Stephanie J. Watts-Williams, Timothy R. Cavagnaro

Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review

Mycorrhiza, 2015; 25(8):587-597

Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review.

Stephanie J Watts-Williams[1*] and Timothy R Cavagnaro[2]

Affiliations:

[1] School of Biological Sciences, Monash University, Clayton, VIC, Australia, 3800.

[2]School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB1 Glen Osmond, SA, Australia, 5064.

*Current address: Boyce Thompson Institute for Plant Research, Tower Rd, Ithaca NY 14853, USA. sjw266@cornell.edu

Abstract

A significant challenge facing the study of arbuscular mycorrhiza is the establishment of suitable non-mycorrhizal treatments that can be compared with mycorrhizal treatments. A number of options are available, including soil sterilisation (physical and chemical), comparison of constitutively mycorrhizal and non-mycorrhizal plant species, comparison of plants grown in soils with different inoculum potential, and the comparison of mycorrhiza-defective mutant genotypes with their mycorrhizal wild-type progenitors. Each option has its inherent advantages and limitations. Here, the potential to use mycorrhiza-defective mutant and wild-type genotype plant pairs as tools to study the functioning of mycorrhiza is reviewed. The emphasis of this review is placed on non-legume plant species, as mycorrhiza-defective plant genotypes in legumes have recently been extensively reviewed. It is concluded that non-legume mycorrhiza-defective mutant and wild-type pairs are useful tools in the study of mycorrhiza. However, the mutant genotypes should be well characterised and, ideally, meet a number of key criteria. The generation of more mycorrhiza-defective mutant genotypes in agronomically important plant species would be of benefit, as would be more research using these genotype pairs, especially under field conditions.

Keywords: Arbuscular mycorrhiza, mycorrhiza-defective mutant genotype, reduced mycorrhizal colonisation (*rmc*), *Solanum lycopersicum* (tomato), Micro-Tom.

Introduction

Arbuscular mycorrhiza are associations formed between the majority (80%) of terrestrial plant species, and arbuscular mycorrhizal (AM) fungi in the soil (Smith and Read 2008). The formation of mycorrhiza can benefit plants through enhanced acquisition of nutrients such as phosphorus (P), nitrogen, (N) and zinc (Zn) (Cavagnaro 2008; Clark and Zeto 2000; Gyaneshwar et al. 2002; Marschner and Dell 1994; Rillig 2004a; Smith and Read 2008). In addition to their beneficial effects on plant nutrition, mycorrhiza provide other ecosystem services: for example, improvement of soil structure (Miller and Jastrow 1990; Rillig 2004b; Rillig and Mummey 2006; Tisdall 1991; Tisdall and Oades 1980), reduction of soil nutrient losses through leaching (Asghari and Cavagnaro 2011; Asghari and Cavagnaro 2012; Asghari et al. 2005; Bender et al. 2015; Bender and van der Heijden 2015; van der Heijden 2010) and the suppression of weeds (Rinaudo et al. 2010; Veiga et al. 2011), improvement of plant acquisition of nutrients from compost (Cavagnaro 2014; Cavagnaro 2015), as well as other benefits in the context of a changing climate and increased abiotic stress (Smith et al. 2010). Consequently, mycorrhiza have an important role in influencing plant communities, ecosystem productivity, and potentially agricultural productivity (Hartnett and Wilson 1999; O'Connor et al. 2002; van der Heijden et al. 1998a; van der Heijden et al. 1998b; Wagg et al. 2011).

In mycorrhizal legume species, where plants can be colonised by mycorrhizal fungi and nodulating bacteria simultaneously, common signalling pathways for the formation of mycorrhizal and rhizobial associations have been well studied (Hirsch and Kapulnik 1998; Horváth et al. 2011; Parniske 2008). This work has resulted in the identification of numerous genotypes defective for AM colonisation (referred to as a ‘mycorrhiza-defective mutants’ hereafter) in model legume species. While the present

review focuses on non-legume mycorrhiza-defective mutant genotypes, it is important to mention that much of the research on the genetic basis of the AM symbiosis has been conducted using legume mutants (Ané et al. 2004; Endre et al. 2002; Imaizumi-Anraku et al. 2005; Lévy et al. 2004; Stracke et al. 2002), and thus they have been invaluable to the study of the AM symbiosis. For example, a symbiotic 'toolkit' has been collated using model legume species, containing 25 molecular components that work in concert to control AM colonisation (Delaux et al. 2013; Table 1). This symbiotic 'toolkit' provides useful information for developing mutant genotypes in non-legume plant species by looking for orthologs of genes in non-legumes that have a known function in AM symbiosis.

The advantage of using non-legume mycorrhiza-defective mutant genotypes is that they do not form associations with nodulating bacteria, thereby avoiding complications of multi-trophic interactions (Barker et al. 1998; Cavagnaro et al. 2004a). As well as being important tools for investigating the molecular basis of AM colonisation (Barker and Larkan 2002), the mutant and wild-type pairs are also useful for studying the functioning of mycorrhiza because it is possible to compare mycorrhizal and non-mycorrhizal plants in native soil without any other experimental manipulation or intervention.

The intention of this review is to explore the potential and advantages of using pairs of mycorrhiza-defective mutants (as non-mycorrhizal controls) and corresponding wild-type genotypes to study the role of mycorrhiza in various aspects of plant and soil ecology, with the aim to stimulate more work using such genotype pairs. In this context, various alternative methods for establishing non-mycorrhizal controls are summarised, before describing different non-legume plant species that have mycorrhiza-defective mutant genotypes characterised and the nature of the

research they are used for. Emphasis is placed on non-legume mycorrhiza-defective mutants as legume mycorrhiza-defective mutants have been reviewed in detail previously (see Barker et al. 2002; Marsh and Schultze 2001; Paszkowski 2006). The review concludes with a brief discussion of research activities that could benefit from the use of mycorrhiza-defective mutant and wild-type pairs of non-legumes.

Non-mycorrhizal treatments in physiological and ecological studies

Most information on the functioning of mycorrhiza has come from studies in which plants colonised by AM fungi are compared to those that are not colonised by AM fungi (Rillig et al. 2008; Smith and Smith 1981b). However, there is no universally accepted method for establishing treatments in which AM fungi are absent but the remainder of the soil biota are present. This is especially challenging under field conditions, where the elimination of a single group of soil biota is extremely difficult (Carey et al. 1992; West et al. 1993). The various techniques used in an attempt to overcome this challenge do have limitations, but in many cases they are the only option available, and are therefore most appropriate.

