Arbuscular Mycorrhiza

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1. INTRODUCTION

The arbuscular mycorrhizal symbiosis is a mutualistic association formed between plants and a wide variety of fungi from the phylum Glomeromycota. The symbiosis is formed by the majority of the vascular flowering plants and is found in ecosystems throughout the world. In general, the symbionts trade nutrients, and the arbuscular mycorrhizal (AM) fungus obtains carbon from the plant while providing the plant with an additional supply of phosphorus (as phosphate). While much research has focused on nutrient exchange, the AM symbiosis is associated with a range of additional benefits for the plant including the acquisition of other mineral nutrients, such as nitrogen and resistance to a variety of stresses. As a consequence, the AM symbiosis is of tremendous significance to life on this planet, in both natural and agricultural ecosystems (69).

The first publications describing the use of *M. truncatula* to study the AM symbiosis emerged in the early 1990's (33, 34). Since then, it has been widely adopted as a reference species for studies of the AM symbiosis and the number of publications has increased exponentially. Many significant advances in our understanding of arbuscular mycorrhiza, particularly the molecular basis of the symbiosis, have been made in *M. truncatula*.

There are many excellent reviews covering the AM symbiosis and *M. truncatula* research is featured in these (2, 5, 8, 24, 25, 27, 29-31, 36, 45, 46, 59, 62-64, 68, 70, 73, 76). The purpose of this chapter is not to repeat earlier reviews, but rather to provide a brief summary and then direct the reader to the primary literature covering the AM symbiosis in *M. truncatula*. The chapter is divided into short sections covering aspects of the symbiosis in which *M. truncatula* research has featured significantly. Each section begins with a short introduction and then highlights research publications that are key to that topic. If available, links to other resources are included.

1.1 Development of the AM symbiosis in *M. truncatula*

In general, the AM symbiosis is considered 'non-specific', that is most plants are capable of forming AM associations with a broad array of AM fungi. However, it is clear that there are differences at the functional level (69). *M. truncatula* is no exception, forming mycorrhizas with different AM fungi (70). The process of colonization of *M. truncatula* roots and the symbiotic structures and interfaces that form, are typical of an AM symbiosis. For detailed information on common aspects in the development of the symbiosis, the reader is referred to the following reviews (9, 24, 26, 29, 61). Briefly, the AM fungus makes contact with the root epidermis and differentiates to form an appressorium, via which it enters the root. Once inside the root cortex, inter-cellular hyphae grow through the root apoplast, and the fungus forms highly branched structures, called arbuscules, within the cortical cells of the root (Figure 1). The arbuscules are assumed to be the site of nutrient exchange between the two symbionts. Their formation induces activation and reorganization of the host cell contents with the proliferation of organelles and membrane systems which results in an extensive symbiotic interface between the plant and fungal cells (70). In *M. truncatula*, arbuscules are formed initially in the cortical cell layers closest to the vascular tissue. Some AM fungi develop vesicles within the roots and in *M. truncatula/G. intraradices* mycorrhizas, prolific vesicle formation can occur. Following the development of hyphae and arbuscules within the root, the fungus also extends its extra-radical hyphae and a network develops in the soil. The external mycelium has a range of functions

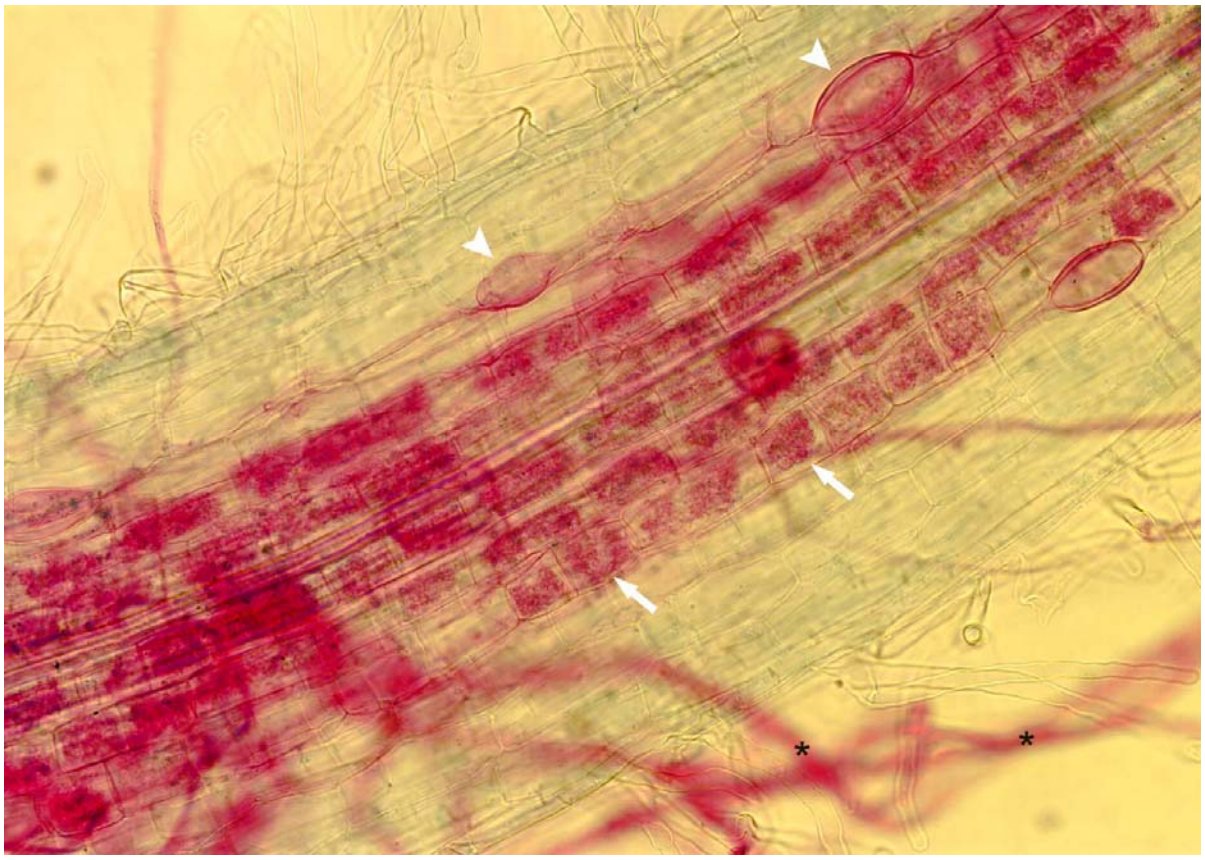

Figure 1. Arbuscular mycorrhiza: *M. truncatula/ Glomus intraradices* (M.J.H)
Arrows indicate arbuscules and arrowheads indicate vesicles. Asterisks indicate extra-radical hyphae (The root is stained with acid fuchsin. A useful overview of approaches for staining mycorrhizal roots can be found in (77)

including nutrient acquisition, the invasion of other roots and the formation of spores.

