Functional diversity of arbuscular mycorrhizas extends to the expression of plant genes involved in P nutrition

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Abstract
This study of functional diversity considers symbiotic associations between two plant species, Medicago truncatula and Lycopersicon esculentum, and seven species of arbuscular mycorrhizal fungi (AMF). The objective was to integrate physiological analyses with molecular techniques to test whether functional diversity between AMF species is not only apparent at the level of mycorrhiza formation, plant nutrient uptake and plant growth, but also at the molecular level as observed by variation in the root expression of plant genes involved in the plant’s P-starvation response. The seven species of AMF varied widely in their influence on the root expression of MtPT2 and Mt4 from M. truncatula and LePT1 and TPSI1 from L. esculentum. At one extreme was Glomus mosseae, whereby its colonization of M. truncatula resulted in the greatest reduction in MtPT2 and Mt4 gene expression and the highest level of P uptake and growth, while at the other extreme was Gigaspora rosea, whereby colonization resulted in the highest levels of MtPT2 and Mt4 gene expression and the lowest P uptake and growth. The expression of LePT1 and TPSI1 within the roots of L. esculentum was low and relatively uniform across the seven mycorrhizas, reflecting the ability of this cultivar to maintain low and constant shoot P levels despite root colonization by a broad selection of AMF. This study extends current understanding of functional diversity and shows that plants can respond differently to AMF, not only at the level of colonization, nutrient uptake and growth, but also at the level of gene expression.

Key words: Arbuscular mycorrhizal fungi, functional diversity, gene expression.

Introduction
Most arbuscular mycorrhizal fungi (AMF) can form mycorrhizas with susceptible plants, regardless of the genetic diversity or geographical distribution of the two symbionts. Still, the formation and function of mycorrhizas can be quite variable between fungal species and even between isolates of the same species (Smith et al., 2000; Smith and Smith, 1997). Functional diversity in AM can refer to the differences observed between fungi in how they help host plants resist biotic and abiotic stress (Smith and Read, 1997), although it is often defined in terms of plant growth responses, which may form ‘a continuum’ from dramatic increases in growth to neutral and even parasitic relationships, depending on the particular fungus–plant combination and environmental conditions (Johnson et al., 1997). While the mechanisms underlying this functional diversity remain obscure, it is becoming clear that it involves the transfer of Pi from the fungus to the plant (Smith et al., 2000; van der Heijden et al., 1998; Ravnskov and Jakobsen, 1995) as well as the transfer of photosynthate from the plant to the fungus (Graham and Abbott, 2000; Johnson et al., 1997; Peng et al., 1993; Son and Smith, 1988). Plant–fungus combinations that promote optimal plant growth are believed to be those that provide the greatest amount of Pi to the plant for the least amount of photosynthate transferred to the fungus.

The molecular aspects of AMF functional diversity have not yet been investigated. Preliminary molecular studies involving a few species of AMF have showed that P-starvation inducible plant genes can be down-regulated by mycorrhizal colonization (Rosewarne et al., 1999; Liu H et al., 1998; Burleigh and Harrison, 1997). The down-regulation of these plant genes could be interpreted as the result of P transfer from the fungus to the plant and the corresponding attenuation of the plant’s P starvation...
response. However, it still remains to be determined whether differences can be observed between AMF species in their ability to down-regulate these plant genes and if their down-regulation is associated with the improved P nutrition of the plant as a result of symbiotic function.

The present work is a study of functional diversity in *Medicago truncatula* and *Lycopersicon esculentum* colonized by seven species of AMF. The objective was to integrate classical physiological approaches with molecular techniques to test whether functional diversity between AMF species is not only apparent at the level of mycorrhiza formation, plant nutrient uptake and plant growth, but also at the molecular level as observed by variation in the expression of plant genes involved in the plant’s P-starvation response.