The most widely used method for establishing non-mycorrhizal control treatments is that of modifying the soil via soil fumigation, disinfection or sterilisation to inactivate the AM fungal propagules (Endlweber and Scheu 2006; Koide and Li 1989; Smith and Smith 1981a; Smith and Smith 1981b). While these methods effectively suppress viable AM fungi, they also adversely affect or eliminate other members of the soil biota. Consequently, such soil manipulation approaches introduce non-target effects into experiments, which may be potentially confounding (Koide and Li 1989; Rillig 2004a) and should be taken into account when interpreting results.

Using a mutant approach to control AM development, by comparing a mycorrhiza-defective mutant plant genotype to its mycorrhizal wild-type counterpart, avoids the need to sterilise or disinfect soil, or compare different plant species (Rillig et al. 2008). A number of mycorrhiza-defective mutant and wild-type genotype pairs have been described, both in legume and non-legume plant species (see Table 1). The present review focuses on non-legume mycorrhiza-defective mutants since the use of legume mutants to compare interactions in mycorrhizal and rhizobial symbioses has been amply reviewed previously (see Barker et al. 2002; Marsh and Schultze 2001; Paszkowski 2006; Shtark et al. 2010; Stacey et al. 2006).

Generation and screening of mycorrhiza-defective mutants

Mycorrhiza-defective mutants can be generated in a number of ways, including *via* fast neutron bombardment (Li et al. 2001) and ethyl methanesulfonate (EMS) generally used to generate mutant plant genotypes (Engvild 1987; Froese-Gertzen et al. 1963; Koornneeff et al. 1982). Whatever the method used, M2 generation mutants are screened in the mutagenised populations for non-mycorrhizal phenotypes by growing the entire population of plants in soil containing AM fungal inoculum, together with the wild-type genotype, in order to compare their AM colonisation phenotype. Potential mycorrhiza-defective mutant genotypes are assessed at the M3 generation and later (up to M9 in David-Schwartz et al. (2001) to ensure that a stable non-mycorrhizal phenotype persists. Paszkowski et al. (2006) screened for mycorrhiza-defective mutant genotypes in maize (*Zea mays*) in a novel manner. Maize roots that are colonised by AM fungi accumulate yellow pigment, which can be detected macroscopically (Klingner et al. 1995). Potential mycorrhiza-defective mutant genotypes from a Mutator-mutagenised population of maize were grown in

soil inoculated with *G. mosseae*. Plants with roots that displayed altered intensity or distribution of yellow pigmentation relative to the wild-type genotype, underwent further microscopic visual screening, ultimately revealing several non-mycorrhizal mutant maize plants.

The fast neutron bombardment method is a classical reverse genetics technique (Li et al. 2001). In consequence, the gene sequence(s) controlling mycorrhizal colonisation is not known until further research is undertaken. Both map-based sequencing and transcriptomic analyses have been used to identify gene sequences that had been disrupted using this approach in mycorrhiza-defective plant mutants (see below). Creation of fast neutron mutagenised seed libraries, and their subsequent screening for desired phenotypes, is a labour-intensive, albeit effective, method for generating and identifying mycorrhiza-defective mutant genotypes.

There are a number of desirable phenotypes that need to be considered when identifying potential mycorrhiza-defective mutant genotypes, and suitable criteria have been summarised by Rillig et al. (2008) as follows: (1) a non-mycorrhizal genotype should not, while the mycorrhizal genotype should, be colonised by AM fungi in the presence of a full suite of soil biota, and (2) the mutant and wild-type genotype pair should have matched growth properties, and similar soil microbial communities, when grown in a soil where AM fungi are absent. With these criteria in mind, currently reported legume mutant genotypes are first briefly listed (see Marsh and Schultze (2001) for details), and non-legume mutant genotypes are reviewed in more detail, including their method of mutagenesis, colonisation phenotype (where relevant), and use in research.

Currently described mycorrhiza-defective mutant genotypes

A number of mycorrhiza-defective mutant and wild-type genotype pairs, in both legume and non-legume species are available, although many more have been characterised in legumes. In legumes, mycorrhiza-defective mutants obtained using different mutagenic approaches have been identified in several plant species including pea (*Pisum sativum*) and fababean (*Vicia faba*) (Duc et al. 1989), lucerne (*M. sativa*) (Bradbury et al. 1991), barrel medic (*Medicago truncatula*) (Sagan et al. 1995), bean (*Phaseolus vulgaris*) (Shirtliffe and Vessey 1996), and *Lotus japonicus* (Senoo et al. 2000), but these are not the focus of this review. In terms of non-legume species, there are currently reports of mycorrhiza-defective mutant and wild-type genotype pairs in tomato (*Solanum lycopersicum*) (Barker et al. 1998; David-Schwartz et al. 2001; David-Schwartz et al. 2003, Kapulnik and Bonfante, unpublished), maize (Paszkowski et al. 2006), rice (*Oryza sativa*) and petunia (*Petunia hybrid*) (Chen et al. 2007; Chen et al. 2008; Gutjahr et al. 2008; Reddy et al. 2007) (see Table 1).

Loss-of-function mycorrhiza-defective mutant genotypes

In addition to the identification and characterisation of mutant genotypes that cannot be colonised by AM fungi, mutants that are defective in an aspect of mycorrhizal functioning have also been characterised. A mutant in *Medicago truncatula* that is defective in the gene encoding for the mycorrhiza-induced phosphate transporter, *MtPT4*, and affected in colonisation by AM fungi (Javot et al. 2007), has been used in a number of studies (Grønlund et al. 2013; Javot et al. 2011, Watts-Williams et al., unpublished). In rice (*Oryza sativa*) and Chinese milk vetch (*Astragalus sinicus*), similar mutants have been characterised for the genes *OsPT11* and *AsPT4*, respectively, orthologues of *MtPT4* (Xie et al. 2013; Yang et al. 2012). Isotope tracer studies, used in conjunction with the *MtPT4* and *OsPT11* mutants, confirmed that the

mycorrhizal pathway of P uptake had been successfully shut down (Yang et al. 2012, Watts-Williams et al., unpublished). Future work using these mutants, and work on developing other loss-of-function mutants in mycorrhiza-induced nutrient transporter genes (including nitrate and ammonium transporters) will contribute considerably to the understanding of plant-AM fungus nutrient relations.