Web Resources

Culture collections of mycorrhizal fungi and information on arbuscular mycorrhizas:
http://invam.caf.wvu.edu/

http://www.kent.ac.uk/bio/beg/englishhomepage.htm

http://mycorrhiza.ag.utk.edu/

2. EARLY SIGNALING EVENTS IN THE AM SYMBIOSIS AND INSIGHTS FROM *M. TRUNCATULA* MUTANTS

Successful colonisation of roots by beneficial micro-organisms and subsequent microbial effects on plant processes depend on early recognition processes, which are most likely coordinated by reciprocal exchange of signals. The molecular dialogue prior to and during initial contact between AM fungi and plant roots has been little studied, mainly due to the difficulties in synchronizing developmental events in the mycorrhizal symbionts. Transcriptome studies and mycorrhiza-defective *M. truncatula* mutants are giving cues as to how biologically active root and fungal factors may regulate cell functions during early interactions. They also point to a role of symbiosis-related plant genes in pathways for specifically sensing fungal signals by the host root.

2.1 Early developmental events and signals

Hyphal growth and transcriptional activity of AM fungi is stimulated by root exudates from host plants including *M. truncatula* (11), and it was shown recently that the active compounds include strigolactones (1, 6). On the plant side, *M. truncatula* genes with predicted functions in signal transduction, transcription and translation are up-regulated (66, 78) as appressoria form on the root surface, and appressoria-contacted cells respond by organising a new apoplastic compartment across the cell lumen into which the fungus penetrates (22). Cell to cell contact is not necessary for AM fungal-driven plant gene responses. Exudates from AM fungal hyphae also induce gene expression in *M. truncatula* roots, leading to the hypothesis that 'Myc' factors may be signals in AM interactions, analogous to Nod factors in the rhizobial symbiosis (47, 78). However, different signal transduction pathways appear to be active in the two symbioses (47, 66, 78).

2.2 Mycorrhiza-defective mutants and Myc factors

Four *M. truncatula* genes common to nodulation and AM formation have so far been identified: *DMI1*, a predicted ion channel, and *DMI2/MtSYM2*, a receptor-like kinase, act upstream of calcium spiking while *DMI3/MtSYM13*, a calcium/calmodulin-dependent protein kinase acts downstream (4, 20, 50, 57), and *SUNN/MtSYM12* encodes a putative leucine-rich repeat receptor-like kinase (67). Genotypes mutated for these genes are either mycorrhiza-defective

(*dmi1, dmi2/Mtsym2, dmi3/Mtsym13*) or autoregulation-defective (*sunn*, hypermycorrhizal phenotype) for the symbiosis (58). However, leaky phenotypes have been reported for *dmi1* and *dmi2/Mtsym2* locus mutants, and they can form associations depending on the AM fungus and plant growth environment (58). All these mutants are also altered in the nodulation symbiosis with *S. meliloti (13).*.

Work with *M. truncatula* mutants indicates that more than one 'Myc' factor is probably produced by AM fungi. Lateral root formation induced by hyphal exudates from AM fungi requires *DM1* and *DMI2*/*MtSYM2* but not *DMI3*/*MtSYM3* (60). *MtENOD11* (encoding a repetitive proline rich protein) is an early nodulation gene which is induced by appressorium and arbuscule formation (42). Appressoria-driven expression of *MtENOD11* is dependent on the *DMI2/MtSYM2* gene (14) but activation of this gene by diffusible AM fungal factors is not *DMI*-dependent (47), suggesting that different Myc factors may activate *MtENOD11* through different molecular pathways. Signal transduction-related gene activation by appressoria and diffusible fungal molecules is *DMI3/MtSYM13*-dependent, as is elicitation of the novel intracellular apparatus for fungal penetration, pointing to related cell perception processes essential to root colonisation (22, 66, 78). Silencing of a calcium-dependent protein kinase1 (CDPK1) in *M. truncatula* also results in significant diminution of mycorrhizal, as well as rhizobial, root colonization (38). Most of these data argue for a role of calcium-sensing in the perception of 'Myc' factors and the induction of plant gene responses during early interactions between *M. truncatula* and AM fungi. This supposition could be verified by the identification of calcium reponses to fungal signals and/or appressoria and of mycorrhiza-specific signalling components involved in 'Myc' factor signal transduction events. Also, on-going work to characterise other *M. truncatula* mutants will widen knowledge of genes involved in the perception of AM fungi by host roots, and should help to identify pathways independent of nodulation events.

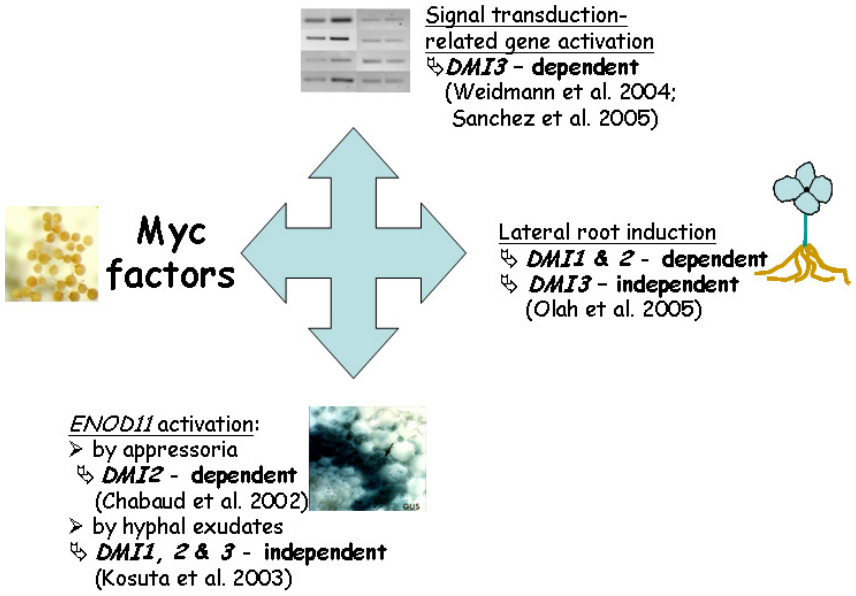

Figure 2. Responses to AM fungal signals in *M. truncatula* (V.G.P).

Key Papers

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

Semi-purified exudates from roots of mycotrophic plant species, including *M. truncatula*, stimulated strong hyphal growth and branching of *Gigaspora gigantea* whilst exudates from non-host roots or from cell cultures were inactive. Chemicals active in other plant-microbe interactions were not elicitors of the branching response.

