates, are expressed at this stage (Lammers et al. 2001). ^{14}C labeling experiments indicate that fatty acid (FA) synthesis does not occur in germinating spores or in extraradical mycelium, suggesting that this metabolic deficit may be related to the fact that AM fungi are obligate symbionts (Trépanier et al. 2005). Unfortunately, at the moment very little is known about genes involved in FA metabolism in AM fungi.

Comparison of transcript profiles between germination hyphae and external mycelium has identified an interesting gene in *G. mosseae*, *GmGIN1*, possibly involved in signaling during spore germination before symbiosis formation. *GmGIN1*, which has a two-domain structure with putative self-splicing activity, is highly expressed during the asymbiotic stage and completely silenced during the symbiosis, both in the intraradical structures and the extraradical mycelium (Requena et al. 2002).

Notwithstanding the widely documented interactions between AM fungi and other soil microorganisms (Bianciotto et al. 2002), only one paper deals with this topic, as regards transcriptome analyses. Requena et al. (1999) monitored changes in *G. mosseae* gene expression in response to *Bacillus subtilis*, a rhizobacterium able to increase mycelial growth. In particular, a highly conserved gene, *GmFOX2*, encoding a multifunctional protein of peroxisomal β-oxidation, was shown to be regulated upon exposure of hyphae to bacterial cells. This is the first indication that AM fungi can respond to external signals other than those produced by host plants.

Early stages of plant–fungus interactions

Development of the AM symbiosis requires both partners to undergo significant morphological changes that are assumed to be coordinated by a reciprocal exchange of signals (Harrison 2005). Little is known about the molecular interactions before and during initial contact between AM fungi and plant roots, mainly due to the difficulties in synchronizing development in the two symbionts (Weidmann et al. 2004). A further step in the plant–fungus dialogue is the formation of appressoria, structures responsible for contact with and penetration of the plant tissues. Root colonization relies on discrete steps that are cell-layer controlled (Bonfante et al. 2000). Appressorium development must be triggered by the topography of epidermal cells, although intact and living cells are necessary for further colonization (Nagahashi and Douds 1997). Several studies using targeted approaches have detected weak and transient activation of plant defense genes during appressorium formation on different plants (Bonanomi et al. 2001; Blilou et al. 2000; Ruíz-Lozano et al. 1999). Nontargeted suppression subtractive hybridization (SSH) has also identified some plant genes expressed in the early stages of *Medicago truncatula–G. mosseae* interactions. Genes encoding germin-like, nodulin 26-like and four other proteins of unknown function were activated at the appressorium stage (Brechenmacher et al. 2004), suggesting a role in this early stage of mycorrhiza formation. Other genes encoding a cell wall protein (PsENOD12A; Albrecht et al. 1998) and a C1p serine protease (Roussel et al. 2001) are activated in pea roots during appressoria formation.

Several papers report the activation of *ENOD11*, a *M. truncatula* gene encoding a putative cell wall repetitive proline-rich protein, during the early stages of root nodulation and of root colonization by AM fungi (Journet et al. 2001; Chabaud et al. 2002; Genre et al. 2005). In a recent work, Genre et al. (2005) have shown that host plant epidermal cells deeply reorganize their cytoplasm upon simple adhesion of the AM fungus to the root surface. The plant cell constructs a novel apoplastic compartment by organizing a pre-penetration apparatus, or PPA, where the fungus is destined to penetrate (Genre et al. 2005). *ENOD11*

has been shown to be activated in epidermal cells both before and during PPA formation. These observations raise questions as to the gene expression scenario associated with PPA formation. Moreover, *MtENOD11* induction was also found in root cortical tissues without cell contact between the partners (Kosuta et al. 2003). This was the first experimental evidence for the existence of AM fungus-derived diffusible signal molecule(s). Hyphal exudates from AM fungi have also been found to stimulate lateral root formation (Olah et al. 2005), but it is not known whether these two different pathways are activated by the same fungal compound.

Other plant genes, which are upregulated both by hyphal exudates when the AM partners are not in direct physical contact and by appressorium formation, have been reported by Weidmann et al. (2004). Among these are genes encoding plant proteins with predicted functions in cell wall modifications and, as might be expected, in signal transduction and translation pathways (i.e., mitogen-activated protein kinase, calcium lipid binding protein, annexin). Perception of diffusible fungal molecules by the host root before appressorium formation is also suggested from the recent observation that an AM fungus can elicit expression of tomato genes during precontact stages (Demartsev et al. 2005). Genes involved in cell wall modification, tissue repair, response to pathogens, and oxidative stress were already regulated 48 h after exposure to fungal inoculants (Demartsev et al. 2005). Interestingly, expansins seem to be involved during initial cell-to-cell contact as indicated by the observations of Weidmann et al. (2004) and Demartsev et al. (2005). This class of extracellular proteins may be operating during epidermal penetration in construction of the interface surrounding the infection hypha and/or may be involved in the generation of signaling molecules from the plant cell wall.

It is worth noting that three genes (*DMI1*, *DMI2*, *DMI3*) involved in the early stages of signal trasduction common to both nodulation and AM formation have been identified in *Medicago* by positional cloning in myc$^-$ mutants, and homologs have been described in other plants (Parniske 2004; Oldroyd et al. 2005). In particular, *DMI2*, a receptor-like kinase, and *DMI1*, a predicted ion channel, act upstream of calcium spiking (a physiological benchmark described in root hairs after Nod factor treatment). Meanwhile, *DMI3*, a Ca^{2+} and calmodulin-dependent protein kinase, acts downstream (Ané et al. 2004; Endre et al. 2002; Lévy et al. 2004; Oldroyd et al. 2005). Although there is no direct evidence of calcium spiking, these data indicate a possible role of calcium also in early cellular interactions between *M. truncatula* and AM fungi (Lévy et al. 2004; Sanchez et al. 2005). *DMI3*-dependent activation of ten plant genes with putative functions in signal transduction, gene expression/RNA metabolism, membrane transport, or responses to abiotic stimuli and development has recently been reported in roots of *M. truncatula* during the early stages of interactions with *Pseudomonas fluorescens* and with *G. mosseae*, thus suggesting common molecular processes in the perception of microbial signals by plant roots (Sanchez et al. 2005). Surprisingly, notwithstanding molecular and genetic similarities in AM and N_2-fixing symbioses, *Sinorhizobium meliloti* only activated one of these genes and inhibited four others. The differences observed may be linked to the degree of host specificity and, at least partly, to the fact that root colonization by N_2-fixing bacteria results in profound cellular reorganization of host-root tissues and in the formation of a new organ (Brewin 2004). This is a clear example of the usefulness of myc$^-$ mutants in dissecting plant–microbe interactions.

Taken as a whole, these results show that the signaling pathway leading to transcriptional changes in mycorrhizal symbiosis is activated in roots by AM fungal molecules and that plant gene activation also occurs without contact between the symbionts. It will be interesting to define the temporal and spatial expression pattern at the root surface challenged with AM fungi or fungal exudates to verify whether this is due to the activity of cell-autonomous or nonautonomous signals.

Meeting the host is a crucial event for the fungal partner. AM fungi recognize their host plants and respond to their proximity. Mosse and Hepper (1975), Giovannetti et al. (1993), Buée et al. (2000), and Nagahashi and Douds (2000) showed that only molecules released from the host plant stimulate hyphal branching in AM fungi, indicating that discrimination between host and non-host already occurs at this stage. The active molecules (branching factors) released by *Lotus japonicus* were recently characterized as sesquiterpenes (Akiyama et al. 2005). So far, only one publication has described the molecular responses of AM fungi to host root exudates (Tamasloukht et al. 2003). The induction of genes encoding mitochondrial proteins occurred within 60 min of exposure before an increase in respiratory activity as measured by oxygen consumption. Hyphal branching becomes evident only later. These findings clearly indicate that the fungus possesses the mechanisms to perceive active root molecules and to switch on specific transcriptional pathways. This signaling cascade appears necessary to induce morphological changes in the fungus and to activate growth.

The differentiation of appressoria is a crucial step for the fungus. Appressorium formation induces in *G. mosseae* an increase in expression of an H^+-ATPase gene (Requena et al. 2003), which correlates with previous studies showing an increase in ATPase activity (Lei et al. 1991) and membrane depolarization (Ayling et al. 2000) when the fungal hypha contacts the root. A number of genes have been identified as upregulated in *G. mosseae* sporocarps

attached to mycelia producing appressoria (Breuninger and Requena 2004). Most of the clones (63%) corresponded to gene fragments with no similarity to sequences listed in the databank and may represent putative novel genes specific for mycorrhizal symbiosis. Ca^{2+} likely plays a role as a second messenger during appressorium formation, since several homologs to genes related to Ca^{2+} signaling were found. This has also been reported for fungal plant pathogens, indicating that pathogens and symbiotic fungi may exploit, at least in part, an evolutionarily conserved signaling cascade.