Mycorrhiza-defective tomato mutants

76R and rmc

The mycorrhiza-defective tomato mutant *rmc* (***r****educed* ***m****ycorrhizal* ***c****olonisation*) was first identified and described by Barker et al. (1998), and it has since been used widely by researchers, alongside its wild-type progenitor 76R, in a number of field and glasshouse studies covering many aspects of soil and plant ecology. Field studies have been undertaken on sites in Australia and the United States, and glasshouse studies have used a range of AM fungal species and soils containing native AM fungal communities (from Europe, Australia and the United States).

The degree to which the 76R and *rmc* genotypes are colonised, and the colonisation phenotypes they express, is highly influenced by fungal identity (Gao et al. 2001). Consequently, a number of different colonisation phenotypes have been described (see Gao et al. 2001 for photos of colonisaiton phenotypes). Before discussing these phenotypes, it is important to note that there has recently been a major revision of the nomenclature of AM fungi (Krüger et al. 2012; Redecker et al. 2013). In this review, for the sake of clarity, the names of the AM fungi are used as in the original publications; however, the revised species names are also provided, for ease of comparison with future work.

Several species of AM fungi that colonise the wild-type 76R genotype normally are unable to colonise the *rmc* genotype, giving the Pen^- phenotype: *Rhizophagus irregularis* (formerly known as *Glomus intraradices* Schenck and Smith [DAOM 181602]), *G. fasciculatum* [Thaxter] Gerd. & Trappe emend. Walker & Koske [LPA7], and *G. etunicatum* Becker and Gerdemann [UT316 A-2]) (Gao et al. 2001; Manjarrez et al. 2008). The *rmc* genotype displays the Coi^- phenotype with other species of AM fungi which can penetrate the root epidermal cells but cannot colonise cortical cells (*Scutellospora calospora* [Nicolson & Gerdemann] Walker & Sanders [WUM 12(2)], *Gigaspora margarita* Becker and Hall, *G. coronatum* Giovannetti [WUM16], formerly known as G. 'City Beach', and *G. mosseae* [Nicholson & Gerdemann] Gerdemann and Trappe [NBR4-1]) (Gao et al. 2001; Manjarrez et al. 2008). For *S. calospora*, the AM fungal symbiosis can be functional (in terms of C transfer from plant to fungi) but colonisation is of an intermediate morphology, and is restricted to root epidermal cells (Gao et al. 2001; Manjarrez et al. 2010; Manjarrez et al. 2008; Poulsen et al. 2005). Interestingly, for *G. intraradices* Schenck and Smith WFVAM23 (referred to as *G. versiforme* [Karsten] Berch in (Gao et al. 2001), see (Gao et al. 2006)), full, functional mycorrhizal development (Myc^+) has been shown to occur in *rmc* roots, although the rate of colonisation is much slower than in 76R roots (Gao et al. 2001; Manjarrez et al. 2008; Poulsen et al. 2005).

Recently, a meta-analysis was conducted on 22 published studies that have compared the 76R and *rmc* genotype pair in terms of growth and tissue nutrient concentrations (Watts-Williams and Cavagnaro 2014). Tissue P concentrations were generally higher (often significantly so) in the 76R genotype than the *rmc* genotype, in both root and shoot tissue, in soils with low and high P concentrations. A similar

242 trend was recorded for tissue copper and sulphur concentrations, with concentrations
243 in the 76R plants higher than that in *rmc* plants. Furthermore, the meta-analysis
244 confirmed that the colonisation phenotype displayed by the AM fungi had a
245 significant influence on the extent to which roots were colonised. The results of the
246 meta-analysis also highlighted that there was no substantial mycorrhizal growth
247 response in either of the two tomato genotypes. It is important to note that, with
248 respect to the criteria for assessing suitable mycorrhiza-defective mutant and wild-
249 type pairs by Rillig et al. (2008), the 76R/*rmc* pair are matched in terms of growth in
250 the absence of AM fungi in all studies (Cavagnaro et al. 2004a; Facelli et al. 2010;
251 Poulsen et al. 2005) except one (Marschner and Timonen 2005).

252 The precise genome location of the *Rmc* locus has been identified and found to
253 include a close match to the *CYCLOPS/IPD3* gene (Larkan et al. 2013). This gene is
254 essential for intracellular regulation of both rhizobial and mycorrhizal symbioses in
255 legumes (Larkan et al. 2013). So far, nearly all cloned legume genes required for
256 nodulation and AM colonisation have their putative orthologs in non-legume plants
257 (Zhu et al. 2006). This is because the two symbioses share some signalling pathways
258 (Zhu et al. 2006), suggesting that the more recent symbiosis between nodulating
259 bacteria and plants may have evolved from the ancient symbiosis between AM fungi
260 and plants (Doyle 1998; Parniske 2008; Wang et al. 2010). Further identification of
261 the gene sequences associated with the *Rmc* locus will be useful information for past
262 and future work using the *rmc* mutant (Larkan et al. 2013).

263 The 76R and *rmc* genotypes continue to be valuable for numerous studies
264 focusing on different aspects of plant nutrition (Cavagnaro et al. 2010; Cavagnaro et
265 al. 2007b; Poulsen et al. 2005; Watts-Williams and Cavagnaro 2012; Watts-Williams
266 et al. 2013; Watts-Williams et al. 2015; Watts-Williams et al. 2014), plant

competition (Cavagnaro et al. 2004a; Facelli et al. 2010; Neumann and George 2005), mycorrhizal formation and colonisation phenotypes (Cavagnaro et al. 2004b; Gao et al. 2001; Manjarrez et al. 2010; Manjarrez et al. 2008; Manjarrez et al. 2009), soil ecology (Cavagnaro et al. 2012; Cavagnaro et al. 2007a; Cavagnaro et al. 2006; Hallett et al. 2009; Marschner and Timonen 2005), soil greenhouse gas emissions (Cavagnaro et al. 2012; Cavagnaro et al. 2008; Lazcano et al. 2014), and plant genetics (Barker et al. 2005; Gao et al. 2006; Larkan et al. 2007; Ruzicka et al. 2013; Ruzicka et al. 2012).