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

The early nodulin gene *MtENOD11* was transiently transcribed in epidermal and outer cortical cells at sites of hyphal penetration by *Gigaspora* into transformed Myc+ root explants., but not in epidermal cells of Myc- (*dmi2-2*) mutant roots in contact with appressoria. A hypothetical 'Myc' signal factor proposed.

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

M. truncatula prepares and organizes AM colonization of the root. *Gigaspora* hyphae induce epidermal cells to construct a novel apoplastic compartment (pre-penetration apparatus), with a novel cytoskeletal organization, where the fungus is destined to penetrate. Its formation is *DMI1*- and *DMI3*-dependent, and is accompanied by activation of *ENOD11*.

Kosuta, S., Chabaud, M., Lougnon, G., Gough, C., Dénarié, J., Barker, D.G., and Bécard, G. (2003). A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *M. truncatula*. *Plant Physiol*. 131**,** 952-962.

Germinating spores of AM fungi separated physically from transformed Myc+ root explants produce a diffusible ('Myc') factor which elicits *MtENOD11* expression in the root cortex, whereas pathogenic fungi do not. *MtENOD11* induction occurs also in three Myc- mutants (*dmi1-1, dmi2-2, dmi3-1)*, blocked in their response to Nod factors, suggesting different signal transduction pathways in the two symbioses.

Morandi, D., Prado, E., Sagan, M., and Duc, G. (2005). Characterisation of new symbiotic *Medicago truncatula* (Gaertn.) mutants, and phenotypic or genotypic complementary information on previously described mutants. *Mycorrhiza* 15: 283-9

Phenotypes are confirmed for *dmi3* (mycorrhiza-defective TRV25) and *sunn* (hypermycorrhizal TR122) mutants. Delayed mycorrhiza development by *G. mosseae* and *G. intraradices* is reported in *dmi1* and *dmi2* mutants. *MtSYM* is proposed to replace *DMI* for *M. truncatula* symbiosis-related gene nomenclature (cf. pea and *L. japonicum*).

Olah, B., Brière C., Bécard, G., Dénarié J., and Gough, C. 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *M. truncatula* via the DMI1/DMI2 signalling pathway. *Plant J*. 44, 195-207.

A diffusible factor from germinating spores of AM fungi stimulated lateral root formation by *M. truncatula* in a similar way to Nod factors. The stimulatory effects are not mimicked by auxin. The root response to AM fungi requires *DMI1* and *DMI2* but not *DMI3*, whilst stimulation of lateral roots by Nod factors requires all three plant genes.

Sanchez, L., Weidmann, S., Arnould, C., Bernard, A.R., Gianinazzi, S., and Gianinazzi-Pearson V. (2005). *P. fluorescens* and *G. mosseae* trigger *DMI3*-dependent activation of genes related to a signal transduction pathway in roots of *M. truncatula*. *Plant Physiol*. 139, 1065-1077.

G. mosseae activates *M. truncatula* genes coding proteins in a putative signal transduction pathway induced during early root colonization by a growth-promoting *P. fluorescens*. Plant gene response to either microorganism requires the DMI3 protein. *S. meliloti* does not activate the same signal transduction pathway.

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

Appressoria-induced *M. truncatula* genes coding proteins with putative functions in signal transduction, transcription or translation are activated in roots by diffusible molecules from germinating spores of *G. mosseae*. Perception of the fungal signals (Myc factors) is *DMI3*-dependent.

3. THE ARBUSCULAR MYCORRHIZA-RELATED TRANSCRIPTOME OF *M. TRUNCATULA*

3.1 Transcriptional responses during development of the AM symbiosis in *M. truncatula*

Development of the AM symbiosis is accompanied by considerable alterations in the cortical cells and to the overall physiology of the root. A variety of transcriptional profiling approaches have been used to gain insight into the molecular events that underlie these changes including EST analyses, cDNA and oligonucleotide-based arrays (10, 28, 37, 43, 49, 52, 53, 56 , 78, 79). There is a rich resource of ESTs from *M. truncatula* mycorrhizal roots with a range of different AM fungal species and these are available through the *M. truncatula* gene index, hosted by TIGR http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=medicago. A list of the *M. truncatula* mycorrhizal cDNA libraries from which ESTs have been generated is shown in Table 1 (54). Key findings from the EST and array analyses include the identification of genes that are expressed specifically, possibly exclusively, in *M. truncatula* AM roots. In addition to providing global overviews of changes in gene expression, the transcriptional studies have identified genes or groups of genes that serve as suitable markers of different stages of the AM symbiosis. These are particularly useful for monitoring the AM symbiosis.

Table 1. *M. truncatula* arbuscular mycorrhizal cDNA libraries available (from (54).

Library name	TIGR cat#	No. of ESTs	Description	Reference
MHAM	T1682	7351	ESTs from roots of *M. truncatula* A17 colonized with *Glomus versiforme*	(52)
MHAM2	#GFS	1679	Same as MHAM	(52)
MtBC	5520	8567	ESTs from roots of *M. truncatula* J5 three weeks after inoculation with *G. intraradices*	(43)
MtAMP	#ARE	3448	ESTs from *M. truncatula* A17 three weeks after inoculation with *G. intraradices*	(21)
MtGIM	#ARB	1686	ESTs from *M. truncatula* A17 three weeks after inoculation with *G. intraradices* (SSH library)	(79)
MtGmEs	#G7D	29	ESTs from *M. truncatula* J5 roots 5 days after inoculation with *G. mosseae* (SSH library)	(78)
MtGmLs	#9CR	37	ESTs from *M. truncatula* J5 roots 20 days after inoculation with *G. mosseae* (SSH library)	(10)

3.2 Spatial expression patterns of mycorrhiza-induced genes in *M. truncatula*

Transcript profiling approaches has enabled the identification of *M. truncatula* genes with AM specific or AM induced expression but these alone cannot provide information about the spatial expression patterns. Since mycorrhiza formation is asynchronous, predictions of location and timing of gene expression based on the expression profiling data are difficult to make. As the symbiosis develops in specific cells within the root, spatial information is particularly important for understanding the role of the gene in the symbiosis. *In situ* hybridization revealed that transcripts of genes of the flavonoid biosynthesis pathway, a proton ATPase and a lectin like gene occurred specifically in cells with arbuscules (17, 34, 48). *In situ* hybridization is time consuming and relatively complicated technique and has been superseded by analysis of promoter-reporter gene fusions. This was facilitated by the development of protocols for transforming *M. truncatula*, particularly for a protocol for the generation of transgenic roots. Additionally, since the *M. truncatula* genome is being sequenced, the identification of promoter sequences of AM specific and induced genes can readily identified. Consequently, the spatial expression patterns of an increasing number of genes are being obtained via analysis of promoter-reporter gene constructs.