The symbiotic phase

In recent years, significant progress has been made in identifying an increasing number of mycorrhiza-induced plant genes. The use of ESTs (Journet et al. 2002; Liu et al. 2003), cDNA arrays (Liu et al. 2003, 2004; Manthey et al. 2004; Grunwald et al. 2004; Hohnjec et al. 2005; Güimil et al. 2005), screening methods to search for differentially expressed transcripts (van Buuren et al. 1999; Grunwald et al. 2004; Wulf et al. 2003; Brechenmacher et al. 2004) and of targeted approaches, has resulted in uncovering the complexity of transcriptional changes during later stages of the AM symbiosis. On the fungal side, the symbiotic phase comprises two distinct compartments: intraradical structures (intercellular hyphae, intracellular coils, arbuscules) and extraradical structures consisting of external mycelium and frequently spores. These compartments are structurally and functionally different, particularly concerning the uptake and transfer of main nutrients (P, N, C) and are therefore characterized by specific gene expression profiles. Data from mycorrhizal root analyses mainly refer to the host plant since fungal RNA is hardly represented even in well-colonized roots (Maldonado-Mendoza et al. 2002).

The transcript profile of *M. truncatula* roots has been investigated in depth during the AM symbiosis (Journet et al. 2002; Liu et al. 2003, 2004; Manthey et al. 2004; Hohnjec et al. 2005). The first *M. truncatula* cDNA macroarray, containing 2,268 genes, was analyzed in a time course experiment (Liu et al. 2003). Two distinct temporal expression patterns were found. Members of one group, i.e., defense and stress-related genes, showed an increase in transcripts during the initial period of contact between the partners and a subsequent decrease during development of the symbiosis. This is in line with previous studies targeting specific genes (Garcia-Garrido and Ocampo 2002 and references therein). In the second group, there were genes showing a sustained increase in transcript levels during colonization, with a significant proportion of new genes similar to signaling proteins (Liu et al. 2003, 2004). Genes potentially involved in signal transduction, i.e., a mitogen-activated protein kinase and a serine/threonine protease, showed the highest induction also in the late stages of AM interactions between *Pisum sativum* and *G. mosseae* (Grunwald et al. 2004).

Novel genes previously not known to be induced in *M. truncatula* arbuscular mycorrhiza have recently been reported (Frenzel et al. 2005). Apart from genes connected to different transport and signaling processes, a novel family of AM-specific lectin genes was identified. Reporter gene fusions revealed an arbuscule-specific expression of two members of this gene family, indicating a possible role for AM-specific lectins during arbuscule formation or functioning. Three different *M. truncatula* lectin-like genes were previously described as induced in mycorrhizal roots (Wulf et al. 2003) and expression of a transcript corresponding to a gene encoding a lectin-like glycoprotein (PsNLEC-1), strongly expressed in root nodules, already demonstrated in *P. sativum* mycorrhizal roots (Balestrini et al. 1999). Identification of numerous different lectin-like sequences, which show increased RNA accumulation in mycorrhizal roots, indicates an important role for these proteins during AM symbiosis (Frenzel et al. 2005).

In 2005 two more comprehensive representations of plant genomes (*M. truncatula* and rice) were considered for microarray analyses during AM symbiosis (Hohnjec et al. 2005; Güimil et al. 2005). They both focused on a mature stage of the association, and early stages may be underrepresented. In detail, a 16086-probe oligo microarray was used to highlight the overlapping genetic program activated in *M. truncatula* roots by two different AM fungi, *G. mosseae* and *G. intraradices* (Hohnjec et al. 2005). A total of 201 plant genes were significantly co-induced in response to both fungi, with more than 160 being reported as AM-induced for the first time, while 176 were downregulated. In agreement with the fact that establishment of an AM symbiosis significantly alters the plant root metabolism, several upregulated genes encoded products related to primary metabolism (i.e., enzymes related to FA metabolism and three different genes encoding different blue copper proteins). There were also enzymes involved in secondary metabolism, components of signal transduction pathways (i.e., receptor kinases), and transcription factors. Interestingly, several hundred genes were additionally upregulated during a single interaction, suggesting that the plant genetic program activated in AM to some extent depends on the colonizing microsymbiont (Hohnjec et al. 2005).

Whole rice transcriptome analyses showed 224 upregulated and 15 downregulated genes (Güimil et al. 2005). Twelve genes, exclusively expressed in mycorrhizal roots, have been proposed as markers for AM symbiosis. Of these, one was found to code for a putative peroxidase

(OsAM1), the gene most strongly induced in mycorrhiza. Interestingly, 43% of mycorrhiza-induced rice genes responded similarly to infection by fungal pathogens, suggesting conservation of transcriptional activation pathways. In addition, about one third of the mycorrhiza-responsive genes of rice were found to match homologous sequences in dicotyledonous plants already reported to be upregulated in AM symbiosis, suggesting a relatively conserved mechanism.

Genes transcriptionally activated in AM roots and encoded proteins associated with different cellular, metabolic, and regulatory processes are discussed in the following sections.

Genes associated with cellular modifications

Intracellular colonization by AM fungi dramatically changes the morphological organization of host cells (Bonfante 2001). In particular, a new interface compartment is created, which contains material similar in composition to the plant cell wall. Investigations on the expression of genes related to cell wall metabolism, as well as information gained from recent functional genomic studies, have led to new ideas on the genesis of the interface compartment (Balestrini and Bonfante 2005 and references therein). Genes encoding both a putative AGP and an HRGP are induced in mycorrhizal roots of *M. truncatula* and maize, respectively, and the transcripts are specifically localized in the cells containing arbuscules (van Buuren et al. 1999; Balestrini et al. 1997). Several of the ESTs identified by Grunwald et al. (2004) belong to gene products involved in cell wall modification. One encodes an extensin-like glycoprotein that belongs to a large protein family characterized by (hydroxy)proline-rich motifs. Members of this family accumulate during both nodule and mycorrhiza development (Journet et al. 2001; Balestrini et al. 1997; Hohnjec et al. 2005). Using cDNA arrays, Liu et al. (2003) observed that the *MtCel1* gene was specifically induced during symbiosis. The *MtCel1* product shares identity with members of the E-type Egase subfamily III (Brummell et al. 1997), which have been associated with expanding tissues and cellulose synthesis (Brummell et al. 1997). In transgenic mycorrhizal roots, *MtCel1* expression was located in arbuscule-containing cells and, considering the membrane domain, it was suggested that *MtCel1* is located in the periarbuscular membrane and involved in assembling the cellulose/hemicellulose matrix at the interface (Liu et al. 2003). Three different endoglucanases with different preferences for sugar bonds and four different pectolytic or polygalacturonate-degrading enzymes were co-induced in *G. mosseae* and *G. intraradices*-colonized *Medicago* roots (Hohnjec et al. 2005). In addition to an alpha-D-xylosidase involved in the degradation of complex carbohydrates, these enzymes could modify the extracellular matrix during fungal colonization and during formation of the periarbuscular matrix (Hohnjec et al. 2005). Cell wall modifications in AM symbiosis are also suggested by the induction of a gene encoding an osmotin/thaumatin-like protein (Hohnjec et al. 2005). These proteins are known to be resistant to proteases and to denaturation, and the corresponding genes are described as being induced in response to osmotic stress or in response to fungi.

Expansins, extracellular proteins with a possible role in wall-loosening, are also activated during intracellular colonization by AM fungi and during early stages of mycorrhizal interactions. Recently, they have been located in cucumber mycorrhizal roots where they could be involved in keeping the interfacial material loose and/or in cell wall loosening and cell enlargement (Balestrini et al. 2005). Preliminary results, obtained by semiquantitative reverse transcription-PCR, suggest that upregulation of expansin genes occurs in mycorrhizal roots (R. Balestrini, unpublished results), indicating that this class of proteins involved in cell wall loosening could be crucial in accommodating the fungus inside cortical cells. Upregulation of an expansin gene in mycorrhizal roots has also been found in *M. truncatula* by *in silico* analysis of ESTs (Journet et al. 2001) and by cDNA array experiments (Liu et al. 2004).

The gene *Mt-XHT1*, induced in *M. truncatula* roots during the associations with both *Glomus* and *Gigaspora* species, has been recently identified (Maldonado-Mendoza et al. 2005). Xyloglucan endotransglucosylases/hydrolases (XHTs) catalyze the hydrolysis and transglycosylation of xyloglucan polymers in plant cell walls. Analysis of transgenic roots expressing an *Mt-XHT1* fusion promoter showed that expression is enhanced not only in the root regions colonized by the fungus, but also at sites distal to these regions. Based on this expression pattern, it was suggested that Mt-XHT is involved in systemic modification of cell wall structure to enable fungal penetration of roots.