Micro-Tom mutants

Micro-Tom, which is a model tomato genotype that has been used extensively in genetic studies because of its small size and rapid life cycle (Carvalho et al. 2011; Meissner et al. 1997), has also been used to create three mycorrhiza-defective mutant genotypes (David-Schwartz et al. 2001; David-Schwartz et al. 2003). The mutants M20 and M161 were obtained by fast-neutron bombardment mutagenesis, whilst the BC1 mutant is an F2 segregant of the cross between wild-type and M161 genotypes. All mycorrhiza-defective mutant genotypes of the Micro-Tom variety are pre-mycorrhizal infection (*pmi*) mutants. Specifically, the M161 mutant displayed the Myc^- phenotype, and was unable to form mycorrhiza when grown in soil inoculated with *G. intraradices* spores (David-Schwartz et al. 2001). However, a low level of AM colonisation (vesicular and arbuscular) occurred when 'whole' inoculum (spores, root segments, external hyphae) was applied to the soil, or when M161 was grown in a field soil (Rillig et al. 2008). When the M161 mutant was grown in the presence of its AM-colonised wild-type progenitor, arbuscules, vesicles and internal hyphae developed in roots at a rate similar to that of the wild-type. Similarly, the M20 mutant displayed the Myc^- colonisation phenotype, and was able to resist AM fungal

colonisation in the presence of spores or (dead) pieces of mycorrhizal root, but was not resistant to colonisation in the presence of a live mycorrhizal wild-type progenitor plant (David-Schwartz et al. 2003). A third Micro-Tom mutant (BC1) has been identified, which is highly resistant to AM fungal colonisation when grown in field soil (1.2% root length colonised) (Rillig et al. 2008). However, this genotype has not yet been tested for resistance to AM fungal colonisation when grown in the presence of the mycorrhizal wild-type plant.

That mycorrhiza-defective Micro-Tom mutants can be colonised when grown in the presence of the wild-type plant needs to be taken into consideration when using these mutant genotypes. In contrast to Micro-Tom mutant genotypes, the *rmc* mutant genotype cannot be colonised in the presence of its wild-type progenitor or other nurse plants (Cavagnaro et al. 2004a). Rillig et al (2008) tested the Micro-Tom mutant genotypes for the selection criteria (see above) for mycorrhiza-defective mutant plants. They found that only the BC1 mutant met all the prescribed criteria while the other two mutants did not, for the following reasons: the M161 plants had a larger root biomass than wild-type when both were grown in the absence of AM fungi, and M20 gave rise to more soil microbial biomass than the wild-type. The Micro-Tom mutant M161 has so far been used in two published studies comparing mycorrhizal and non-mycorrhizal plants for root exudates involved in signal exchange between host plants and AM fungi (Gadkar et al. 2003; Sun et al. 2012). These mycorrhiza-defective mutant and wild-type Micro-Tom pairs could be of considerable utility in the study of mycorrhizal functioning.

Other non-legume mutants

316 In maize, Mutator-mutagenised F2 families of the normal line W64A were screened
317 by Paszkowski et al. (2006) for alteration in yellow root pigmentation compared to
318 wild-type roots (see above for detail on screening). From this screen, the authors
319 described seven mycorrhiza-defective mutants in maize and categorised them into
320 three colonisation phenotype classes: *nope1* (***no pe****rception **1***) mutants, which showed
321 a marked reduction in intraradical colonisation by *G. mosseae*, but displayed
322 occasional root sectors containing normal mycorrhizal structures (appressoria and
323 arbuscules), *taci1* (***taci****turn **1***) mutants, which had lower colonisation levels than the
324 wild-type genotype (45% compared to 86% root length colonised) and slightly
325 modified fungal structures, and *Pram1* (***Pr****ecocious **a**rbuscular **m**ycorrhiza **1***)
326 mutants, which are in fact colonised more rapidly and intensely than the wild-type,
327 becoming saturated with intraradical fungal structures (arbuscules and vesicles) much
328 earlier. These maize mutants represent the first mycorrhiza-defective mutant plants to
329 be characterised in an agronomically important cereal crop. Future research using
330 these mutants will be very useful for research into nutrient uptake in cereal crops,
331 especially in field trials. However, to our knowledge, no such studies have yet been
332 reported in the literature.

333 Reddy et al. (2007) used a transposon-mutagenised population of petunia
334 (W138, Gerats et al. 1990) in order to identify and characterise a mycorrhiza-
335 defective genotype in this line. The resulting mycorrhiza-defective petunia genotype,
336 *pam1* (***p****enetration and **a**rbuscule **m**orphogenesis**1***), displayed a strong decrease in
337 AM fungal colonisation compared to its wild-type progenitor. *G. intraradices* formed
338 complex appressoria on *pam1* roots but could not easily penetrate the epidermal cells.
339 Where the fungus did penetrate epidermal cells, the resulting hyphae were distorted
340 compared to those in the wild-type roots, and did not progress further except in the

rare instance where hyphae produced lateral branches between cells and small lateral appendages. In addition, two stabilised *pam1* mutant lines (*pam1S1* and *pam1S2*) were isolated and characterised. The two stabilised mutant lines displayed reduced extra- and intra-radical AM fungal colonisation compared to *pam1*, and thus the *pam1S1* line was used for subsequent experiments (Reddy et al. (2007). As with the Micro-Tom mutants, the *pam1S1* mutant could be colonised by AM fungi when grown in the presence of a nurse plant. However, intraradical colonisation lacked arbuscule formation, and there was no contribution to the plant's shoot P or Cu nutrition as a result of root colonisation.

In rice, a large number of mycorrhiza-defective mutant genotypes were characterised by Gutjahr et al. (2008), who were interested in identifying rice lines that were defective in one of a number of signalling steps in the common SYM pathway both upstream and downstream of Ca^{2+} spiking (see Parniske (2008) for recent review). The authors searched for relevant insertion lines in both T-DNA and Tos17 databases and found one insertion in CASTOR, three in POLLUX, two in CCMAK, and three in CYCLOPS (IPD3, see Table 1). The nine *sym* mutants were then grown in soil inoculated with spores of *G. intraradices* and assessed for colonisation phenotype. Root colonisation in all the mutants was restricted to hyphal colonisation in the epidermal cells, with no cortical colonisation and thus no arbuscules or vesicles forming in any of the mutants.