A list of localized AM induced genes in this model plant is presented in Table 2. Expression of some of these genes (*MtPt4*, *MtGst1*, *MtGlp1*, *MtCel1*, *MtHa1*, *MtLec5*, *MtLec7*) are localized exclusively in arbuscule-containing cells, whereas expression of AM specific genes such as *MtScp1*, *MtBcp1*, *MtTi1*, *MtTub1*, and *MtAnn2* is detected in arbuscule-containing cells and cells surrounding them. Many of these genes are useful markers of cortical invasion and arbuscule formation in the AM symbiosis.

Table 2. Mycorrhiza-induced genes for which spatial expression pattern have been described in *M. truncatula*. These genes are useful molecular markers for the AM symbiosis

Gene	Annotation	Cellular localization technique	Localization	Reference
*MtPt*4	Phosphate transporter	Promoter-GUS[1] Immunolocalization	Arbuscules	(32)
*MtHa*1	H+-ATPase	*In situ* hybridization	Arbuscules	(48)
*MtGlp*1	Germin-like protein	*In situ* hybridization	Arbuscules	(17)
*MtGst*1	Glutathione-S-transferase	Promoter-GUS	Arbuscules and adjacent cells	(79)
*MtCel*1	Cellulase	Promoter-GUS	Arbuscules	(52)
*MtScp*1	Serine carboxy peptidase	Promoter-GUS	Arbuscules and adjacent cells	(52)
*MtTi*1	Trypsin inhibitor	Promoter-GUS	Arbuscules and adjacent region	(28)
*MtAnn*2	Annexin	Promoter-GUS	Arbusculess and adjacent cells	(56)
*MtTubb*1	B-Tubulin	Promoter-GUS	Arbuscule and adjacent cells	(56)
Blue copper binding protein (*MtBcp*1)		Promoter-GUS	Arbuscule exclusively	(37)
Lectin (*MtLec*5)	Lectin	Promoter-GUS	Arbuscules	(21)
Lectin (*MtLec*7)	Lectin	Promoter-GUS	Arbuscules	(21)

[1] GUS, ß-glucuronidase (*uidA*) gene

Key papers

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

Suppressive subtractive hybridization was used to identify genes unregulated in the *M. truncatula*/G. mosseae interaction. *M. truncatula* genes induced during appressoria formation were identified and are useful as molecular markers of early stages of the symbiosis.

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

Transcriptional profiling using a *M. truncatula* 6K array reveals AM induced genes, 75 of which are induced in both AM symbiosis and root nodule symbiosis. Analysis of annexin and B-tubulin promoter Gus fusions revealed strong expression in cells with arbuscules. These genes are useful markers of arbuscule formation.

Hohnjec, N., Vieweg, M.F., Puhler, A., Becker, A., and Kuster, H. (2005). Overlaps in the Transcriptional Profiles of *M. truncatula* Roots Inoculated with Two Different Glomus Fungi Provide Insights into the Genetic Program Activated during Arbuscular Mycorrhiza. *Plant Physiol*. 137**,** 1283-1301.

Transcriptional profiling using the *M. truncatula* 16K oligonucleotide-based array identifies over 200 AM induced genes. Promoter analysis of a blue copper binding protein gene reveals strong expression in cells with arbuscules.

Liu, J.Y., Blaylock, L.A., Endre, G., Cho, J., Town, C.D., VandenBosch, K.A., and Harrison, M.J. (2003). Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. *Plant Cell* 15**,** 2106-2123

Transcriptional profiling with a cDNA arrays revealed AM-induced genes. A serine carboxypeptidase shows expression in cells containing arbuscules and in adjacent cortical cells, while a mycorrhiza-specific cellulase is expressed only in cells with arbuscules. These genes are useful markers of an AM symbiosis.

Weidmann, S., Sanchez, L., Descombin, J., Chatagnier, O., Gianinazzi, S., and Gianinazzi-Pearson, V. (2004). Fungal elicitation of signal transduction related plant genes precedes mycorrhiza establishment and requires the DMI 3 gene in *M. truncatula*. *Mol. Plant-Microbe Interact.* 17**,** 1385-1393.

Suppressive subtractive hybridization identified AM induced genes. Subsequent analysis shows that some of these are induced without physical contact between the symbionts and are useful markers for the presence of a putative 'myc' factor. Furthermore the DMI3 protein is required for their expression.

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

Suppressive subtractive hybridization identifies AM induced genes. The library is the MtGIM library indicated in Table 1. A *M. truncatula* glutathione-S-transferase promoter Gus fusion shows induction in the cortex of colonized roots.

Harrison, M.J., Dewbre, G.R., and Liu J. (2002) A phosphate transporter from *M. truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14, 2413–2429.

M. truncatula phosphate transporter (*MtPt4*) was identified by searches on EST databases. It was shown that *MtPt4* is a low affinity phosphate transporter immunodetected in the membrane fraction of mycorrhizal roots. A genomic fragment containing *MtPt4* promoter was isolated from a genomic library and fused to GUS gene. GUS staining was exclusively expressed in arbuscule containing cells. Additional immunolocalization and confocal microscopy showed that *MtPt4* protein is present in the periarbuscular membrane of the branches of mature arbuscules. *MtPt4* is useful as a molecular marker for AM symbiosis.

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

In silico analysis was performed on 5,464 ESTs generated out of two *M. truncatula* cDNA libraries, and by which additional AM-specific genes were identified. A novel family of AM-specific lectin genes was analyzed in more detailed, and the expression of two members of this gene family were localized in arbuscules by promoter-reporter gene fusions in transgenic roots.

Web Resources

Useful information about *M. truncatula* arrays and other transcriptional profiling resources can be found at the following sites.

http://www.genetik.uni-bielefeld.de/MolMyk/

http://www.medicago.org/

http://medicago.toulouse.inra.fr/Mt/EST/

4. THE ARBUSCULAR MYCORRHIZA-RELATED PROTEOME OF *M. TRUNCATULA*

Proteins are key effectors of plant responses to environmental stimuli. Relative little is known about modifications in protein expression induced by cell to cell interactions between AM symbionts, as compared to the considerable amount of data generated at the transcript level. Developments in high throughput and subcellular proteomics, combining 2-D electrophoresis, mass spectrometry (MALDI-TOF, LC-MS/MS), bioinformatics and the use of plant mutants, are providing opportunities for large-scale profiling of AM symbiosis-related proteins. Research has consequently progressed from targeted approaches, focussing on individual proteins, to wide display approaches monitoring differentially regulated proteins (3, 7) and subcellular analyses focussing on membrane compartments to identify the AM symbiosome (74, 75). The challenge is also to forge the link between transciptomics and proteomics in order to understand the consequences of gene expression for protein activity (18). A large number of *M. truncatula* genes have no assigned function, and information obtained by transcriptomics cannot directly predict co-/post-transcriptional events, post-translational modifications or protein-protein interactions. Systematic proteomics have begun to provide more direct insight into cell functions that are regulated during AM processes in *M. truncatula*, as well as an understanding of events that are involved in key cell compartments.