During intracellular colonization, the cytoskeleton of invaded cortical cells undergoes massive transient rearrangement, presumably to enable development of the arbuscule interface (Genre and Bonfante 2005). In addition, recent data suggest that such reorganization may be induced by signals before the fungus penetrates the cell (Blancaflor et al. 2001; Genre et al. 2005). The activation of an α-tubulin promoter in arbuscule-containing cells (Bonfante et al. 1996) and enhanced mRNA accumulation of a β-tubulin gene (Grunwald et al. 2004) in AM symbioses have been demonstrated. The induction of a β-tubulin gene predominantly in arbuscule-containing cells was also detected in *M. truncatula* using a promoter gene strategy (Manthey et al. 2004). On the fungal side,

investigation on β-tubulin genes from AM fungi indicates that they are modulated during symbiosis (Rhody et al. 2003; Delp et al. 2003). These results clearly show that plant and fungal tubulins cannot be used as housekeeping genes in gene expression analysis. As alternatives, elongation factor-1-alpha (*EF-1α*), glyceraldehyde phosphate dehydrogenase (*Gapdh*, *Mtgap1*), and ribosomal genes have been used (i.e., Weidmann et al. 2004; Grunwald et al. 2004; Maldonado-Mendoza et al. 2005; Sanchez et al. 2005; Liu et al. 2003; Breuninger and Requena 2004; Lanfranco et al. 2005).

Modifications in nuclear ploidy have also been observed in AM-colonized root cells (Berta et al. 2000). The formation of 8C nuclei might be related to the upregulation of a gene belonging to the cullin family, which has been associated with control of endoreduplication (Tahiri-Alaoui et al. 2002).

Genes associated with defense

Defense-related gene expression has been extensively documented in AM associations by different experimental approaches. Bulk sample analyses of mycorrhizal roots show that defense-response genes usually have transiently increased expression especially during the early stages, and then levels decline (Spanu et al. 1989; Harrison and Dixon 1993; Kapulnik et al. 1996; Liu et al. 2003; Ruíz-Lozano et al. 1999). However, in situ experiments reveal that some transcripts related to plant defense responses are exclusively detected in arbuscule-containing cells (Balestrini et al. 1997; Blee and Anderson 2000; Harrison and Dixon 1993, 1994; Lambais and Mehdy 1993, 1998; Salzer et al. 2000), suggesting a very localized response. In *M. truncatula*, the class III chitinase gene family was expressed in cells containing active arbuscules (Salzer et al. 2000; Bonanomi et al. 2001), whereas pathogen-induced chitinase genes were not transcribed. These enzymes may play a role during arbuscule formation in cleaving fungal elicitors, so reducing/inactivating their eventual induction of a defense response.

GmarCuZnSOD, a CuZn superoxide dismutase-encoding gene, has recently been characterized in the AM fungus *G. margarita* (Lanfranco et al. 2005). SODs rapidly convert superoxide to hydrogen peroxide and act as a primary defense against reactive oxygen species (ROS) during oxidative stress. Accumulation of ROS was in fact reported in AM roots, and both plant and fungal cells seem to be involved (Salzer et al. 1999; Fester and Hause 2005; Lanfranco et al. 2005). Fungal ROS scavenging systems such as SOD might be required to control and overcome the plant defense responses at a site that is crucial to AM symbiotic function. On the plant side, AM symbiosis downregulates the expression of SOD genes in lettuce under well-watered conditions. In contrast, AM colonization in combination with drought stress increases expression of the *Mn-sodII* gene, suggesting that this may mediate the protection against drought exerted by AM fungi on host plants (Ruiz-Lozano et al. 2001). Interestingly, several AM-specific transcripts coding for H_2O_2-producing and scavenging enzymes (i.e., germin-like proteins) were found in rice (Güimil et al. 2005) and *M. truncatula* (Doll et al. 2003), confirming the role of ROS during the interaction.

The host plant cell also contributes in maintaining arbuscule function. A gene encoding a plant leghemoglobin, *VfLb29*, was recently shown to be specifically upregulated in arbuscule-containing cells (Vieweg et al. 2004; Fehlberg et al. 2005). Besides their role in oxygen supply, plant leghemoglobins are thought to bind nitric oxide (NO), a molecule crucial to plant defense against pathogens (Delledonne et al. 2001). It has been speculated that the *VfLb29* gene may encode NO-scavenging activity and therefore help to suppress defense responses in arbuscule-containing cells (Vieweg et al. 2004).

A number of genes identified during arbuscule development by Grunwald et al. (2004) have been reported in other systems as stress-related genes (i.e., metallothioneins). It was proposed that accumulation of the corresponding gene products could contribute to enhanced resistance of mycorrhizal roots to soil-borne fungal pathogens, as this type of bioprotection has been shown to be linked to the development of functional arbuscules (Slezack et al. 2000). These genes could also be involved in the control of fungal development and could be operating during arbuscule degradation where detoxification and removal of the fungal material is necessary (Grunwald et al. 2004).

Glutathione S-transferase transcripts have been found to accumulate in the arbuscule stage of mycorrhiza (Brechenmacher et al. 2004; Wulf et al. 2003). Although this gene could be involved in defense responses against the fungus, it may also be involved in arbuscule senescence (Brechenmacher et al. 2004). This may also be true for the metallothioneins, which are also expressed during pathogenesis and senescence (Grunwald et al. 2004). In contrast, an MT gene from *G. margarita* is expressed at a very low level during the symbiotic phase (Lanfranco et al. 2002).

Increased transcription of genes involved in carotenoid synthesis has been related to mycorrhizal colonization in several plants (Walter et al. 2000; Fester et al. 2002a; Hans et al. 2004; Hohnjec et al. 2005). This coincides with the accumulation in mycorrhizal roots of several apocarotenoids such as mycorradicin and cyclohexenones (Fester et al. 2002a; Hans et al. 2004 and references therein), the synthesis of which probably takes place via the plastid-located non-mevalonate methylerythritol phosphate pathway (Walter et al. 2000). Carotenoids are potent antioxidants and

they could provide efficient protection against ROS generated during the life cycle of arbuscules (Hans et al. 2004; Fester et al. 2002b; Fester and Hause 2005). Interestingly, the mutation of a gene encoding a protein localized in plastids results in a myc$^-$ phenotype in *L. japonicus* (Imaizumi-Anraku et al. 2005).

Genes associated with nutrient uptake

Morphological changes during AM colonization mirror physiological ones crucial for symbiosis, that is, for nutrient exchange between the partners. The characterization in *Glomus versiforme* of a high affinity Pi transporter (PT), expressed in extraradical hyphae (Harrison and van Buuren 1995) and thus involved in the uptake of P from the soil, was a milestone in AM research. Detailed gene expression data have been obtained for the homologous gene in *G. intraradices* using an in vitro culture system (Maldonado-Mendoza et al. 2001). The gene showed a regulation pattern typical of fungal and plant high-affinity PT, with induction at the transcriptional level by low amounts of Pi, comparable to the micromolar concentration found in soil. Another PT homolog (*GmosPT*) has recently been described in *G. mosseae*. *GmosPT* showed an expression pattern similar to the *G. intraradices* PT gene. Interestingly, a relatively high expression level, independent of external Pi concentrations, was observed in intraradical fungal structures (Benedetto et al. 2005). This presence of PT transcripts suggests that inside the root the fungus may exert control over the amount of phosphate delivered to the plant. This would agree with the idea that Pi fluxes between the two symbionts rely on complex mechanisms. According to these hypotheses, *G. intraradices* gene expression also responds to overall mycorrhiza Pi content (Maldonado-Mendoza et al. 2001), and uptake of Pi by the fungus and its transfer to the host in axenic cultures of transformed mycorrhizal roots has been shown to be stimulated by plant carbon availability (Bucking and Shachar-Hill 2005). In addition to phosphate transporters, genes encoding alkaline phosphatases have been described in *G. intraradices* and *G. margarita*. In detail, levels of the corresponding transcripts were higher in mycorrhizal roots than in germinating spores and external hyphae, suggesting that they may play a role in nutrient exchanges with host plants (Aono et al. 2004). These data support previous investigations that have led to this enzyme being used as an efficiency marker (Tisserant et al. 1993; Gianinazzi-Pearson et al. 1995).

On the plant side, phosphate transporters operating at the root–soil interface are downregulated, and the plant relies largely on phosphate delivered by the fungal symbiont (Smith et al. 2003). Investigations performed on host plants such as potato, rice, and *M. truncatula* highlighted the presence of Pi transporters exclusively expressed during symbiosis (Rosewarne et al. 1999; Rausch et al. 2001; Harrison et al. 2002; Paszkowski et al. 2002; Karandashov and Bucher 2005). In particular, *MtPT4* from *M. truncatula* was shown to be located in the periarbuscular membrane, at the interface between arbuscules and the invaginated plant cell membrane, generally assumed as the site of nutrient exchanges (Harrison et al. 2002; Bago et al. 2000). Here, it could be involved in the uptake of phosphate released from the arbuscule. It is also worth mentioning that a gene (*Mtha1*) for a plasma membrane H^+ATPase is upregulated in *M. truncatula* during AM symbiosis (Manthey et al. 2004), and *Mtha1* transcripts accumulate in arbuscule-containing cells (Krajinski et al. 2002). In tobacco, corresponding protein has been located at the plant membrane in the symbiotic interface (Gianinazzi-Pearson et al. 2000). A possible role in sustaining transport processes across the periarbuscular membrane is strongly suggested.