A gene required for mycorrhizal colonisation in rice, *OsDMI3* (***d****oes not* ***m****ake* ***i****nfections* **3**), has been identified. Chen et al. (2007) searched a rice Tos17 mutant database for *OsDMI3* insertion lines, identifying two, but ultimately using just one (NF8513) for subsequent experiments. When grown in soil inoculated with *G. intraradices*, the *OsDMI3* mutant roots showed occasional penetration of the cortical

366 cells, without any arbuscule formation. However, most observed fungal growth
367 (appressoria and external hyphae) was restricted to the root surface.

368 Similarly, Chen et al. (2008) characterised three knockout mutants defective in
369 AM fungal colonisation (NC0263, NC2713, NC2794), by searching for putative
370 *Tos17* insertion lines available for *OsIPD3*, another gene required for mycorrhiza
371 formation in rice. When inoculated with *G. intraradices*, the root epidermal cells of
372 the three *OsIPD3* mutants could not be penetrated, and there was no intraradical
373 colonisation of the roots by the AM fungi (i.e., no hyphae, arbuscules or vesicles)
374 except in one root segment of a NC2713 mutant, that displayed aborted intracellular
375 fungal hyphae. There is no explanation given for this observation in NC2713, but it is
376 assumed that the observed aborted hyphae did not confer functionality of the
377 symbiosis. Chen et al. (2008) noted that the colonisation phenotype displayed by the
378 *OsIPD3* mutants was comparable to that of the previously identified *OsDMI3* mutant
379 genotype (Chen et al. 2007).
380
381 Future directions
382 Mycorrhiza-defective plant mutant genotypes have the potential to be used in a broad
383 range of studies. Future uses of both legume and non-legume mutant genotypes may
384 be extended to areas of study where mycorrhiza have previously been shown to
385 improve plant or soil health but hypotheses have not yet been tested using a
386 mycorrhiza-defective mutant, such as soil nutrient cycling (Jeffries and Barea 1994;
387 Read and Perez-Moreno 2003) and interactions with foliar-feeding insects (Gange and
388 West 1994; Gehring and Whitham 1994; Wamberg et al. 2003) and foliar pathogens
389 (Campos-Soriano et al. 2012; Nair et al. 2015; West 1997). Research that directly
390 compares plant nutrient uptake via the direct (i.e., *via* root epidermal cells) and

mycorrhizal pathways could utilise appropriate mycorrhiza-defective mutant and wild-type genotype pairs (Poulsen et al. 2005), in conjunction with the use of stable or radioactive isotopes (Merrild et al. 2013; Watts-Williams et al. 2015). It would also be useful for future studies using mycorrhiza-defective mutant and wild-type pairs to continue to integrate molecular biology methods (e.g., quantification of gene expression) with more commonly reported physiological variables (e.g., plant nutrient concentration).

The intention of this review was to explore the potential to use mycorrhiza-defective mutant genotypes to study the formation and functioning of mycorrhizas in non-legumes. This approach has both strengths and limitations. Nevertheless, such mutant and wild-type genotype pairs are proving to be useful tools in the study of arbuscular mycorrhiza, and it is hoped that this review will stimulate and inform further research using this approach.

Acknowledgements

We wish to thank Prof. Sally Smith for comments on a very early draft of the manuscript. We also gratefully acknowledge Prof. Sally Smith and A/Prof. Susan Barker for continued access to the *rmc* and 76R genotypes of tomato. TRC also thanks Prof. Louise Jackson and members of her group for many valuable discussions over the years. TRC also wishes to acknowledge the Australian Research Council for financial support (FT120100463). SJWW wishes to acknowledge support received from the Monash University Postgraduate Publications Award.

References

Ané J-M, Kiss GB, Riely BK, Penmetsa RV, Oldroyd GED, Ayax C, Lévy J, Debellé F, Baek J-M, Kalo P, Rosenberg C, Roe BA, Long SR, Dénarié J, Cook DR (2004) Medicago truncatula DMI1 required for bacterial and fungal symbioses in legumes. Science 303:1364-1367 doi:10.1126/science.1092986

Asghari HR, Cavagnaro TR (2011) Arbuscular mycorrhizas enhance plant interception of leached nutrients. Funct Plant Biol 38:219-226 doi:10.1071/fp10180

Asghari HR, Cavagnaro TR (2012) Arbuscular mycorrhizas reduce nitrogen loss via leaching. Plos One 7:151-155 doi:e29825 10.1371/journal.pone.0029825

Asghari HR, Chittleborough DJ, Smith FA, Smith SE (2005) Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores. Plant Soil 275:181-193 doi:10.1007/s11104-005-1328-2

Barker S, Duplessis S, Tagu D (2002) The application of genetic approaches for investigations of mycorrhizal symbioses. In: Smith S, Smith FA (eds) Diversity and Integration in Mycorrhizas, vol 94. Developments in Plant and Soil Sciences. Springer Netherlands, pp 85-95. doi:10.1007/978-94-017-1284-2_9

Barker SJ, Edmonds-Tibbett TL, Forsyth LM, Klingler JP, Toussaint JP, Smith FA, Smith SE (2005) Root infection of the reduced mycorrhizal colonization (rmc) mutant of tomato reveals genetic interaction between symbiosis and parasitism. Physiol Mol Plant Pathol 67:277-283 doi:10.1016/j.pmpp.2006.03.003

Barker SJ, Larkan NJ (2002) Molecular approaches to understanding mycorrhizal symbioses. Plant Soil 244:107-116 doi:10.1023/a:1020211624849

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998) A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. Plant J 15:791-797 doi:10.1046/j.1365-313X.1998.00252.x

Bender SF, Conen F, Van der Heijden MGA (2015) Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. Soil Biol Biochem 80:283-292 doi:10.1016/j.soilbio.2014.10.016

Bender SF, van der Heijden MGA (2015) Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. J Appl Ecol 52:228-239 doi:10.1111/1365-2664.12351

Bradbury SM, Peterson RL, Bowley SR (1991) Interactions between 3 alfalfa nodulation genotypes and 2 Glomus species. New Phytol 119:115-120 doi:10.1111/j.1469-8137.1991.tb01014.x