Key papers

Amiour, N., Recorbet, G., Robert, F., Gianinazzi, S., Dumas-Gaudot, E. (2006). Mutations in DMI3 and SUNN modify the appressorium-responsive root proteome in arbuscular mycorrhiza. *Mol. Plant-Microbe Interact*. 19, 988-997.

A detailed study of *G. intraradices* appressorium formation shows changes are triggered in soluble protein profiles of *M. truncatula* roots prior to fungal penetration. Root proteome responses to the AM fungus are differentially affected in mycorrhiza- or autoregulation-defective *M. truncatula* genotypes, mutated for *DMI3* or *SUNN* respectively.

Bestel-Corre, G., Dumas-Gaudot, E., Poinsot, V., Dieu, M., Dierick, J-F., van Tuinen, D., Remacle, J., Gianinazzi-Pearson, V., and Gianinazzi, S. (2002). Proteome analysis and identification of symbiosis-related proteins from *Mediago truncatula* Gaertn. By two-dimensional electrophoresis and mass spectrometry. *Electrophoresis* 23, 122-137.

Time course analysis of *M. truncatula* soluble root protein profiles after inoculation with *G. mosseae* or *S. meliloti* revealed numerous modifications which were mainly newly induced proteins. None were common to both

symbioses. Identified mycorrhiza-induced proteins were defence- or respiration-related. First **evidence for** appressorium-induced plant proteins is presented.

Valot, B., Dieu, M., Recorbet, G., Raes, M., Gianinazzi, S., and Dumas-Gaudot, E. (2005). Identification of membrane-associated proteins regulated by the arbuscular symbiosis. *Plant Mol. Biol*. 59, 565-580.

Comparative proteomics of soluble and insoluble microsomal fractions revealed differential protein regulation in membranes of non-mycorrhizal and mycorrhizal *M. truncatula* roots. Most of the identified proteins were novel to the AM symbiosis and not regulated by phosphate. They may be candidate markers of symbiotic interactions.

Valot, B., Negroni, L., Zivy, M., Gianinazzi, S., and Dumas-Gaudot, E. (2006). A mass spectrometric approach to identify arbuscular mycorrhiza-related proteins in root plasma membrane fractions. *Proteomics* 6, 145-155.

Proteome analyses of enriched root plasma membrane extracts identified major membrane intrinsic proteins in non-mycorrhizal and mycorrhizal *M. truncatula* roots. A plasma membrane efflux-type ATPase and a blue copper binding protein were only detected in *G. intraradices*-colonized roots, consistent with previously reported gene expression in arbuscule-containing cells.

Web Resources

The symbiotic proteome of *M. truncatula* can be found at the following site.
http://www.mtproteomics.com/

5. PHOSPHATE PHYSIOLOGY AND TRANSPORT IN *M. TRUNCATULA* ARBUSCULAR MYCORRHIZA

Plants and fungi in AM associations have co-evolved for over 450 million years producing adaptations in both symbionts that sustain the development and function of this symbiosis. The basis for a mutualistic interaction relies on the exchange of nutrients. The plant provides carbon for the fungus and the fungus acquires, and makes available to the plant, soil nutrients, mainly phosphorus (as orthophosphate, Pi) but also nitrogen. In many environments, these essential nutrients are present at levels that are limiting for plant growth; consequently, the additional source of nutrients delivered via the fungus is generally beneficial for the plant. In the arbuscular mycorrhiza, plants can obtain Pi from the soil directly through transport systems in the root epidermis and root hairs, and through the mycorrhizal pathway via fungal external hyphae and the arbuscules (44, 71, 72). Recently, 33P transfer studies in different *M. truncatula*-fungus interactions showed that, in some cases, the fungus provides the majority of the Pi supply. In addition, some Pi is delivered via the fungus even when overall plant growth responses or Pi uptake remain unaffected (71, 72).

Pi transport mechanisms have been studied not only at the physiological, but also at the molecular level in *M. truncatula.* Some of the proteins that are required for Pi transport, both in roots and in mycorrhizas, have been identified. These include MtPT1, and MtPT2 (15, 51), phosphate transporters which are expressed in root epidermis and root hairs (15), and a *M. truncatula* mycorrhiza-specific phosphate transporter, MtPT4 (32). MtPT4 is expressed in the periarbuscular membrane at the symbiotic interface surrounding arbuscules inside cortical cells

and is predicted to play a role in Pi transport in the symbiosis (32). Studies in *M. truncatula* also revealed mycorrhizal effects on the expression of Pi-starvation inducible genes. For example, *Mt4*, a phosphate starvation-inducible gene of unknown function, is rapidly down-regulated by AM colonization, possibly prior to the delivery of significant quantities of Pi (12).

In addition to plant Pi transporters, studies of mycorrhizal symbioses in *M. truncatula* have enabled the identification of some of the fungal Pi transporters including GvPT, a phosphate transporter from *G. versiforme* and GiPT, a Pi transporter from *G. intraradices* (35, 55). During the AM symbiosis in *M. truncatula*, Pi transporters expressed in the root epidermis, such as MtPT1 and 2, are down-regulated, indicating that *M. truncatula* reorganizes its Pi acquisition strategies to take advantage of Pi delivery via the fungus (51). In the near future, the work being conducted in *M. truncatula* in genomics, transcriptomics, and proteomics will help us to gain insight on the mechanisms involved in phosphate transport on this symbiosis.

Key papers

Liu, H., Trieu, A.T., Blaylock, L.A., and Harrison, M.J. (1998). Cloning and characterization of two phosphate transporters from *M. truncatula* roots: Regulation in response to phosphate and to colonization by arbuscular mycorrhizal (AM) fungi. *Mol. Plant-Microbe Interact.* 11, 14-22.

Cloning of two transporters from *M. truncatula* roots is described and the first indication provided that expression of phosphate transport systems in the root are negatively regulated during the AM symbiosis.

Harrison, M.J., Dewbre, G.R., and Liu J. (2002) A phosphate transporter from *M. truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *Plant Cell* 14, 2413–2429.

This paper describes the characterization of *MtPt4*, a phosphate transporter expressed in mycorrhizal roots. Sequence motifs derived of the analysis of previously reported plant phosphate transporters were used to screen *M. truncatula* EST databases to identify it. *MtPt4* localizes to the arbuscules on the periarbuscular membrane.