Karandashov and Bucher (2005) have recently shown that the potato phosphate transporter gene *StPT3* is expressed in root cells harboring various mycorrhizal structures, including thick-coiled hyphae, and that cell–cell contact between the partners is required to induce phosphate transport. In addition, the data suggest that signal perception and the intracellular transduction pathway involved in mycorrhiza-specific regulation of phosphate transport are conserved in different orders of eudicot host plants (Karandashov and Bucher 2005). Novel and additional mycorrhiza-inducible Pi transporters from solanaceous species (tomato and potato) and rice have recently been described (Nagy et al. 2005; Güimil et al. 2005). Redundancy within mycorrhiza-inducible Pi transport pathways might ensure that symbiotic Pi transfer will be evolutionarily robust and relatively insensitive to mutation (Nagy et al. 2005).

At present, *MtPT4* is the only *M. truncatula* PT gene strongly induced by AM colonization. In accordance with the hypothesis that all inorganic phosphate is delivered via the mycorrhizal fungus (Smith et al. 2003), *MtPT1* and *MtPT2* genes, coding for components of the direct pathway of inorganic phosphate uptake, were downregulated during both *G. versiforme* or *G. intraradices* AM symbiosis (Versaw et al. 2002; Hohnjec et al. 2005). Interestingly, these genes were slightly induced or not regulated during the interaction with *G. mossae*, suggesting possible differences in the regulation of phosphate transporters in different AM interactions. In addition, another co-repressed PT gene (TC84790), which could be representative of the direct uptake system, has also been identified (Hohnjec et al. 2005).

After identification of a gene encoding a mycorrhiza-responsive acid phosphatase in *Tagetes patula*, a new mechanism for acquisition of P in arbuscular mycorrhizal

Table 1 List of genes reported to be transcriptionally induced or regulated in a specific stage of AM symbiosis development

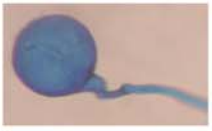
	Specific stage/ structure	Metabolic pathway/ functional category	Genes/Gene analyses	References
Fungal asymbiotic phase	- Spores		**EST**[a]	Stommel et al., 2001
	- *In vitro* germinating spores		**EST**[b]	Lanfranco et al., 2000, 2002;
		Stress defence	Metallothionein[b]	Podila & Lanfranco, 2004
		Lipid utilization	Acyl-CoA dehydrogenase[c]	Bago et al., 2002
			EST[c]	Lammers et al., 2001
		Glyoxylate cycle	Isocitrate lyase[c]	
			Malate synthase[c]	
		C metabolism	Glycogen synthase[c]	Bago et al., 2003
		Cell cycle	TOR homolog[d]	Requena et al., 2000
		Signalling ?	GmGIN1[d]	Requena et al., 2002
	- *In vitro* germinating spores interacting with rhizosphere bacteria	Peroxisomal β-oxidation	FOX[d]	Requena et al., 1999
Early stages of plant-fungus interaction	- Before contact	Cell-wall modification, signal transduction, translation pathways	EST[f]	Weidmann et al., 2004
		Cell wall/signalling	*MtENOD11*	Kosuta et al., 2003
	- Germinating spores exposed to root exudates	Mitochondria functioning	Pyruvate carboxylase[a]	Tamasloukht et al., 2003
	- Appressorium /contact	Cell-wall modification, signal transduction, translation pathways	EST[f]	Weidmann et al., 2004
		Transduction pathway, gene expression/RNA metabolism, membrane transport, responses to abiotic stimuli, development	SSH[f]	Sanchez et al., 2005
		Defence Defence and stress-related genes	Germin-like protein	Brechenmacher et al. 2004 Bonanomi et al., 2001; Blilou et al., 2000; Ruiz-Lozano et al., 1999; Spanu et al., 1989 ; Ruiz-Lozano et al., 1999 ; Harrison and Dixon 1993 ; Kapulnik et al., 1996
		Water channel protein	Nodulin 26-like aquaporin	Brechenmacher et al. 2004
		Cell wall	*PsENOD12A*	Albrecht et al., 1998
		Cell wall/signalling	*MtENOD11*	Chabaud et al., 2002
		Signalling	C1p Serine protease	Roussel et al., 2001
		Metabolic pump	H+ATPase[d]	Requena et al., 2003
		Signalling	Ca^{2+} related proteins[d]	Breuninger & Requena 2004
Symbiotic phase	Arbuscule-containing plant cells /symbiotic interface	Cell wall modification, signalling, nutrient transfer	Sequences from microarrays analysis[f]	Liu et al., 2003, 2004
		Cell wall modification,protein degradation and plant defence,nutrient transport, primary metabolism, secondary metabolism, signal transduction and transduction factor	Sequences from microarrays analysis[f]	Hohnjec et al., 2005
		Cell wall modification, signal transduction	SSH and macroarray analysis[g]	Grunwald et al., 2004
		Plant genes regulated in response to fungal pathogen infection and to increased P_i availability, genes expressed exclusively in mycorrhiza	Sequences from microarrays analysis[h]	Güimil et al., 2005
		Transport, signalling processes, lectin genes	EST[f]	Frenzel et al., 2005
		Cell wall/cell wall modification	*Mt-XHT1*	Maldonado–Mendoza et al.,

Table 1 (continued)

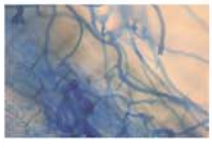
				2005
			Expansin	Liu et al., 2004
			Endo-β-1,4-glucanase (*MtCel1*)	Liu et al., 2003
			Endoglucanases/Pectinases	Hohnjec et al., 2005
			Osmotin/thaumatin-like protein	Hohnjec et al., 2005
			MtENOD11	Journet et al., 2001
			Arabinogalactan protein	van Buuren et al., 1999
			HRGP	Balestrini et al., 1997;Grunwald et al., 2004 ; Hohnjec et al., 2005
		Cytoskeleton	α-tubulin	Bonfante et al., 1996
			β-tubulin	Manthey et al., 2004
		Sugar transport	Hexose transporters	Harrison et al., 1996 ; Liu et al., 2003
			Sugar transporter (*Mtst1*)	Hohnjec et al., 2005
		P transport	P transporters	Raush et al., 2001; Harrison et al., 2002; Paszkowski et al., 2002 ; Nagy et al., 2005
		C metabolism	Sucrose synthase	Ravnskov et al., 2003 ; Hohnjec et al., 2003
		Nitrate acquisition	Nitrate transporter	Hohnjec et al., 2005 ; Wulf et al., 2003
		N assimilation	Glutamine synthetase	Brechenmacher et al. 2004
		H+ATPase	Metabolic pump	Krajinski et al., 2002 ; Gianinazzi-Pearson et al., 2000
		Defence		Blee and Anderson, 2000; Harrison and Dixon, 1993; 1994; Lambais and Mehdy, 1993, 1998
			PR10	Brechenmacher et al. 2004
			Wound-induced protein	Brechenmacher et al. 2004
			Germin-like protein	Doll et al., 2003 ; Güimil et al., 2005 ; Frenzel et al., 2005
			Glutathione S-transferase	Wulf et al., 2003 ; Brechenmacher et al. 2004
			Class III chitinase	Salzer et al., 2000; Bonanomi et al., 2001
		Signaling	Ser carboxypeptidase (*MtScp1*)	Liu et al., 2003
			Alpha-fucosidase	Liu et al., 2004
		Response to oxidative stress? Recognition processes/arbuscule formation ?	Leghemoglobin	Vieweg et al., 2004
			Lectin-like gene (*PsNLEC-1*)	Balestrini et al., 1999
			Lectins	Wulf et al., 2003 ; Frenzel et al., 2005
		Cell cycle	Cullin	Tahiri-Alaoui et al., 2002
		Mediator of electron transfer processes	Blue copper binding protein (*MtBcp1)*	Hohnjec et al., 2005
		Oxidative stress	Putative peroxidase	Güimil et al., 2005
			Mn-sod II	Ruiz-Lozano et al., 2001
		Carotenoid synthesis	Phytoene desaturase	Fester et al., 2002a
			DXR	Hans et al., 2004
	- Fungal intraradical structures	Response to oxidative stress	Superoxide dismutase[b]	Lanfranco et al., 2005
		N metabolism	Urease accessory protein[c], ornithine amino transferase[c]	Govindarajulu et al., 2005
			NH^{4+} transporter[c]	Karldorf et al., 1998
			Nitrate reductase[c]	
		C metabolism	Glycogen synthase[c]	Bago et al., 2003
		Unknown function	GmMtLs17	Brechenmacher et al., 2004
		Hydrolysis of phosphorus compounds	Alkaline phosphatase[b,c]	Aono et al., 2004
	- Fungal extraradical structures	P transport	P transporter[c,d,e]	Harrison & van Buuren 1995; Maldonado-Mendoza et al., 2001; Benedetto et al., 2005
		Lipid utilization		
		N metabolism	Acyl-CoA dehydrogenase[c]	Bago et al., 2002

Table 1 (continued)

		Glyoxylate cycle	Glutamine synthase[c,d]	Govindarajulu et al., 2005 Breuninger et al., 2004
		C metabolism	Isocitrate lyase[c] Malate synthase[c]	Lammers et al., 2001
		Regulatory role ?	Glycogen synthase[c] Ginmyc1[c] Ginhb1[c]	Bago et al., 2003 Delp et al., 2003
			EST[c]	Sawaki & Saito 2001

For untargeted approaches (EST, arrays, SSH), only the functional categories have reported. Fungal genes are indicated in bold.