Campos-Soriano L, GarcÍA-MartÍNez J, Segundo BS (2012) The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. Mol Plant Pathol 13:579-592 doi:10.1111/j.1364-3703.2011.00773.x

Carey P, Fitter A, Watkinson A (1992) A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. Oecologia 90:550-555 doi:10.1007/BF01875449

Carvalho RF, Campos ML, Pino LE, Crestana SL, Zsogon A, Lima JE, Benedito VA, Peres LEP (2011) Convergence of developmental mutants into a single tomato model system: 'Micro-Tom' as an effective toolkit for plant development research. Plant Methods 7 doi:10.1186/1746-4811-7-18

Cavagnaro TR (2008) The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. Plant Soil 304:315-325 doi:10.1007/s11104-008-9559-7

Cavagnaro TR (2014) Impacts of compost application on the formation and functioning of arbuscular mycorrhizas. Soil Biol Biochem 78 doi:10.1016/j.soilbio.2014.07.007

Cavagnaro TR (2015) Biologically regulated nutrient supply systems: compost and arbuscular mycorrhizas – a review. Advances in Agronomy 129:in press

Cavagnaro TR, Barrios-Masias FH, Jackson LE (2012) Arbuscular mycorrhizas and their role in plant growth, nitrogen interception and soil gas efflux in an organic production system. Plant Soil 353:181-194 doi:10.1007/s11104-011-1021-6

Cavagnaro TR, Dickson S, Smith FA (2010) Arbuscular mycorrhizas modify plant responses to soil zinc addition. Plant Soil 329:307-313 doi:10.1007/s11104-009-0158-z

Cavagnaro TR, Jackson LE, Scow KM, Hristova KR (2007a) Effects of arbuscular mycorrhizas on ammonia oxidizing bacteria in an organic farm soil. Microb Ecol 54:618-626 doi:10.1007/s00248-007-9212-7

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006) Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282:209-225 doi:10.1007/s11104-005-5847-7

Cavagnaro TR, Langley AJ, Jackson LE, Smukler SM, Koch GW (2008) Growth, nutrition, and soil respiration of a mycorrhiza-defective tomato mutant and its mycorrhizal wild-type progenitor. Funct Plant Biol 35:228-235 doi:10.1071/fp07281

Cavagnaro TR, Smith FA, Hay G, Carne-Cavagnaro VL, Smith SE (2004a) Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-defective tomato mutant to colonisation but inoculation does change competitive interactions with wild-type tomato. New Phytol 161:485-494 doi:10.1046/j.1469-8137.2004.00967.x

Cavagnaro TR, Smith FA, Smith SE (2004b) Interactions between arbuscular mycorrhizal fungi and a mycorrhiza-defective mutant tomato: does a noninfective fungus alter the ability of an infective fungus to colonise the roots - and vice versa? New Phytol 164:485-491 doi:10.1111/j.1469-8137.2004.01210.x

493 Cavagnaro TR, Sokolow SK, Jackson LE (2007b) Mycorrhizal effects on growth and
494 nutrition of tomato under elevated atmospheric carbon dioxide. Funct Plant Biol
495 34:730-736 doi:10.1071/fp06340

496 Chen C, Gao M, Liu J, Zhu H (2007) Fungal symbiosis in rice requires an ortholog of
497 a legume common symbiosis gene encoding a Ca2+/Calmodulin-dependent protein
498 kinase. Plant Physiol 145:1619-1628 doi:10.1104/pp.107.109876

499 Chen CY, Ané JM, Zhu HY (2008) OsIPD3, an ortholog of the *Medicago truncatula*
500 DMI3 interacting protein IPD3, is required for mycorrhizal symbiosis in rice. New
501 Phytol 180:311-315 doi:10.1111/j.1469-8137.2008.02612.x

502 Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J
503 Plant Nutr 23:867-902 doi:10.1080/01904160009382068

504 David-Schwartz R, Badani H, Smadar W, Levy AA, Galili G, Kapulnik Y (2001)
505 Identification of a novel genetically controlled step in mycorrhizal colonization: plant
506 resistance to infection by fungal spores but not extra-radical hyphae. Plant J 27:561-
507 569 doi:10.1046/j.1365-313X.2001.01113.x

508 David-Schwartz R, Gadkar V, Wininger S, Bendov R, Galili G, Levy AA, Kapulnik
509 Y (2003) Isolation of a premycorrhizal infection (pmi2) mutant of tomato, resistant to
510 arbuscular mycorrhizal fungal colonization. Mol Plant-Microbe Interact 16:382-388
511 doi:10.1094/mpmi.2003.16.5.382

512 Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M (2013) Evolution of the plant–
513 microbe symbiotic 'toolkit'. Trends Plant Sci 18:298-304
514 doi:10.1016/j.tplants.2013.01.008

515 Doyle JJ (1998) Phylogenetic perspectives on nodulation: evolving views of plants
516 and symbiotic bacteria. Trends Plant Sci 3:473-478 doi:10.1016/S1360-
517 1385(98)01340-5

518 Duc G, Trouvelot A, Gianinazzi-Pearson V, Gianinazzi S (1989) 1st report of non-
519 mycorrhizal plant mutants (myc-) obtained in pea (*Pisum-sativum*-L) and fababean
520 (*Vicia-faba* L). Plant Sci 60:215-222 doi:10.1016/0168-9452(89)90169-6

521 Endlweber K, Scheu S (2006) Establishing arbuscular mycorrhiza-free soil: A
522 comparison of six methods and their effects on nutrient mobilization. Applied Soil
523 Ecology 34:276-279 doi:10.1016/j.apsoil.2006.04.001

524 Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB (2002) A receptor kinase
525 gene regulating symbiotic nodule development. Nature 417:962-966
526 doi:doi.org/10.1038/nature00842

527 Engvild KC (1987) Nodulation and nitrogen fixation mutants of pea, *Pisum sativum*.
528 Theor Appl Genet 74:711-713 doi:10.1007/BF00247546

529 Facelli E, Smith SE, Facelli JM, Christophersen HM, Smith FA (2010) Underground
530 friends or enemies: model plants help to unravel direct and indirect effects of
531 arbuscular mycorrhizal fungi on plant competition. New Phytol 185:1050-1061
532 doi:10.1111/j.1469-8137.2009.03162.x

533 Froese-Gertzen EE, Konzak C, Foster R, Nilan R (1963) Correlation between some
534 chemical and biological reactions of ethyl methanesulphonate. Nature 198:447-448