**Materials and methods**

*Culture of plants and fungi*

*Medicago truncatula* Gaertn ‘Jemalong’ (A17), *Lycopersicon esculentum* Mill. (76R) and the fungal isolates *Glomus versiforme* Karsten & Berch (BEG47), *Glomus intraradices* Schenck & Smith (BEG87), *Glomus caledonium* Nicol. & Gerd. Trappe & Gerdemann (BEG15), *Glomus claroideum* Schenck & Smith (BEG14), *Glomus mosseae* (unregistered Finnish isolate V294 obtained from Mauritz Vestergaard), *Scutellospora calospora* Nicol. & Gerd. Walker & Sanders (BEG43) and *Gigaspora rosea* Vestberg were used for the experiments. The fungi were propagated in pot cultures with *Trifolium subterraneum* as described by Ravnskov et al. (1999). Treatments consisted of three replicate pots each containing 350 g of a quartz sand±soil mix (1:1, v:v; sterilized by irradiation 1594 Burleigh 16 h light (400 μm) for 5 weeks prior to harvest.

*Intracellular colonization including the abundance of arbuscules, arbusculate coils, hyphal coils, and vesicles* was measured as described in Carter et al. (2001). Total RNA was isolated from the roots of each treatment using the FastRNA-Green® kit from Bio101. Northern blotting of RNA (10 μg per treatment), 32P-labelling of the probes by random priming and hybridization by the formamide method was carried out as described by Sambrook et al. (1989). For prehybridization, blots were incubated in 50% formamide, 5 × SSPE, 1% SDS, 0.5 × Denhardt’s solution, and 100 μg ml−1 salmon sperm DNA at 42 °C for 2 h. The full-length cDNAs of the Pi transporter MtPT2 (Liu H et al., 1998) and the P starvation-inducible gene Mt4 (Burleigh and Harrison, 1997) from *M. truncatula* and the Pi transporter LePT1 (Liu C et al., 1998) and the P starvation-inducible gene TPS1 (Liu et al., 1997) from *L. esculentum* were used as probes. A 600 bp reverse-transcribed fragment of 18S rRNA from *M. truncatula* (unpublished data) was used as load control. Denatured, 32P-labelled probe was added to the prehybridization solution and blots were hybridized for 16 h. The final washing conditions were 1 × SSPE and 1% SDS at 65 °C. RNA blot results were duplicated in a second, independent hybridization using RNA extracted from a different set of replicate plants. To illustrate how Mt4 expression was correlated with shoot P concentration, its signal intensity in each treatment was quantified using a Biorad Molecular Imager FX™ and the Quantity One™ software package and to account for loading error, adjusted relative to the signal intensity of each treatment’s respective ribosomal control.

**Results**

*Colonization*

Both *M. truncatula* and *L. esculentum* were colonized by the seven species of AMF after 5 weeks of growth. *M. truncatula* had high and relatively uniform levels of root colonization, whereas *L. esculentum* had considerably lower and more variable levels (Fig. 1A, B). The average proportion of root length colonized was 70±12% for *M. truncatula* and 47±24% for *L. esculentum*. However, while *M. truncatula* supported higher levels of colonization relative to tomato, the fungi colonized a proportionally similar amount of root in both plant species (r2=0.74), whereby *G. intraradices* and *G. claroideum* colonized the highest proportion of root length (Fig. 1A, B). The formation of arbuscules, arbusculate coils, hyphal coils, and vesicles within the roots of the two host plants was examined. Table 1 shows that all of the fungi formed intracellular structures within the roots of their host plants. Arbuscules were formed in all of the mycorrhizas of *M. truncatula*, which were relatively numerous and uniform across the seven mycorrhizas (an average of 53±10% of the colonized root length). In *L. esculentum*, *G. versiforme*, *G. intraradices*, *G. claroideum*, and *G. mosseae* also formed arbuscules, although less numerous in most cases relative to *M. truncatula*. Hyphal and/or arbusculate coils were formed in *L. esculentum* when colonized by all but *G. versiforme*, with *S. calospora* and *G. rosea* forming an abundance of these structures. *G. intraradices* and *G. claroideum* formed vesicles within the roots of both host species.