[a] *Gigaspora rosea*
[b] *Gigaspora margarita*
[c] *Glomus intraradices*
[d] *Glomus mosseae*
[e] *Glomus versiforme*
[f] *Medicago truncatula*
[g] *Pisum sativum*
[h] *Oryza sativa*

associations was proposed (Ezawa et al. 2005). The authors suggest that the fungal partner activates part of the low-P adaptation system of the plant partner, phosphatase secretion, and improves the overall efficiency of P uptake.

Advances have also been made in understanding the movement of nitrogen in the AM symbiosis, another nutrient whose availability frequently limits plant growth. AM fungi directly take up and transfer N to their host plants (Bago et al. 1996; Johansen et al. 1993; He et al. 2002) and increase the utilization of different forms of N by plants (Hodge et al. 2001). Elegant isotope labeling experiments in combination with gene expression data (Govindarajulu et al. 2005) recently confirmed a model previously proposed by Bago et al. (2002). Inorganic N (nitrate and ammonium) is taken up by the extraradical mycelium, incorporated into amino acids and translocated to the intraradical mycelium mainly as arginine. N is then transferred from the fungus to the plant as ammonium without any loss of C skeleton, thanks to the catabolic arm of the urea cycle that converts arginine into ammonium. This proposed mechanism requires that enzymes for N assimilation are expressed differently in the extraradical and the intraradical mycelium. In fact, quantitative real-time PCR assays have shown that a gene of primary nitrogen assimilation (glutamine synthase) was preferentially expressed in the extraradical hyphae, whereas genes involved in arginine breakdown (urease accessory protein and ornithine amino transferase) and NH_4^+ transfer (ammonium transporter) were more highly expressed in the intraradical mycelium (Govindarajulu et al. 2005). It is worth mentioning that different N sources modulated the activity but not the expression of the fungal glutamine synthase (Breuninger et al. 2004). Another fungal gene (*Gi-1*) possibly involved in the synthesis of nitrogen compounds was shown to be expressed only during the symbiotic phase and upregulated under N fertilization (Ruiz-Lozano et al. 2002).

On the plant side, induction of four genes corresponding to the first AM-related nitrate transporters identified in *M. truncatula* was recently reported (Hohnjec et al. 2005). These findings, together with a report on an AM-induced nitrate transporter in tomato (Hildebrandt et al. 2002), suggest mechanisms not only supporting ammonium uptake but also nitrate acquisition during AM symbiosis. Repression of an *M. truncatula* nitrate transporter, previously observed (Burleigh 2001) in roots colonized by *G. mosseae*, was also confirmed in *G. intraradices*-colonized roots.

A similar situation of up- and downregulation of members belonging to the same gene family is also reported for hexose transporters. During the symbiotic phase, C metabolism of both partners is significantly modified, as seen at the level of gene expression. In AM-colonized roots, enhanced gene expression of sucrose synthase was observed (Ravnskov et al. 2003; Hohnjec et al. 2003), leading to the hypothesis that sucrose synthase is involved in generating a sink-strength. In the 1996, Harrison reported high expression of a sugar transporter gene (*Mtst1*) in arbuscule-containing cells and in cortical cells surrounding colonized areas. The induction of *Mtst1* was also reported in *G. mosseae*-colonized roots by Hohnjec et al. (2005), in addition to another sugar transporter (TC77798); upregulation of a plant sugar transporter gene (AW584546) was also identified in *M. truncatula* and *G. versiforme* AM symbioses using macroarray hybridization (Liu et al. 2003). Surprisingly, the same gene was found to be downregulated by AM fungi by Hohnjec et al. (2005), suggesting the possibility that different AM fungi might activate different members of the hexose transporter gene family or different sugar transporters might be recruited during specific stages of the symbiosis.

C metabolism of AM fungi has also been investigated. Intraradical fungal structures (presumably the arbuscules)

are known to take up photosynthetically fixed plant C as hexoses. Unfortunately, no fungal hexose transporter-coding gene has been characterized yet in AM fungi. Triacylglycerols and glycogen are also synthesized by the fungus inside the root and then transferred to the extraradical mycelium (Bago et al. 2002, 2003). Gene expression studies performed on a putative acyl-coenzyme A dehydrogenase (Bago et al. 2002) and a glycogen synthase (Bago et al. 2003) were consistent with the results obtained with ^{13}C labeling assays. In contrast, genes coding for enzymes of the glyoxylate cycle (isocitrate lyase and malate synthase) were found to be actively expressed in the extraradical mycelium where the fungus, unable to take up hexoses, converts lipids into carbohydrates via this metabolic pathway (Lammers et al. 2001). However, detailed expression studies on a wider spectrum of genes are needed to improve our knowledge on C fluxes in AM fungi.

Conclusions and perspectives

An array of experimental evidence has shown that AM fungi have a deep impact on plant gene expression. Transcripts of a number of genes expressed in mycorrhizal roots occur in cortical cells containing the arbuscules. A mycorrhiza-specific signal may be responsible for the activation of these genes (Harrison 2005). In contrast, some other genes are expressed in arbuscule-containing cells and in cortical cells in the vicinity of colonized cells, suggesting the presence of a second mobile signal acting in the colonized region of the root (Liu et al. 2003; Wulf et al. 2003; Harrison 2005). Moreover, AM symbiosis can also influence gene expression elsewhere in the plant since modulation of gene expression has been observed in the leaves of mycorrhizal plants (García-Rodríguez et al. 2005). It remains to be defined whether this is due to a long distance signal or is a response to physiological changes in the mycorrhizal plant.

Technical advances are making it possible to explore transcript profiles in the fungal partner. Indirect information about coding sequences in AM fungi (that will represent a platform for gene expression studies based on array technology) will be available soon with the complete genome sequence of *G. intraradices* (http://darwin.nmsu.edu/~fungi/).

Methods such as in situ hybridization and reporter gene visualization have permitted cell-specific analysis of individual gene expression. New methods for cell-specific gene expression analysis in AM symbiosis will be very useful since the mycorrhizal process is asynchronous and gene induction might be localized only in specific cell types such as those containing arbuscules. For this purpose, laser-assisted microdissection is a powerful and attractive tool (Day et al. 2005).

Finally, information from transcriptome analyses will surely be added to that gained from other promising approaches such as proteomic studies (Bestel-Corre et al. 2003 and references therein), forward and reverse genetics (i.e., RNAi, Ivashuta et al. 2005), and the use of transgenic plants expressing green fluorescent protein-tagged markers allowing in vivo observations (Genre et al. 2005).

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References

Albrecht C, Geurts R, Bisseling T (1998) Legume nodulation and mycorrhizae formation; two extremes in host specificity meet. EMBO J 18:281–288

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Ané JM, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GE, Ayax C, Levy J, Debelle F, Baek JM, Kalo P et al (2004) *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. Science 303:1364–1367

Aono T, Maldonado-Mendoza IE, Dewbre GR, Harrison MJ, Saito M (2004) Expression of alkaline phosphatase genes in arbuscular mycorrhizas. New Phytol 162:525–553

Ayling SM, Smith SE, Smith FA (2000) Transmembrane electric potential difference of germ tubes of arbuscular mycorrhizal fungi responds to external stimuli. New Phytol 147:631–639

Bago B, Chamberland H, Goulet A, Vierheilig H, Lafontaine JG, Piché Y (1996) Effect of nikkomycin Z, a chitin-synthase inhibitor, on hyphal growth and cell wall structure of two arbuscular–mycorrhizal fungi. Protoplasma 192:80–92

Bago B, Shachar-Hill Y, Pfeffer PE (2000) Dissecting carbon pathways in arbuscular mycorrhizas with NMR spectroscopy. In: Podila GK, Douds DD (eds) Current advances in mycorrhizae research. APS, St. Paul, USA, pp 111–126

Bago B, Zipfel W, Williams RC, Jun J, Arreola R, Pfeffer PE, Lammers PJ, Shachar-Hill Y (2002) Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. Plant Physiol 128:108–124

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol 131:1496–1507

Balestrini R, Bonfante P (2005) The interface compartment in arbuscular mycorrhizae: a special type of plant cell wall? Plant Biosyst 139:8–15

Balestrini R, Josè-Estanyol M, Puigdomènech P, Bonfante P (1997) Hydroxyproline rich glycoprotein mRNA accumulation in maize root cells colonized by the arbuscular mycorrhizal fungus as revealed by in situ hybridization. Protoplasma 198:36–42

Balestrini R, Perotto S, Gasverde E, Dahiya P, Guldmann LL, Brewin NJ, Bonfante P (1999) Transcription of a gene encoding a lectin like glycoprotein is induced in root cells harbouring arbuscular mycorrhizal fungi in *Pisum sativum*. Mol Plant-Microb Interact 12:785–791

Balestrini R, Cosgrove DJ, Bonfante P (2005) Differential location of alpha-expansin proteins during the accommodation of root cells to an arbuscular mycorrhizal fungus. Planta 220:889–899