535 Gadkar V, David-Schwartz R, Nagahashi G, Douds DD, Wininger S, Kapulnik Y
536 (2003) Root exudate of pmi tomato mutant M161 reduces AM fungal proliferation in
537 vitro. FEMS Microbiol Lett 223:193-198 doi:10.1016/s0378-1097(03)00357-4

538 Gange AC, West HM (1994) Interactions between arbuscular mycorrhizal fungi and
539 foliar-feeding insects in *Plantago lanceolata* L. New Phytol 128:79-87
540 doi:10.1111/j.1469-8137.1994.tb03989.x

541 Gao LL, Delp G, Smith SE (2001) Colonization patterns in a mycorrhiza-defective
542 mutant tomato vary with different arbuscular-mycorrhizal fungi. New Phytol 151:477-
543 491 doi:10.1046/j.0028-646x.2001.00193.x

544 Gao LL, Smith FA, Smith SE (2006) The rmc locus does not affect plant interactions
545 or defence-related gene expression when tomato (*Solanum lycopersicum*) is infected
546 with the root fungal parasite, *Rhizoctonia*. Funct Plant Biol 33:289-296
547 doi:10.1071/fp05153

548 Gehring CA, Whitham TG (1994) Interactions between aboveground herbivores and
549 the mycorrhizal mutualists of plants. Trends Ecol Evol 9:251-255 doi:10.1016/0169-
550 5347(94)90290-9

551 Gerats AG, Huits H, Vrijlandt E, Maraña C, Souer E, Beld M (1990) Molecular
552 characterization of a nonautonomous transposable element (dTph1) of petunia. The
553 Plant Cell 2:1121-1128 doi:10.1105/tpc.2.11.1121

554 Grønlund M, Albrechtsen M, Johansen IE, Hammer EC, Nielsen TH, Jakobsen I
555 (2013) The interplay between P uptake pathways in mycorrhizal peas: a combined
556 physiological and gene-silencing approach. Physiol Plant 149:234-248
557 doi:10.1111/ppl.12030

558 Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-
559 Anraku H, Paszkowski U (2008) Arbuscular mycorrhiza–specific signaling in rice
560 transcends the common symbiosis signaling pathway. The Plant Cell 20:2989-3005
561 doi:10.1105/tpc.108.062414

562 Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms
563 in improving P nutrition of plants. Plant Soil 245:83-93
564 doi:10.1023/a:1020663916259

565 Hallett PD, Feeney DS, Bengough AG, Rillig MC, Scrimgeour CM, Young IM
566 (2009) Disentangling the impact of AM fungi versus roots on soil structure and water
567 transport. Plant Soil 314:183-196 doi:10.1007/s11104-008-9717-y

568 Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure
569 and diversity in tallgrass prairie. Ecology 80:1187-1195 doi:10.1890/0012-
570 9658(1999)080[1187:mipcsa]2.0.co;2

571 Hirsch AM, Kapulnik Y (1998) Signal transduction pathways in mycorrhizal
572 associations: comparisons with the *Rhizobium*–legume symbiosis. Fungal Genet Biol
573 23:205-212

574 Horváth B, Yeun LH, Domonkos Á, Halász G, Gobbato E, Ayaydin F, Miró K,
575 Hirsch S, Sun J, Tadege M, Ratet P, Mysore KS, Ané J-M, Oldroyd GED, Kaló P
576 (2011) *Medicago truncatula* IPD3 is a member of the common symbiotic signaling
577 pathway required for rhizobial and mycorrhizal symbioses. Mol Plant-Microbe
578 Interact 24:1345-1358 doi:10.1094/MPMI-01-11-0015

579 Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi
580 H, Murakami Y, Mulder L, Vickers K, Pike J, Allan Downie J, Wang T, Sato S,
581 Asamizu E, Tabata S, Yoshikawa M, Murooka Y, Wu G-J, Kawaguchi M, Kawasaki
582 S, Parniske M, Hayashi M (2005) Plastid proteins crucial for symbiotic fungal and
583 bacterial entry into plant roots. Nature 433:527-531 doi:10.1038/nature03237

584 Javot H, Penmetsa RV, Breuillin F, Bhattarai KK, Noar RD, Gomez SK, Zhang Q,
585 Cook DR, Harrison MJ (2011) *Medicago truncatula* mtpt4 mutants reveal a role for
586 nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal
587 symbiosis. The Plant Journal 68:954-965 doi:10.1111/j.1365-313X.2011.04746.x

588 Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ (2007) A *Medicago*
589 *truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal
590 symbiosis. PNAS 104:1720-1725

591 Jeffries P, Barea JM (1994) Biogeochemical cycling and arbuscular mycorrhizas in
592 the sustainability of plant-soil systems. In: Gianinazzi S, Schüepp H (eds) Impact of
593 Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems.
594 Birkhäuser, Basel, pp 101-115. doi:10.1007/978-3-0348-8504-1_9

595 Klingner A, Bothe H, Wray V, Marner FJ (1995) Identification of a yellow pigment
596 formed in maize roots upon mycorrhizal colonization. Phytochemistry 38:53-55
597 doi:10.1016/0031-9422(94)00538-5

598 Koide RT, Li MG (1989) Appropriate controls for vesicular arbuscular mycorrhiza
599 research. New Phytol 111:35-44 doi:10.1111/j.1469-8137.1989.tb04215.x

600 Koornneeff M, Dellaert LWM, van der Veen JH (1982) EMS- and relation-induced
601 mutation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh. Mutat Res
602 93:109-123

603 Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic
604 reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi
605 from phylum to species level. New Phytol 193:970-984 doi:10.1111/j.1469-
606 8137.2011.03962.x

607 Larkan N, Ruzicka D, Edmonds-Tibbett T, Durkin JH, Jackson L, Smith FA,
608 Schachtman D, Smith S, Barker S (2013) The reduced mycorrhizal colonisation (rmc)
609 mutation of tomato disrupts five gene sequences including the CYCLOPS/IPD3
610 homologue. Mycorrhiza:1-12 doi:10.1007/s00572-013-0498-7

611 Larkan NJ, Smith SE, Barker SJ (2007) Position of the reduced mycorrhizal
612 colonisation (Rmc) locus on the tomato genome map. Mycorrhiza 17:311-318
613 doi:10.1007/s00572-007-0106-9