Karandashov, V., and Bucher, M. (2005) Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends in Plant Sc*. 10, 22-29.

This recent review dedicated to phosphate transport in AM symbiosis contains relevant references to work conducted in *M. truncatula* as well as other plant species.

Smith S.E., Smith F.A., and Jakobsen I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.* 133, 16-20.

This paper explores P uptake and transport in combinations of three different plants (flax, tomato and medic) associated with three different fungal species. When *M. truncatula* is associated to *Glomus intraradices*, or *G. caledonium* all the phosphate acquired is delivered though the mycorrhizal pathway. When *Gigaspora roseae* was the fungal partner the plant received only 40% of P from the fungus. Effects were also distinct in plant growth between the three associations. A demonstration of the different pathways of P uptake in AM plants is shown.

Smith, S.E., Smith F. A., and Jakobsen, I. (2004). Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytol*. 162, 511–524.

Phosphate delivery via the fungus is further examined in mycorrhizal flax, tomato and medic and results indicate that even in the absence of any visible growth stimulation, the plants tested were receiving Pi via the AM fungus.

6. NON-NUTRITIONAL STRESS ALLEVIATION BY ARBUSCULAR MYCORRHIZA IN *M. TRUNCATULA*

Improved tolerance of AM plants to biotic (pathogenic microbes) and abiotic (pollutants, salt, drought) stresses is a well-known phenomenon in many plant species, but the mechanisms through which the symbiosis acts have not yet been clearly elucidated. AM protective effects against root pathogens are thought to act via different pathways, including sensitization through priming of defence-related plant pathways. In this context, AM fungi weakly or transiently elicit defence-related pathways in *M. truncatula* roots, with cell type-specific differential gene expression at sites of arbuscule formation (33, 34, 65). Attenuated defence responses may be relevant to priming of a protective mechanism in pathogen-challenged mycorrhizal roots (19). Recent proteome analyses of root interactions with *Aphanomyces euteiches* point to possible molecular similarities between mycorrhiza-induced and constitutive genotype tolerance in *M. truncatula* (16).

M. truncatula is also exploited as a model plant to investigate how the AM symbiosis impacts on, or is affected by, organic or inorganic soil pollutants. *M. truncatula* has the ability to accumulate trace (heavy) metals from contaminated substrates, and uptake is reduced in AM plants in the case of cadmium (40) (Gollotte et al. unpublished). Overall, AM have little or no effect on *M. truncatula* growth in polluted sludges but sensitivity of the AM fungi involved can vary (39, 41). Ecotoxicological tests to evaluate substrate/soil quality have been developed based on *M. truncatula* interactions with *G. mosseae* (23).

Key papers

Colditz, F., Braun, H-P., Jacquot, C., Niehaus, K., and Krajinski, F. (2005) Protemoic profiling unravels insights into the molecular background underlying increased *Aphanomyces euteiches*-tolerance of *M. truncatula*. *Plant Mol. Biol.* **59**, 387-406.

Bioprotection against *A. euteiches* in mycorrhizal in *M. truncatula* roots is partly due to competition for infection sites. Similar protein patterns in mycorrhizal tissues and in roots of a relatively resistant *M. truncatula* line point also to a protective effect induced by establishment of the symbiosis prior to challenge by the pathogen.

Jacquot, E., van Tuinen, D., Gianinazzi, S., and Gianinazzi-Pearson, V. (2000). Monitoring species of arbuscular mycorrhizal fungi in planta and in soil by nested PCR: application to the study of the impact of sewage sludge. *Plant Soil* 226, 179-188.

A biological system for evaluating toxicity of non-composted sewage sludge is proposed based on *in planta* sensitivity of different mycorrhizal fungi in interactions with *M. truncatula*.

Jacquot-Plumey, E., Caussanel, J-P., Gianinazzi, S., van Tuinen, D., and Gianinazzi-Pearson, V. (2003). Heavy metals in sewage sludges contribute to their adverse effects on the arbuscular mycorrhizal fungus *Glomus mosseae*. *Fol. Geobot*. 38, 167-176.

The depressive effect of dehydrated or composted urban sewage sludge on mycorrhizal development by *M. truncatula* was related to the metallic pollutant contents rather than antagonistic organisms or P.

Gianinazzi, S., Plumey-Jacquot, E., Gianinazzi-Pearson, V., and Leyval, C. (2005). Contribution of arbuscular mycorrhiza to soil quality and terrestrial ecology. In: Microbiological Methods for Assessing Soil Quality, J. Bloem, D.W. Hopkins, A. Benedetti (eds). CABI Publishing, Cambridge, USA, pp. 248-256.

Description of two bioassays, normalised as national standards, of ecotoxicological effects of wastes and for soil quality based on *G. mosseae* spore germination and colonization of *M. truncatula* roots.

Salzer, P. , Bonanomi, A., Beyr, K., Vögeli-Lange, R., Aeschbacher, R. A., Lange, J., Wiemken, A., Kim, D., Cook, D.R., and Boller, T. (2000). Differential expression of eight chitinase genes in *M. truncatula* during mycorrhiza formation, nodulation, and pathogen infection. *Mol. Plant-Microbe Inter*. 13, 763-777

Pathogenesis-related Class II, III-1 and IV chitinase genes are weakly up-regulated at early mycorrhizal stages whilst Class III-2, -3 and -4 chitinases are uniquely expressed in mycorrhizal tissues at later stages. The function of specific chitinases in attenuating defence responses to AM fungi is hypothesized.

GENERAL REFERENCES

1. Akiyama K, Matsuzaki K-I, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824-7
2. Allen MF, Swenson W, Querejeta JI, Egerton-Warburton LM, Treseder KK. 2003. Ecology of mycorrhizae: A conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology* 41: 271-303
3. Amiour N, Recorbet G, Robert F, Gianinazzi S, Dumas-Gaudot E. 2006. Mutations in DMI3 and SUNN modify the appressorium-responsive root proteome in arbuscular mycorrhiza. *Molecular Plant-Microbe Interactions* 19: 988-97
4. Ane JM, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, et al. 2004. *Medicago truncatula* DMI1 required for bacterial and fungal symbioses in legumes. *Science* 303: 1364-7
5. Bago B, Pfeffer PE, Shachar-Hill Y. 2000. Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiology* 124: 949-57
6. Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, et al. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *Plos Biology* 4: 1239-47
7. Bestel-Corre G, Dumas-Gaudot E, Poinsot V, Dieu M, Dierick JF, et al. 2002. Proteome analysis and identification of symbiosis-related proteins from *Medicago truncatula* Gaertn. by two-dimensional electrophoresis and mass spectrometry. *Electrophoresis* 23: 122-37
8. Bonfante P, Perotto S. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. *New Phytologist* 130: 3-21
9. Bonfante-Fasolo P. 1984. Anatomy and morphology of VA mycorrhizae. In *VA Mycorrhizae*, ed. CL Powell, DJ Bagyaraj, pp. 5-33. Boca Raton, Florida: CRC Press