*Plant growth*

Plant growth can be measured in a variety of ways and in this study root length and shoot dry weight were selected to illustrate the functional diversity that can be observed between different plant–fungus combinations. The root length of *M. truncatula* and *L. esculentum* was elevated in most cases in response to mycorrhizal colonization, although root length was considerably lower in the
mycorrhizal plants when compared to their respective non-mycorrhizal controls receiving P fertilization (NM+P) (Fig. 1A, B). The average root length was 38±16 m pot⁻¹ for mycorrhizal *M. truncatula* and 42±14 m pot⁻¹ for mycorrhizal *L. esculentum*. Mycorrhizal root lengths were highest in plants colonized by *G. mosseae* (or *G. intraradices* in the case of *M. truncatula* and *G. versiforme* in the case of *L. esculentum*) and lowest in plants colonized by *G. rosea* (Fig. 1A, B).

Shoot dry weight of *M. truncatula* and *L. esculentum* was elevated in most cases by AMF colonization, although the growth response of the mycorrhizal plants was lower than that of their respective NM+P controls (Fig. 2). Mycorrhizal colonization increased the shoot dry weight of *M. truncatula* considerably more than that of *L. esculentum*—the mycorrhizas of *M. truncatula* had an average shoot dry weight of 3.5 times that of their non-mycorrhizal control (NM), whereas the mycorrhizas of *L. esculentum* had an average shoot dry weight of only 1.4 times that of their NM control. *M. truncatula* colonized by *G. mosseae*, *G. intraradices* or *G. claroideum* had the highest shoot dry weights, whereas in *L. esculentum*, plants colonized by *G. mosseae* or *G. versiforme* had the highest shoot dry weights (Fig. 2). *M. truncatula* colonized by *G. rosea* had no growth benefit, while *L. esculentum* colonized by *G. rosea* or *G. caledonium* had a growth depression (Fig. 2). *G. caledonium* and *G. claroideum* elevated the shoot dry weight of *M. truncatula*, but not that of *L. esculentum*. Responses similar to those described above were also observed for root and shoot fresh weight in both plant species (data not shown).

**Shoot P content and concentration**

The shoot P content of *M. truncatula* and *L. esculentum* was elevated by AMF colonization, although the mycorrhizal plants contained considerably less P than that of their respective NM+P controls (Fig. 3). Colonization by AMF elevated the shoot P content of *M. truncatula* considerably more than that of *L. esculentum*—the mycorrhizas of *M. truncatula* had an average shoot P content 6.7 times that of their NM control, whereas the mycorrhizas of *L. esculentum* had an average shoot P content 4.5 times that of their NM control. The shoot P content of *M. truncatula* was elevated in most cases by AMF colonization, although the mycorrhizal plants contained considerably less P than that of their respective NM+P controls (Fig. 3).

### Table 1. The internal root colonization of *M. truncatula* and *L. esculentum* by seven species of AMF

<table>
<thead>
<tr>
<th>AMF</th>
<th><em>M. truncatula</em></th>
<th><em>L. esculentum</em></th>
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<tr>
<td></td>
<td>A</td>
<td>V</td>
</tr>
<tr>
<td>Gv</td>
<td>41±7</td>
<td>0±0</td>
</tr>
<tr>
<td>Gi</td>
<td>68±5</td>
<td>65±11</td>
</tr>
<tr>
<td>Gca</td>
<td>62±7</td>
<td>0±0</td>
</tr>
<tr>
<td>Gcl</td>
<td>60±6</td>
<td>62±1</td>
</tr>
<tr>
<td>Gm</td>
<td>47±9</td>
<td>0±0</td>
</tr>
<tr>
<td>Sc</td>
<td>51±6</td>
<td>0±0</td>
</tr>
<tr>
<td>Gr</td>
<td>43±10</td>
<td>0±0</td>
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Fig. 1. Total and colonized root length of *M. truncatula* (A) and *L. esculentum* (B) grown without mycorrhizas or in association with seven AMF. Colonized root length is presented both as a percentage (% RLC) and as absolute values (dark columns). Treatments: NM, non-mycorrhizal control; NM+P, P-fertilized, non-mycorrhizal control; Gv, *Glomus versiforme*; Gi, *Glomus intraradices*; Gca, *Glomus caledonium*; Gcl, *Glomus claroideum*; Gm, *Glomus mosseae*; Sc, *Scutellospora calospora*, and Gr, *Gigaspora rosea* mycorrhizas. Abbreviation: % RLC, percentage root length colonized. Values presented ±SE, n=3. The offset bar represents the LSD at P=0.05.
content only 1.6 times that of their NM control. Shoot P content was not elevated in either plant species when colonized by *G. rosea*. There were also differences between plant species in their response to certain AMF. *G. caledonium* and *G. claroideum* increased the shoot P content of *M. truncatula*, but not that of *L. esculentum* (Fig. 3). Shoot P content was highly positively correlated with shoot dry weight in both plant species (*M. truncatula*, $r^2=0.94$; *L. esculentum*, $r^2=0.98$) (Fig. 4).