Beilby JP, Kidby DK (1980) Biochemistry of ungerminated and germinated spores of the vesicular–arbuscular mycorrhizal fungus *Glomus caledonium*: changes in neutral and polar lipids. J Lipid Res 21:739–750

Benedetto A, Magurno F, Bonfante P, Lanfranco L (2005) Expression profiles of a phosphate transporter gene (*GmosPT*) from the endomycorrhizal fungus *Glomus mosseae*. Mycorrhiza 15:620–627

Berta G, Fusconi A, Sampo S, Lingua G, Perticone S, Repetto O (2000) Polyploidy in tomato roots as affected by arbuscular mycorrhizal colonization. Plant Soil 226:37–44

Bestel-Corre G, Dumas-Gaudot E, Gianinazzi S (2003) Proteomics as a tool to monitor plant–microbe endosymbioses in the rhizosphere. Mycorrhiza 14:1–10

Bianciotto V, Barbiero G, Bonfante P (1995) Analysis of the cell-cycle in an arbuscular mycorrhizal fungus by flow-cytometry and bromodeoxyuridine labelling. Protoplasma 188:161–169

Bianciotto V, Perotto S, Ruiz-Lozano JM, Bonfante P (2002) Arbuscular mycorrhizal fungi and soil bacteria: from cellular investigations to biotechnological perspectives. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture. Birknäuser Verlag, Switzerland, pp 19–31

Blancaflor EB, Zhao L, Harrison MJ (2001) Microtubule organization in root cells of *Medicago truncatula* during development of an arbuscular mycorrhizal symbiosis with *Glomus versiforme*. Protoplasma 217:154–165

Blee KA, Anderson AJ (2000) Defense responses in plants to arbuscular mycorrhizal fungi. In: Podila GK, Douds DD (eds) Current advances in mycorrhizae research. APS, St. Paul, USA, pp 27–44

Blilou I, Bueno P, Ocampo JA, Garcia-Garrido J (2000) Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal *Glomus mosseae*. Mycol Res 104:722–725

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 Phytol 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. Springer, Berlín Heidelberg New York, pp 45–61

Bonfante P, Balestrini R, Mendgen K (1994) Storage and secretion processes in the spore of *Gigaspora margarita* Becker and Hall as revealed by high-pressure freezing and freeze substitution. New Phytol 128:93–101

Bonfante P, Bergero R, Uribe X, Romera C, Rigau J, Puigdoménech P (1996) Transcriptional activation of a maize-tubulin gene in mycorrhizal maize and transgenic tobacco plants. Plant J 9:737–743

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. Mol Plant-Microb Interact 13:1109–1120

Brechenmacher L, Weidmann S, van Tuinen D, Chatagnier O, Gianinazzi S, Franken P, Gianinazzi-Pearson V (2004) Expression profiling of up-regulated plant and fungal genes in early and late stages of *Medicago truncatula–Glomus mosseae* interactions. Mycorrhiza 14:253–262

Breuninger M, Requena N (2004) Recognition events in AM symbiosis: analysis of fungal gene expression at the early appressorium stage. Fungal Genet Biol 41:794–804

Breuninger M, Trujillo CG, Serrano E, Fischer R, Requena N (2004) Different nitrogen sources modulate activity but not expression of glutamine synthetase in arbuscular mycorrhizal fungi. Fungal Genet Biol 41:542–552

Brewin NJ (2004) Plant cell wall remodelling in the Rhizobium–legume symbiosis. Crit Rev Plant Sci 23:293–316

Brummell DA, Bird CR, Schuch W, Bennett AB (1997) An endo-1, 4-β-glucanase expressed at high levels in rapidly expanding tissues. Plant Mol Biol 33:87–95

Bucking H, Shachar-Hill Y (2005) Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. New Phytol 165:899–912

Buée M, Rossignol M, Jauneau A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol Plant-Microb Interact 13:693–698

Burleigh SH (2001) Relative quantitative PCR to study nutrient transport processes in arbuscular mycorrhizas. Plant Sci 160:899–904

Burleigh SH, Harrison MJ (1997) A novel gene whose expression in *Medicago truncatula* roots is suppressed in response to colonization by vesicular arbuscular mycorrhizal (VAM) fungi and to phosphate nutrition. Plant Mol Biol 34:199–208

Chabaud M, Venard C, Defaux-Petras A, Becard G, Barker DG (2002) Targeted inoculation of *Medicago truncatula* in vitro root cultures reveals *MtENOD11* expression during early stages of infection by arbuscular mycorrhizal fungi. New Phytol 156:265–273

Day RC, Grossniklaus U, Macknight RC (2005) Be more specific! Laser-assisted microdissection of plant cells. Trends Plant Sci 10:340–397

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA 98:13454–13459

Delp G, Timonen S, Rosewarne GM, Barker SJ, Smith S (2003) Differential expression of *Glomus intraradices* genes in external mycelium and mycorrhizal roots of tomato and barley. Mycol Res 107:1083–1093

Demartsev V, Aussenberg ER, Gadkar V, Koltai H, Zilberstein A, Kapulnik Y (2005) Alteration in tomato (*Lycopersicon esculentum*) gene expression during early stages of the interaction with *Glomus intraradices*. In: Management committee and final meeting on "Achievements and Future Landscape for Arbuscular Mycorrhiza Research", Dijon, France, 2–4 June 2005

Doll J, Hause B, Demchenko K, Pawlowski K, Krajinski F (2003) A member of the germin-like protein family is a highly conserved mycorrhiza-specific induced gene. Plant Cell Physiol 44:1208–1214

Duc G, Trouvelet A, Gianinazzi-Pearson V, Gianinazzi S (1989) First report of nonmycorrhizal plant mutants (Myc-) obtained in pea (*Pisum sativum* L.) and fababean (*Vicia faba* L.). Plant Sci 60:215–222

Endre G, Kereszt A, Kevei Z, Mihacea S, Kaló, P, Kiss GB (2002) A receptor kinase gene regulating symbiotic nodule development. Nature 417:962–966

Ezawa T, Hayatsu M, Saito M (2005) A new hypothesis on the strategy for acquisition of phosphorus in arbuscular mycorrhiza: up-regulation of secreted acid phosphatase gene in the host plant. Mol Plant-Microb Interact 18:1046–1053

Fehlberg V, Vieweg MF, Dohmann EMN, Hohnjec N, Pühler A, Perlick AM, Küster H (2005) The promoter of the leghaemoglobin gene *VfLb29*: functional analysis and identification of modules necessary for its activation in the infected cells of root nodules and in the arbuscule-containing cells of mycorrhizal roots. J Exp Bot 56:799–806

Fester T, Hause G (2005) Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. Mycorrhiza 15:373–379

Fester T, Schmidt D, Lohse S, Walter MH, Giuliano G, Bramley PM, Fraser PD, Hause B, Strack D (2002a) Stimulation of carotenoid metabolism in arbuscular mycorrhizal roots. Planta 216:148–154

Fester T, Hause B, Schmidt D, Halfmann K, Schmidt J, Wray V, Hause G, Strack D (2002b) Occurrence and localization of apocarotenoids in arbuscular mycorrhizal plant roots. Plant Cell Physiol 43: 256–265

Frenzel A, Manthey K, Perlick AM, Meyer F, Puhler A, Kuster H, Krajinski F (2005) Combined transcriptome profiling reveals a novel family of arbuscular mycorrhizal-specific *Medicago truncatula* lectin genes. Mol Plant-Microb Interact 18:771–782

Garcia-Garrido JM, Ocampo JA (2002) Regulation of the plant defence response in arbuscular mycorrhizal symbiosis. J Exp Bot 53:1377–1386

García-Rodríguez S, Pozo MJ, Azcón-Aguilar C, Ferrol N (2005) Expression of a tomato sugar transporter is increased in leaves of mycorrhizal or *Phytophthora parasitica*-infected plants. Mycorrhiza 15:489–496

Gaspar L, Pollero RJ, Cabello M (1994) Triacylglycerol consumption during spore germination of vesicular–arcuscular mycorrhizal fungi. J Am Oil Chem Soc 71:449–452

Genre A, Bonfante P (2005) Building a mycorrhizal cell: How to reach compatibility between plants and arbuscular mycorrhizal fungi. J Plant Interact 1:3–13

Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG (2005) Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell 17:3489–3499

Gianinazzi-Pearson V, Denarié J (1997) Red carpet genetic programmes for root endosymbioses. Trends Plant Sci 10:371–372

Gianinazzi-Pearson V, Gollotte A, Lherminier J, Tisserant B, Franken P, Dumas-Gaudot E, Lemoine MC, van Tuinen D, Gianinazzi S (1995) Cellular and molecular approaches in the characterization of symbiotic events in functional arbuscular mycorrhizal associations. Can J Bot 73:S526–S532

Gianinazzi-Pearson V, Arnould C, Oufattole M, Arango M, Gianinazzi S (2000) Differential activation of H^+-ATPase genes by an arbuscular mycorrhizal fungus in root cells of transgenic tobacco. Planta 211:609–613

Giovannetti M, Sbrana C, Avio L, Citernesi AS, Logi C (1993) Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-infection stages. New Phytol 125:587–594