10. Brechenmacher L, Weidmann S, van Tuinen D, Chatagnier O, Gianinazzi S, et al. 2004. Expression profiling of up-regulated plant and fungal genes in early and late stages of *Medicago truncatula-Glomus mosseae* interactions. *Mycorrhiza* 14: 253-62
11. Buee 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. *MPMI* 13: 693-8
12. Burleigh SH, Harrison MJ. 1999. The down-regulation of *Mt4*-like genes by phosphate fertilization occurs systemically and involves phosphate translocation to the shoots. *Plant Physiology* 119: 241-8
13. Catoira R, Galera C, de Billy F, Penmetsa RV, Journet E, et al. 2000. Four genes of *Medicago truncatula* controlling components of a Nod factor transduction pathway. *Plant Cell* 12: 1647-65
14. 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 Phytologist* 156: 265-73
15. Chiou TJ, Liu H, Harrison MJ. 2001. The spatial expression patterns of a phosphate transporter (MtPT1) from *Medicago truncatula* indicate a role in phosphate transport at the root/soil interface. *Plant Journal* 25: 1-15
16. Colditz F, Braun HP, Jacquet C, Niehaus K, Krajinski F. 2005. Proteomic profiling unravels insights into the molecular background underlying increased *Aphanomyces euteiches* tolerance of *Medicago truncatula*. *Plant Molecular Biology* 59: 387-406
17. 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 and Cell Physiology* 44: 1208-14
18. Dumas-Gaudot E, Amiour N, Weidmann S, Bestel-Correl G, Valot B, et al. 2004. A technical trick for studying proteomics in parallel to transcriptomics in symbiotic root-fungus interactions. *Proteomics* 4: 451-3
19. Dumas-Gaudot E, Gollote A, Cordier C, Gianinazzi S, Gianinazzi-Pearson V. 2000. Modulation of host defence systems. In *Arbuscular Mycorrhizas: Physiology and Function*, ed. Y Kapulnik, D Douds, pp. 173-200. Dordecht: Kluwer Academic Publishers
20. Endre G, Kereszt A, Kevel Z, Mihacea S, Kaló P, Kiss G. 2002. A receptor kinase gene regulating symbiotic nodule development. *Nature* 417: 962-6
21. Frenzel A, Manthey K, Perlick AM, Meyer F, Puhler A, et al. 2005. Combined transcriptome profiling reveals a novel family of arbuscular mycorrhizal-specific *Medicago truncatula* lectin genes. *Molecular Plant-Microbe Interactions* 18: 771-82
22. 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: 3489-99
23. Gianinazzi S, Plumey-Jacquot E, Gianinazzi-Pearson V, Leyval C. 2005. Contribution of arbuscular mycorrhizal to soil quality and terrestrial ecology. In *Microbiological Methods for Assessing Soil Quality*, ed. J Bloem, DW Hopkins, A Benedetti, pp. 248-56. Cambridge, USA: CABI publishing
24. Gianinazzi-Pearson V. 1996. Plant cell responses to arbuscular myorrhiza fungi: Getting to the roots of the symbiosis. *Plant Cell* 8: 1871-83

25. Gianinazzi-Pearson V, Denarie J. 1997. Red carpet genetic programmes for root endosymbioses. *Trends in Plant Science* 2: 371-2
26. Gianinazzi-Pearson V, Gianinazzi S. 1988. Morphological integration and functional compatibility between symbionts in vesicular-arbuscular endomycorrhizal associations. In *Cell to Cell Signals in Plant, Animal and Microbial Symbiosis*, ed. S Scannerini, DC Smith, P Bonfante-Fasolo, V Gianinazzi-Pearson, pp. 73-84. Berlin: Springer-Verlag
27. Gohre V, Paszkowski U. 2006. Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* 223: 1115-22
28. Grunwald U, Nyamsuren O, Tamasloukht MB, Lapopin L, Becker A, et al. 2004. Identification of mycorrhiza-regulated genes with arbuscule development-related expression profile. *Plant Molecular Biology* 55: 553-66
29. Harrison MJ. 1997. The arbuscular mycorrhizal symbiosis: an underground association. *Trends in Plant Science* 2: 54-6
30. Harrison MJ. 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 361-89
31. Harrison MJ. 2005. Signaling in the arbuscular mycorrhizal symbiosis. *Annual reviews of Microbiology* 59: 19-42
32. 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: 2413-29
33. 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*. *Molecular Plant-Microbe Interactions* 6: 643-54
34. Harrison MJ, Dixon RA. 1994. Spatial patterns of expression of flavonoid/isoflavonoid pathway genes during interactions between roots of *Medicago truncatula* and the mycorrhizal fungus *Glomus versiforme*. *Plant Journal* 6: 9-20
35. Harrison MJ, van Buuren ML. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* 378: 626-9
36. Hart MM, Reader RJ, Klironomos JN. 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. *Trends in Ecology & Evolution* 18: 418-23
37. Hohnjec N, Vieweg MF, Puhler A, Becker A, Kuster 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: 1283-301
38. Ivashuta S, Liu J, Liu J, Lohar DP, Haridas S, et al. 2005. RNA interference identifies a calcium-dependent protein kinase involved in *Medicago truncatula* root development. *Plant Cell* 17: 2911-21
39. Jacquot E, van Tuinen D, Gianinazzi S, Gianinazzi-Pearson V. 2000. Monitoring species of arbuscular mycorrhizal fungi in planta and in soil by nested PCR: application to the study of the impact of sewage sludge. *Plant and Soil* 226: 179-88
40. Jacquot-Plumey E, Caussanel JP, Gianinazzi S, Van Tuinen D, Gianinazzi-Pearson V. 2003. Heavy metals in sewage sludges contribute to their adverse effects on the arbuscular mycorrhizal fungus Glomus mosseae. *Folia Geobotanica* 38: 167-76
41. Jacquot-Plumey E, van Tuinen A, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V. 2001. 25S rDNA-based molecular monitoring of glomalean fungi in sewage sludge-treated field plots. *Environmental Microbiology* 3: 525-31