The concentration of P within the shoots of *M. truncatula* and *L. esculentum* was elevated in response to colonization (Fig. 5), although shoot P concentration was higher overall in mycorrhizas of *M. truncatula* (1.8 ± 0.4 mg kg$^{-1}$) than in the mycorrhizas of *L. esculentum* (1.3 ± 0.1 mg kg$^{-1}$). In *M. truncatula*, plants colonized by *G. versiforme*, *G. caledonium* or *S. calospora* had the highest concentration of P in their shoots, whereas plants colonized by *G. rosea* had the lowest levels. By contrast, the shoot P concentration of mycorrhizal *L. esculentum* was relatively low, uniform and only marginally higher than that of its NM control (Fig. 5).

### Expression of P-starvation plant genes in arbuscular mycorrhizas

P starvation-inducible plant genes were used as molecular markers to study the ability of AMF to attenuate the plant’s P-starvation response. The expression of MtPT2 and Mt4 in the roots of *M. truncatula* was highest in the NM control and essentially absent in the NM+P control (Fig. 6A). A similar trend was observed for the expression of TPSI1 in the NM controls of *L. esculentum*, but not for LePT1, which, in contrast to the other three genes, had a high level of expression in the *L. esculentum* NM+P control (Fig. 6B). The expression patterns of MtPT2 and Mt4 in the roots of mycorrhizal *M. truncatula* varied considerably in response to AMF colonization, whereby expression was...
lowest in roots colonized by *G. mosseae* and the highest in roots colonized by *G. rosea* (Fig. 6A). Their expression was negatively, linearly correlated with the concentration of P within shoots of *M. truncatula*. This association was strongest for Mt4 ($r^2=0.79$) (Fig. 7) and lowest for MtPT2 ($r^2=0.39$). The expression of LePT1 and TPSI1 was quite uniform across the seven mycorrhizas and essentially uninfluenced by colonization (Fig. 6B).

**Discussion**

This study combines classical physiological analyses with molecular techniques to extend previous studies on functional diversity in the AM symbiosis to the level of gene expression. The 14 different fungus–plant combinations examined had responses ranging from functionally compatible interactions as measured by increased root and shoot growth, shoot P content and shoot P concentration, to essentially neutral and even incompatible responses, which exhibited physiological symptoms of P starvation. The goal was to test whether such diversity seen among mycorrhizal associations at the level of host plant physiology (van der Heijden *et al.*, 1998; Ravnskov and Jakobsen, 1995) could also be observed at the molecular level as indicated by alterations in the expression of P starvation-inducible plant genes within mycorrhizal roots. The expression of Mt4 and MtPT2 from *M. truncatula* and LePT1 and TPSI1 from *L. esculentum* in roots from plants grown without mycorrhizas or in association with seven AMF. Northern blots of total RNA isolated from mycorrhizal roots of *M. truncatula* probed with either $^{32}$P-labelled MtPT2 cDNA (upper panel), Mt4 cDNA (middle panel) or 18S rDNA (lower panel) (A) and RNA isolated from mycorrhizal roots of *L. esculentum* probed with either $^{32}$P-labelled LePT1 cDNA (upper panel), TPSI1 cDNA (middle panel) or 18S rDNA (lower panel) (B). Treatments: NM, non-mycorrhizal control; NM+P, P-fertilized, non-mycorrhizal control; Gv, Glomus versiforme; Gi, Glomus intraradices; Gca, Glomus caledonium; Gcl, Glomus claroideum; Gm, Glomus mosseae; Sc, Scutellospora calospora, and Gr, Gigaspora rosea mycorrhizas. Values presented $\pm$ SE, $n=3$. The offset bar represents the LSD at $P=0.05$.