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435:819–823

Grunwald U, Nyamsuren O, Tamasloukht M, Lapopin L, Becker A, Mann P, Gianinazzi-Pearson V, Krajinski F, Franken P (2004) Identification of mycorrhiza-regulated genes with arbuscule development-related expression profile. Plant Mol Biol 55:553–566

Güimil S, Chang H-S, Zhu T, Sesma A, Osbourn A, Roux C, Ioannidis V, Oakeley EJ, Docquier M, Descombes P, Briggs SP, Paszkowski U (2005) Comparative transcriptomics of rice reveals an ancient pattern of response to microbial colonization. Proc Natl Acad Sci USA 102:8066–8070

Hans J, Hause B, Strack D, Walter MH (2004) Cloning, characterization, and immunolocalization of a mycorrhiza-inducible 1-deoxy-D-xylulose 5-phosphate reductoisomerase in arbuscule-containing cells of maize. Plant Physiol 134:614–624

Harrison MJ (1996) A sugar transporter from *Medicago truncatula*: altered expression pattern in roots during vesicular–arbuscular (VA) mycorrhizal associations. Plant J 9:491–503

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

Harrison MJ, Dixon RA (1993) Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular–arbuscular mycorrhizal associations in roots of *Medicago truncatula*. Mol Plant-Microb Interact 6:643–654

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*. Plant J 6:9–20

Harrison MJ, van Buuren ML (1995) A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. Nature 378:626–629

Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14:2413–2429

Hause B, Fester T (2005) Molecular and cell biology of arbuscular mycorrhizal symbiosis. Planta 221:184–196

He XL, Mouratov S, Steinberger Y (2002) Temporal and spatial dynamics of vesicular–arbuscular mycorrhizal fungi under the canopy of *Zygophyllum dumosum* Boiss. in the Negev Desert. J Arid Environ 52:379–387

Hildebrandt U, Schmelzer E, Bothe H (2002) Expression of nitrate transporter genes in tomato colonized by an arbuscular mycorrhizal fungus. Physiol Plant 115:125–136

Hirsch AM, Kapulnik Y (1998) Signal transduction pathways in mycorrhizal associations: comparisons with the *Rhizobium*–legume symbiosis. Fungal Genet Biol 23:205–212

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299

Hohnjec N, Perlick AM, Puhler A, Kuster H (2003) The *Medicago truncatula* sucrose synthase gene *MtSucS1* is activated both in the infected region of root nodules and in the cortex of roots colonized by arbuscular mycorrhizal fungi. Mol Plant-Microb Interact 16:903–915

Hohnjec N, Vieweg MF, Pühler A, Becker A, Küster H (2005) Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. Plant Physiol 137:1283–1301

Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi H, Murakami Y, Mulder L, Vickers K, Pike J, Downie JA, Wang T, Sato S, Asamizu E, Tabata S, Yoshikawa M, Murooka Y, Wu GJ, Kawaguchi M, Kawasaki S, Parniske M, Hayashi M (2005) Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. Nature 433:527–531

Ivashuta S, Liu J, Liu J, Lohar DP, Haridas S, Bucciarelli B, VandenBosch KA, Vance CP, Harrison MJ, Gantt JS (2005) RNA interference identifies a calcium dependent protein kinase involved in *Medicago truncatula* root development. Plant Cell 17:2911–2921

Johansen A, Jakobsen I, Jensen ES (1993) Hyphal transport by a vesicular–arbuscular mycorrhizal fungus of N applied to the soil as ammonium or nitrate. Biol Fertil Soils 16:66–70

Journet EP, El-Gachtouli N, Vernoud V, de Billy F, Pichon M, Dedieu A, Arnould C, Morandi D, Barker DG, Gianinazzi-Pearson V (2001) *Medicago truncatula ENOD11*: a novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells. Mol Plant-Microb Interact 14:737–748

Journet EP, van Tuinen D, Gouzy J, Crespeau H, Carreau V, Farmer MJ, Niebel A, Schiex T, Jaillon O, Chatagnier O et al (2002) Exploring root symbiotic programs in the model legume *Medicago truncatula* using EST analysis. Nucleic Acids Res 30:5579–5592

Jun J, Abubaker J, Rehrer C, Pfeffer PE, Shachar-Hill Y, Lammers PJ (2002) Expression in an arbuscular mycorrhizal fungus of genes putatively involved in metabolism, transport, the cytoskeleton and the cell cycle. Plant Soil 244:141–148

Kapulnik Y, Volpin H, Itzhaki H, Ganon D, Galili S, David R, Shaul O, Elad Y, Chet I, Okon Y (1996) Suppression of defence responses in mycorrhizal alfalfa and tobacco roots. New Phytol 133:59–64

Karandashov V, Bucher M (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. Trends Plant Sci 10:22–29

Kistner C, Parniske M (2002) Evolution of signal transduction in intracellular symbiosis. Trends Plant Sci 7:511–518

Kistner C, Winzer T, Pitzschke A, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Webb KJ, Szczyglowski K, Parniske M (2005) Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. Plant Cell 17:2217–2229

Kosuta S, Chabaud M, Lougnon G, Gough C, Denarie J, Barker DG, Becard G (2003) A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis specific *MtENOD11* expression in roots of *Medicago truncatula*. Plant Physiol 131:952–962

Krajinski F, Martin-Laurent F, Gianinazzi S, Gianinazzi-Pearson V, Franken F (1998) Cloning and analysis of *psam2*, a gene from *Pisum sativum* L. regulated insymbiotic arbuscular mycorrhiza and pathogenic root–fungus interactions. Physiol Plant Mol Pathol 52:297–307

Krajinski F, Hause B, Gianinazzi-Pearson V, Franken P (2002) *Mtha1*, a plasma membrane H^+-ATPase gene from *Medicago truncatula*, shows arbuscule specific induced expression in mycorrhizal tissue. Plant Biol 4:754–761

Kuster H, Hohnjec, Krajinski F, El Yahyaoui F, Manthey K, Gouzy J, Dondrup M, Meyer F, Kalinowski J, Brechenmacher L, van Tuinen D, Gianinazzi-Pearson V, Puhler A, Gamas P, Becker A (2004) Construction and validation of cDNA-based Mt6k-RIT macro- and microarrays to explore root endosymbioses in the model legume *Medicago truncatula*. J Biotechnol 108:95–113

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. Mol Plant-Microb Interact 6:75–83

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 Phytol 140:33–42

Lammers PJ, Jun J, Abubaker J, Arreola R, Gopalan A, Bago B, Hernandez-Sebastia C, Allen JW, Douds DD, Pfeffer PE et al (2001) The glyoxylate cycle in an arbuscular mycorrhizal fungus: gene expression and carbon flow. Plant Physiol 127:1287–1298

Lanfranco L, Gabella S, Bonfante P (2000) EST as a useful tool for studying gene expression in arbuscular mycorrhizal fungi. In: Weber H, Imhof S, Zeuske D (eds) Abstract and papers of the Third International Congress on Symbiosis. University of Marburg, 13–19 August 2000, Marburg, Germany, pp 108–114

Lanfranco L, Bolchi A, Ros EC, Ottonello S, Bonfante P (2002) Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol 130:58–67

Lanfranco L, Novero M, Bonfante P (2005) The mycorrhizal fungus *Gigaspora margarita* possesses a CuZn superoxidedismutase which is up-regulated during the symbiosis with legume hosts. Plant Physiol 137:1319–1330

Lei J, Bécard G, Catford JG, Piché Y (1991) Root factors stimulate ^{32}P uptake and plasmalemma ATPase activity in vesicular–arbuscular mycorrhizal fungus, *Gigaspora margarita*. New Phytol 118:289–294

Lévy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet EP, Ane JM, Lauber E, Bisseling T et al (2004) A putative Ca^{2+} and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. Science 303:1361–1364

Liu J, Blaylock LA, Endre G, Cho J, Town CD, Vanden Bosch KA, Harrison MJ (2003) Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of the arbuscular mycorrhizal symbiosis. Plant Cell 15:2106–2123

Liu J, Blaylock LA, Harrison MJ (2004) cDNA arrays as a tool to identify mycorrhiza-regulated genes: identification of mycorrhiza-induced genes that encode or generate signaling molecules implicated in the control of root growth. Can J Bot 82:1177–1185

Maldonado-Mendoza IE, Dewbre GR, Harrison MJ (2001) A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment. Mol Plant-Microb Interact 14:1140–1148

Maldonado-Mendoza IE, Dewbre GR, van Buuren ML, Versaw WK, Harrison MJ (2002) Methods to estimate the proportion of plant and fungal RNA in an arbuscular mycorrhiza. Mycorrhiza 12:67–74

Maldonado-Mendoza IE, Dewbre GR, Blaylock L, Harrison MJ (2005) Expression of a xyloglucan endotransglucosylase/hydrolase gene, *Mt-XTH1*, from *Medicago truncatula* is induced systemically in mycorrhizal roots. Gene 345:191–197

Manthey K, Krajinski F, Hohnjec N, Firnhaber C, Puhler A, Perlick AM, Kuster H (2004) Transcriptome profiling in root nodules and arbuscular mycorrhiza identifies a collection of novel genes induced during *Medicago truncatula* root endosymbioses. Mol Plant-Microb Interact 17:1063–1077