42. Journet EP, El-Gachtouli N, Vernoud V, de Billy F, Pichon M, et al. 2001. *Medicago truncatula* ENOD11: A novel RPRP-encoding early nodulin gene expressed during mycorrhization in arbuscule-containing cells. *Molecular Plant-Microbe Interactions* 14: 737-48
43. Journet EP, van Tuinen D, Gouzy J, Crespeau H, Carreau V, et al. 2002. Exploring root symbiotic programs in the model legume *Medicago truncatula* using EST analysis. *Nucleic Acids Research* 30: 5579-92
44. Karandashov V, Bucher M. 2005. Symbiotic phosphate transport in arbuscular mycorrhizas. *Trends in Plant Science* 10: 22-9
45. Kiers ET, van der Heijden MGA. 2006. Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypotheses of evolutionary cooperation. *Ecology* 87: 1627-36
46. Koide R, Li M. 1990. On host regulation of the vesicular-arbuscular mycorrhizal symbiosis. *New Phytologist* 114: 59-74
47. Kosuta S, Chabaud M, Lougnon G, Gough C, Denarie J, et al. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. *Plant Physiol.* 131: 952-62
48. 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 Biology* 4: 754-61
49. Kuster H, Hohnjec N, Krajinski F, El Yahyaoui F, Manthey K, et al. 2004. Construction and validation of cDNA-based Mt6k-RIT macro- and microarrays to explore root endosymbioses in the model legume *Medicago truncatula*. *Journal of Biotechnology* 108: 95-113
50. Levy J, Bres C, Geurts R, Chalhoub B, Kulikova O, et al. 2004. A putative Ca2+ and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. *Science* 303: 1361-4
51. Liu H, Trieu AT, Blaylock LA, Harrison MJ. 1998. Cloning and characterization of two phosphate transporters from *Medicago truncatula* roots: Regulation in response to phosphate and to colonization by arbuscular mycorrhizal (AM) fungi. *Molecular Plant-Microbe Interactions* 11: 14-22
52. Liu J, Blaylock L, Endre G, Cho J, Town CD, et al. 2003. Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of the arbuscular mycorrhizal symbiosis. *Plant Cell* 15: 2106-23
53. Liu J, Blaylock L, Harrison MJ. 2004. cDNA arrays as tools to identify mycorrhiza-regulated genes: identification of mycorrhiza-induced genes that encode or generate signaling molecules implicated in the control of root growth. *Canadian Journal of Botany* 82: 1177-85
54. Liu J, Lopez-Meyer M, Maldonado-Mendoza IE, Harrison MJ. 2006. Development of the arbuscular mycorrhizal symbiosis: insights from genomics. In *Fungi in the Environment*, ed. GM Gadd, SC Watkinson, P Dyer, pp. 201-23. Cambridge: Cambridge University Press
55. Maldonado-Mendoza IE, Dewbre GR, Harrison MJ. 2001. Expression of a *Glomus intraradices* phosphate transporter gene (*GiPT*) in the extra-radical mycelium of an arbuscular mycorrhiza: regulation in response to phosphate. *Molecular Plant-Microbe Interactions* 14: 1140-8

56. Manthey K, Krajinski F, Hohnjec N, Firnhaber C, Puhler A, et al. 2004. Transcriptome profiling in root nodules and arbuscular mycorrhiza identifies a collection of novel genes induced during *Medicago truncatula* root endosymbioses. *Molecular Plant-Microbe Interactions* 17: 1063-77
57. Mitra RM, Gleason CA, Edwards A, Hadfield J, Downie JA, et al. 2004. A Ca2+/calmodulin-dependent protein kinase required for symbiotic nodule development: Gene identification by transcript-based cloning. *Proceedings of the National Academy of Sciences of the United States of America* 101: 4701-5
58. Morandi D, Prado E, Sagan M, Duc G. 2005. Characterisation of new symbiotic *Medicago truncatula* (Gaertn.) mutants, and phenotypic or genotypic complementary information on previously described mutants. *Mycorrhiza* 15: 283-9
59. Newsham KK, Fitter AH, Watkinson AR. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. *Trends in Ecology & Evolution* 10: 407-11
60. Olah B, Briere C, Becard G, Denarie 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 Journal* 44: 195-207
61. Parniske M. 2004. Molecular genetics of the arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology* 7: 414-21
62. Paszkowski U. 2006. A journey through signaling in arbuscular mycorrhizal symbioses 2006. *New Phytologist* 172: 35-46
63. Paszkowski U. 2006. Mutualism and parasitism: the yin and yang of plant symbioses. *Current Opinion in Plant Biology* 9: 364-70
64. Rillig MC, Mummey DL. 2006. Mycorrhizas and soil structure. *New Phytologist* 171: 41-53
65. Salzer P, Bonanomi A, Beyer K, Vogeli-Lange R, Aeschbacker R, et al. 2000. Differential expression of eight chitinase genes in *Medicago truncatula* roots during mycorrhiza formation, nodulation and pathogen infection. *Molecular Plant-Microbe Interactions* 7: 763-78
66. 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 Physiology* 139: 1065-77
67. Schnabel E, Journet EP, de Carvalho-Niebel F, Duc G, Frugoli J. 2005. The *Medicago truncatula* SUNN gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Molecular Biology* 58: 809-22
68. Smith SE, Gianinazzi-Pearson V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual Review of Plant Physiology Plant Molecular Biology* 39: 221-44
69. Smith SE, Read DJ, eds. 1997. *Mycorrhizal Symbiosis*. San Diego, CA: Academic Press, Inc.
70. Smith SE, Smith FA. 1990. Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytologist* 114: 1-38
71. Smith SE, Smith FA, Jakobsen I. 2003. Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiology* 133: 16-20

72. Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* 162: 511-24
73. Strack D, Fester T. 2006. Isoprenoid metabolism and plastid reorganization in arbuscular mycorrhizal roots. *New Phytologist* 172: 22-34
74. Valot B, Dieu M, Recorbet G, Raes M, Gianinazzi S, Dumas-Gaudot E. 2005. Identification of membrane-associated proteins regulated by the arbuscular mycorrhizal symbiosis. *Plant Molecular Biology* 59: 565-80
75. Valot B, Negroni L, Zivy M, Gianinazzi S, Dumas-Gaudot E. 2006. A mass spetrometric approach to identify arbuscular mycorrhiza-related proteins in root plasma membrane fractions. *Proteomics* 6: 145-55
76. Vierheilig H. 2004. Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany* 82: 1166-76
77. Vierheilig H, Schweiger P, Brundrett M. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum* 125: 393-404
78. Weidmann S, Sanchez-Calderon 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 DMI 3 gene in *Medicago truncatula*. *Molecular Plant Microbe Interactions* 17: 1385-93
79. Wulf A, Manthey K, Doll J, Perlick AM, Linke B, et al. 2003. Transcriptional changes in response to arbuscular mycorrhiza development in the model plant *Medicago truncatula*. *Molecular Plant-Microbe Interactions* 16: 306-14