**Mycorrhizal responsiveness in *M. truncatula* and *L. esculentum***

The two plant species used in this study displayed different, but well-described physiological alterations as a result of mycorrhizal colonization. *M. truncatula* behaved as a typical mycorrhiza-responsive plant species, having relatively high levels of root colonization, considerable growth benefits as measured by increased root length and elevated shoot dry weight and improved P nutrition as measured by elevations in shoot P content and shoot P concentration. *L. esculentum*, in contrast, was overall less responsive to mycorrhizal colonization, whereby it had lower levels of root colonization, limited growth responses and limited P uptake in the mycorrhizal plants when compared to *M. truncatula’s* mycorrhizal responsiveness. This limited response of *L. esculentum* to AMF colonization has been recently observed in some
other tomato cultivars (Bryla and Koide, 1998). Thus, the marked difference between the two host plants enabled the study of AMF functional diversity in both a mycorrhizal responsive and relatively unresponsive host plant.

**Diversity between AMF at the level of root colonization**

This study demonstrated that AMF differ in their ability to colonize their host plants in terms of both colonized root length and the fungal structures formed within the root. Two remarkable AMF were *G. intraradices* and *G. claroideum*, which colonized the greatest root length in both plant species and also were the only fungi to form vesicles within their hosts. This ability of some AMF to colonize their host plants in terms of both colonized root length and the fungal structures formed within the root. Two remarkable AMF were *G. intraradices* and *G. claroideum*, which colonized more of the root surface of its host and extracted Pi from the soil closer to the root than *G. caledonium*, which tended to colonize less and extract Pi from the soil further from the root. While in this study *G. intraradices* and *G. claroideum* were able to achieve high levels of root colonization in both plant species, it is important to note that these two fungi were not any better at providing their host plants with Pi, which supports previous findings that mycorrhizal function is not linked to the degree of colonization (Graham and Eissenstat, 1998).

The high frequency of arbuscules (41–68% of the root length) and intercellular hyphae in the mycorrhizal roots of *M. truncatula* classifies this host as a typical Arum-type AM (Smith and Smith, 1997). By contrast, the AMF structures within the roots of *L. esculentum* were much more diverse, which ranged from arbuscules only (*G. versiforme*) and mainly arbuscules (*G. intraradices, G. claroideum* and *G. mosseae*) to hyphal/arthrobacular coils (*G. caledonium, S. calospora* and *G. rosea*). Hence, the AMF colonizing *L. esculentum* exhibited both Arum- and Paris-type morphology. Such variation in AMF morphology, confirms recent work by Cavagnaro et al. (2001), who recently showed that both the genotype of the AMF and that of the host plant determines these morphological features. The switch from Arum-type in *M. truncatula* to Paris-type in *L. esculentum* was most pronounced for the Gigasporaceae isolates, but also one Glomus species, *G. caledonium*, produced Paris-type structures only. The ability to produce abundant vesicles appears to be a more conservative character, which, as discussed, was observed in roots of both plant species colonized by *G. intraradices* and *G. claroideum*. The causal relationships behind the diversity in morphology of root-internal fungal structures is not yet clear (Cavagnaro et al., 2001).