Marsh JF, Schultze M (2001) Analysis of arbuscular mycorrhizas using symbiosis defective plant mutants. New Phytol 150:525–532

Martin-Laurent F, van Tuinen D, Dumas-Gaudot E, Gianinazzi-Pearson V, Gianinazzi S, Franken P (1997) Differential display analysis of RNA accumulation in arbuscular mycorrhiza of pea and isolation of a novel symbiosis-regulated plant gene. Mol Gen Genet 256: 37–44

Morandi D, Prado E, Sagan M, Duc G (2005) Characterization of new symbiotic *Medicago truncatula* (Gaertn.) mutants, and phenotypic or genotypic complementary information on previously described mutants. Mycorrhiza 15:283–289

Mosse B, Hepper CM (1975) Vesicular–arbuscular mycorrhizal infections in root organ cultures. Physiol Plant Pathol 5:215–223

Murphy PJ, Langridge P, Smith SE (1997) Cloning plant genes differentially expressed during colonization of roots of *Hordeum vulgare* by the vesicular arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytol 135:291–301

Nagahashi G, Douds DD (1997) Appressorium formation by AM fungi on isolated cell walls of carrot roots. New Phytol 136:299–304

Nagahashi G, Douds DD (2000) Partial separation of the root exudates components and their effects upon the growth of germinated spores of AM fungi. Mycol Res 104:1453–1464

Nagy R, Karandashov V, Chague V, Kalinkevich K, Tamasloukht MB, Xu G, Jakobsen I, Levy AA, Amrhein N, Bucher M (2005) The characterization of novel mycorrhiza specific phosphate transporters from *Lycopersicon esculentum* and *Solanum tuberosum* uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. Plant J 42:236–250

Olah B, Brière C, Bécard G, Dénarie' J, Gough C (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant J 44:195–207

Oldroyd GED, Harrison MJ, Udvardi M (2005) Peace talks and trade deals. Keys to long-term harmony in legume–microbe symbioses. Plant Physiol 137:1205–1210

Parniske M (2004) Molecular genetics of the arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 7:414–421

Paszkowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 99:13324–13329

Podila GK, Lanfranco L (2004) Genomics approaches to unravel mycorrhizal symbiosis. In: Varma A, Werner H (eds) Plant surface microbiology. Springer, Berlin Heidelberg New York, pp 561–592

Rausch C, Daram P, Brunner S, Jansa J, Laloi M, Leggewie G, Amrhein N, Bucher M (2001) A phosphate transporter expressed in arbuscule-containing cells in potato. Nature 414:462–466

Ravnskov S, Wu Y, Graham JH (2003) Arbuscular mycorrhizal fungi differentially affect expression of genes coding for sucrose synthases in maize roots. New Phytol 157:539–545

Requena N, Fuller P, Franken P (1999) Molecular characterization of *Gm-FOX2*, an evolutionarily highly conserved gene from the mycorrhizal fungus *Glomus mosseae*, down-regulated during interaction with rhizobacteria. Mol Plant-Microb Interact 12:934–942

Requena N, Mann P, Franken P (2000) A homologue of the cell cycle check point TOR2 from *Saccharomyces cerevisiae* exists in the arbuscular mycorrhizal fungus *Glomus mosseae*. Protoplasma 212:89–98

Requena N, Mann P, Hampp R, Franken P (2002) Early developmentally regulated genes in the arbuscular mycorrhizal fungus *Glomus mosseae*: identification of *GmGIN1*, a novel gene with homology to the C-terminus of metazoan hedgehog proteins. Plant Soil 244:129–139

Requena N, Breuninger M, Franken P, Ocón A (2003) Symbiotic status, phosphate, and sucrose regulate the expression of two plasma membrane H+-ATPase genes from the mycorrhizal fungus *Glomus mosseae*. Plant Physiol 132:1–10

Rhody D, Stommel M, Roeder C, Mann P, Franken P (2003) Differential RNA accumulation of two β-*tubulin* genes in arbuscular mycorrhizal fungi. Mycorrhiza 13:137–142

Rosewarne G, Barker SJ, Smith SE, Smith FA, Schachtman DP (1999) A *Lycopersicon esculetum* phosphate transporter (*LePT1*) involved in phosphorus uptake from a vesicular–arbuscular mycorrhizal fungus. New Phytol 144:507–516

Roussel H, van Tuinen D, Franken P, Gianinazzi S, Gianinazzi-Pearson V (2001) Signalling between arbuscular mycorrhizal fungi and plants: identification of a gene expressed during early interactions by differential RNA display analysis. Plant Soil 232:13–19

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. Mol Plant-Microb Interact 12:976–984

Ruiz-Lozano JM, Collados C, Barea JM, Azcon R (2001) Cloning of cDNAs encoding SODs from lettuce plants which show differential regulation by arbuscular mycorrhizal symbiosis and by drought stress. J Exp Bot 52:2241–2242

Ruiz-Lozano JM, Collados C, Porcel R, Azcon R, Barea JM (2002) Identification of a cDNA from the arbuscular mycorrhizal fungus *Glomus intraradices* that is expressed during mycorrhizal symbiosis and up-regulated by N fertilization. Mol Plant-Microb Interact 15:360–367

Salzer P, Corbière H, Boller T (1999) Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the arbuscular mycorrhiza-forming fungus *Glomus intraradices*. Planta 208:319–325

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. Mol Plant-Microb Interact 13:763–777

Sanchez L, Weidmann S, Arnould C, Bernard AR, Gianinazzi S, Gianinazzi-Pearson V (2005) *Pseudomonas fluorescens* and *Glomus mosseae* trigger DMI3-dependent activation of genes related to a signal transduction pathway in roots of *Medicago truncatula*. Plant Physiol 139:1065–1077

Sawaki H, Saito M (2001) Expressed genes in the extraradical hyphae of an arbuscular mycorrhizal fungus, *Glomus intraradices*, in the symbiotic phase. FEMS Microbiol Lett 195:109–113

Slezack S, Dumas-Gaudot E, Paynot M, Gianinazzi S (2000) Is a fully established arbuscular mycorrhizal symbiosis required for bioprotection of *Pisum sativum* roots against *Aphanomyces euteiches*? Mol Plant-Microb Interact 13:238–241

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London, UK

Smith SE, Smith AF, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol 133:16–20

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

Stommel M, Mann P, Franken P (2001) EST-library construction using spore RNA of the arbuscular mycorrhizal fungus *Gigaspora rosea*. Mycorrhiza 10:281–285

Stougaard J (2001) Genetics and genomics of root symbiosis. Curr Opin Plant Biol 4:328–335

Tahiri-Alaoui A, Lingua G, Avrova A, Sampò S, Fusconi A, Antoniw J, Berta G (2002) A cullin gene is induced in tomato roots forming arbuscular mycorrhizae. Can J Bot 80:607–616

Tamasloukht MB, Sejalon-Delmas N, Kluever A, Jauneau A, Roux C, Becard G, Franken P (2003) Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*. Plant Physiol 131:1468–1478

Tisserant B, Gianinazzi-Pearson V, Gianinazzi S, Gollotte A (1993) In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. Mycol Res 97:245–250

Trépanier M, Bécard G, Moutoglis P, Willemot C, Gagné S, Avis TJ, Rioux JA (2005) Dependence of arbuscular mycorrhizal fungi on their plant host for palmitic acid synthesis. Appl Environ Microbiol 71:5341–5347

van Buuren ML, Maldonado-Mendoza IE, Trieu AT, Blaylock LA, Harrison MJ (1999) Novel genes induced during an arbuscular mycorrhizal (AM) symbiosis formed between *Medicago truncatula* and *Glomus versiforme*. Mol Plant-Microb Interact 12:171–181

Versaw WK, Chiou TJ, Harrison MJ (2002) Phosphate transporters of *Medicago truncatula* and arbuscular mycorrhizal fungi. Plant Soil 244:239–245

Vieweg MF, Fruhling M, Quandt HJ, Heim U, Baumlein H, Puhler A, Kuster H, Perlick AM (2004) The promoter of the *Vicia faba* L. leghemoglobin gene *VfLb29* is specifically activated in the infected cells of root nodules and in the arbuscule-containing cells of mycorrhizal roots from different legume and non legume plants. Mol Plant-Microb Interact 17:62–69

Walter MH, Fester T, Strack D (2000) Arbuscular mycorrhizal fungi induce the nonmevalonate methylerythritol phosphate pathway of isoprenoid biosynthesis correlated with accumulation of the 'yellow pigment' and other apocarotenoids. Plant J 21: 571–578

Weidmann S, Sanchez L, Descombin J, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V (2004) Fungal elicitation of signal transduction-related plant genes precedes mycorrhiza establishment and requires the dmi3 gene in *Medicago truncatula*. Mol Plant-Microb Interact 17:1385–1393

Wulf A, Manthey K, Doll J, Perlick AM, Linke B, Bekel T, Meyer F, Franken P, Kuster H, Krajinski F (2003) Transcriptional changes in response to arbuscular mycorrhiza development in the model plant *Medicago truncatula*. Mol Plant-Microb Interact 16:306–314