**Functional diversity at the level of growth and P accumulation**

In accordance with the aim of the experimental design, which had set soil Pi as the growth-limiting factor, a strong positive, linear correlation was observed between the shoot dry weight and the shoot P content of the mycorrhizal plants. *M. truncatula* and *L. esculentum* colonized by *G. mosseae* had the highest P contents and growth responses, while plants colonized by *G. rosea* had the lowest, which suggested that particular AMF had a tendency to alter the plant’s P nutrition and growth, regardless of plant species. Ravnskov and Jakobsen (1995) noted a similar trend in a study involving cucumber, wheat and flax plants colonized by two species of *Glomus*. The inherent ability of certain fungi to supply high amounts of Pi to their host plants might be explained by fungal traits involved in the uptake and transport of P from the soil to the plant, perhaps including the abundance and spatial distribution of external hyphae in the soil, the uptake efficiency of hyphal Pi transporters at the fungus–soil interface, the translocation efficiency of Pi in the hyphae, the abundance of arbuscules in the root or perhaps the efficiency of Pi transfer from the fungus to the host. Some of these fungal attributes associated with P supply may be eventually identifiable by molecular means and could ultimately be useful as markers for predicting AMF function. In this study, the abundance of intracellular fungal structures (arbuscules,
hyphal coils or arbuscule coils) within the root was not a good indicator of mycorrhizal function, since both *M. truncatula* and *L. esculentum* colonized by *G. mosseae* had relatively low to moderate arbuscule numbers, yet these mycorrhizal plants had the greatest growth benefits and the highest shoot P contents.

The differences between AMF in their ability to increase the growth of their host plants might also depend on factors not directly associated with the efficient transfer of Pi from the fungus to the host. For example, excessive carbohydrate demand by particular AMF, which can account for up to 20% of the plant’s photosynthate production (Jakobsen and Rosendahl, 1990), has been suggested to influence the ability of these fungi to promote plant growth (Graham and Eissenstat, 1998; Johnson *et al.*, 1997; Peng *et al.*, 1993). One sign of excessive photosynthate demand might be mycorrhizal plants that have reduced growth relative to other mycorrhizal plants, while maintaining elevated shoot P concentrations. Such elevations can occur if another nutrient is limiting plant growth more than that of Pi, which can then lead to a build-up of Pi within the plant’s tissues. In the present study, for example, *M. truncatula* colonized by *G. versiforme*, *G. caleionium* and *S. calospora* had reduced growth relative to plants colonized by *G. mosseae*, despite having elevated shoot P concentrations. While it is only speculation that carbon demand was responsible, it can at least be concluded that the macro- and micronutrients added to the substrate prior to the start of the experiment were not responsible for limiting growth in these mycorrhizas, since the NM+P control grew quite well in the same substrate.

Aside from fungal traits associated with efficient P transfer and possible demands for photosynthate by the fungus, it has been shown that plant species can also have a profound effect on whether a particular mycorrhiza is functional or not (Ravnksov and Jakobsen, 1995). *M. truncatula* colonized by *G. caleionium* or *G. claroideum* in the present study had a notable growth benefit and elevated shoot P content, whereas *L. esculentum* was not responsive to these fungal species. These results support evidence for a plant-influenced component to functional diversity (Ravnksov and Jakobsen, 1995; Graham and Eissenstat, 1994).

**Functional diversity at the molecular level**

The expression of four P starvation-inducible plant genes from two plant species was studied to determine whether the diversity of physiological response by plants to AMF colonization could likewise be observed at the molecular level. While previous studies have shown that a few species of AMF can down-regulate P starvation-inducible plant genes within mycorrhizal roots (Rosewarne *et al.*, 1999; Liu *H et al.*, 1998; Burleigh and Harrison, 1997), it remained to be determined whether differences can be observed between AMF in their influence on the expression of these plant genes and if their down-regulation is associated with the improved P nutrition of the plant as a result of symbiotic function. In theory, AM fungi might have developed a mechanism over approximately 460 million years of co-evolution with host plants (Redecker *et al.*, 2000) to down-regulate plant P transporters within roots by some uncharacterized means independent of P transfer.

Seven species of AMF were shown here to vary widely in their influence on the root expression of plant genes involved in the P-starvation response. At one extreme was *G. mosseae*, whereby its colonization of *M. truncatula* resulted in the greatest reduction in MtPT2 and Mt4 gene expression and the highest level of P uptake and growth, while at the other extreme was *G. rosea*, whereby colonization resulted in the highest levels of MtPT2 and Mt4 gene expression and the lowest P uptake and growth. This link between gene expression and shoot P concentration was strongest for Mt4, which has been shown to be highly regulated by P fertilization in uncolonized plants (Burleigh and Harrison, 1998). While the experimental design made it impossible to elucidate the mechanism by which these plant genes were down-regulated in the seven mycorrhizas, this study nonetheless linked their reduced expression with the improved P nutrition of the mycorrhizal plants. A similar association was found with the root P concentration of the *M. truncatula* mycorrhizas, however, since a portion of the Pi in mycorrhizal roots is derived from fungal tissues, this complex relationship was not studied further.

In the case of *L. esculentum*, little variation was observed in the expression of LePT1 and TPS1 across the roots of the seven mycorrhizas, this pattern of expression was associated with low and uniform shoot P concentrations in the plants, which has been noted previously for this cultivar of *L. esculentum* in another study involving AMF colonization (Lingling Gao, University of Adelaide, South Australia, personal communication). These results may reflect this cultivar’s relatively strong control over its P nutrition, since it was able to maximize the utilization of shoot Pi for growth purposes, even when colonized by seven diverse species of AMF. Whatever the case, these results demonstrate that large differences can exist between plant species in how they respond at the molecular level to AMF colonization.

As stated in two recent reviews on molecular aspects of this symbiosis (Harrison, 1999; Barker *et al.*, 1998), little is known about how AMF influence plant gene expression, especially in *Paris*-type mycorrhizas, which form intracellular arbuscule and hyphal coils. This study addressed this need by examining plant gene expression in both *Arum*- and for the first time, *Paris*-type mycorrhizas of *L. esculentum*. However, despite the rather large morphological differences found within the seven mycorrhizas of *L. esculentum*, little variation was observed in the expres-
sion patterns of the two P starvation-inducible plant genes, LePT1 and TPSII. Likewise, while the mycorrhizas of *M. truncatula* had considerable variation in the root expression of MtPT2 and Mt4, they nonetheless had relatively high and uniform numbers of arbuscules within their roots. Hence, it was concluded that alterations in the expression of these plant genes were not closely associated with the AMF’s morphological features. The lack of any trends associated with the presence or absence of these features and plant growth, P nutrition and the root expression of P starvation-inducible genes further strengthens the idea that these different fungal structures may be similar in their function, at least with respect to the transfer of Pi from the fungus to the plant (Cavagnaro et al., 2001).

Since the probes used in this expression study were full-length clones, it was possible that the signal observed on the Northern blots represented a number of gene family members, at least in the case of the P-transporters (Liu C et al., 1998, Liu H et al., 1988). The presence of family members might explain why LePT1 from *L. esculentum* was not down-regulated in the NM+P control, despite the findings of Liu C et al. (1998), that this gene was downregulated within uncolonized tomato roots by Pi fertilization. Loading error cannot explain this result, since the same blot was used in the analysis of TPSII gene expression, which showed strong down-regulation in the NM+P control, and a similar result was observed in another independently replicated blot. A similar pattern of expression using the full-length clone of LePT1 was likewise noted by Lingling Gao of the University of Adelaide, South Australia in an expression study involving this cultivar’s defence responses during AMF colonization (personal communication).

Conclusion

This study extends current understanding of functional diversity and shows that plants can respond differently to AMF, not only at the level of colonization, nutrient uptake and growth, but also at the level of gene expression. In some cases the plant’s physiological and molecular responses to particular AMF could be explained by the ability of the fungus to supply its host with Pi. However, the ability of some AMF to elevate the shoot P concentration, but not improve the growth of their host plants, may alternatively be reflective of what can be referred to as ‘the other half’ of nutrient transport in mycorrhizas, namely carbon flow from the plant to the fungus (Smith and Read, 1997). Furthermore, this work demonstrated that although there can be considerable morphological differences between the AM formed by *L. esculentum*, there was no obvious effect on plant growth, P nutrition or the root expression of two P starvation-inducible plant genes. Finally, it was shown that plant genotype plays a central role in how AMF influence their host plants, both at the physiological and molecular level: a mycorrhizal-dependent plant species was more strongly influenced by AMF than a less mycorrhizal-dependent species in terms of alterations in shoot P concentration and in root expression of P starvation-inducible plant genes.

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References