614 Lazcano C, Barrios-Masias FH, Jackson LE (2014) Arbuscular mycorrhizal effects on
615 plant water relations and soil greenhouse gas emissions under changing moisture
616 regimes. Soil Biol Biochem 74:184-192 doi:10.1016/j.soilbio.2014.03.010

617 Lévy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet E-P, Ané J-M,
618 Lauber E, Bisseling T, Dénarié J, Rosenberg C, Debellé F (2004) A putative Ca2+
619 and calmodulin-dependent protein kinase required for bacterial and fungal symbioses.
620 Science 303:1361-1364 doi:10.1126/science.1093038

621 Li X, Song YJ, Century K, Straight S, Ronald P, Dong XN, Lassner M, Zhang YL
622 (2001) A fast neutron deletion mutagenesis-based reverse genetics system for plants.
623 Plant J 27:235-242 doi:10.1046/j.1365-313x.2001.01084.x

624 Manjarrez M, Christophersen HM, Smith SE, Smith FA (2010) Cortical colonisation
625 is not an absolute requirement for phosphorus transfer to plants in arbuscular
626 mycorrhizas formed by *Scutellospora calospora* in a tomato mutant: evidence from
627 physiology and gene expression. Funct Plant Biol 37:1132-1142
628 doi:10.1071/FP09248

629 Manjarrez M, Smith FA, Marschner P, Smith SE (2008) Is cortical root colonization
630 required for carbon transfer to arbuscular mycorrhizal fungi? Evidence from
631 colonization phenotypes and spore production in the reduced mycorrhizal
632 colonization (rmc) mutant of tomato. Botany 86:1009-1019 doi:10.1139/b08-043

633 Manjarrez M, Wallwork M, Smith SE, Smith FA, Dickson S (2009) Different
634 arbuscular mycorrhizal fungi induce differences in cellular responses and fungal
635 activity in a mycorrhiza-defective mutant of tomato (rmc). Funct Plant Biol 36:86-96
636 doi:10.1071/fp08032

637 Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil
638 159:89-102

639 Marschner P, Timonen S (2005) Interactions between plant species and mycorrhizal
640 colonization on the bacterial community composition in the rhizosphere. Applied Soil
641 Ecology 28:23-36 doi:10.1016/j.apsoil.2004.06.007

642 Marsh JF, Schultze M (2001) Analysis of arbuscular mycorrhizas using symbiosis-
643 defective plant mutants. New Phytol 150:525-532 doi:10.1046/j.1469-
644 8137.2001.00140.x

645 Meissner R, Jacobson Y, Melamed S, Levyatuv S, Shalev G, Ashri A, Elkind Y, Levy
646 A (1997) A new model system for tomato genetics. Plant J 12:1465-1472
647 doi:10.1046/j.1365-313x.1997.12061465.x

648 Merrild MP, Ambus P, Rosendahl S, Jakobsen I (2013) Common arbuscular
649 mycorrhizal networks amplify competition for phosphorus between seedlings and
650 established plants. New Phytol 200:229-240 doi:10.1111/nph.12351

651 Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions
652 with soil aggregation. Soil Biol Biochem 22:579-584 doi:10.1016/0038-
653 0717(90)90001-g

654 Nair A, Kolet SP, Thulasiram HV, Bhargava S (2015) Systemic jasmonic acid
655 modulation in mycorrhizal tomato plants and its role in induced resistance against
656 *Alternaria alternata*. Plant Biol:n/a-n/a doi:10.1111/plb.12277

657 Neumann E, George E (2005) Does the presence of arbuscular mycorrhizal fungi
658 influence growth and nutrient uptake of a wild-type tomato cultivar and a mycorrhiza-
659 defective mutant, cultivated with roots sharing the same soil volume? New Phytol
660 166:601-609 doi:10.1111/j.1469-8137.2005.01351.x

661 O'Connor PJ, Smith SE, Smith FA (2002) Arbuscular mycorrhizas influence plant
662 diversity and community structure in a semiarid herbland. New Phytol 154:209-218
663 doi:10.1046/j.1469-8137.2002.00364.x

664 Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses.
665 Nature Reviews Microbiology 6:763-775 doi:10.1038/nrmicro1987

666 Paszkowski U (2006) A journey through signaling in arbuscular mycorrhizal
667 symbioses 2006. New Phytol 172:35-46 doi:10.1111/j.1469-8137.2006.01840.x

668 Paszkowski U, Jakovleva L, Boller T (2006) Maize mutants affected at distinct stages
669 of the arbuscular mycorrhizal symbiosis. Plant J 47:165-173 doi:10.1111/j.1365-
670 313X.2006.02785.x

671 Poulsen KH, Nagy R, Gao LL, Smith SE, Bucher M, Smith FA, Jakobsen I (2005)
672 Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a
673 reduced mycorrhizal colonization mutant in tomato associated with a compatible
674 fungus. New Phytol 168:445-453 doi:10.1111/j.1469-8137.2005.01523.x

675 Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a
676 journey towards relevance? New Phytol 157:475-492 doi:10.1046/j.1469-
677 8137.2003.00704.x

678 Reddy SDMR, Schorderet M, Feller U, Reinhardt D (2007) A petunia mutant affected
679 in intracellular accommodation and morphogenesis of arbuscular mycorrhizal fungi.
680 Plant J 51:739-750 doi:10.1111/j.1365-313X.2007.03175.x

681 Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An
682 evidence-based consensus for the classification of arbuscular mycorrhizal fungi
683 (Glomeromycota). Mycorrhiza:1-17 doi:10.1007/s00572-013-0486-y

684 Rillig MC (2004a) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol
685 Lett 7:740-754 doi:10.1111/j.1461-0248.2004.00620.x

686 Rillig MC (2004b) Arbuscular mycorrhizae, glomalin, and soil aggregation. Can J
687 Soil Sci 84:355-363

688 Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41-
689 53 doi:10.1111/j.1469-8137.2006.01750.x

690 Rillig MC, Ramsey PW, Gannon JE, Mummey DL, Gadkar V, Kapulnik Y (2008)
691 Suitability of mycorrhiza-defective mutant/wildtype plant pairs (*Solanum*
692 *lycopersicum* L. cv Micro-Tom) to address questions in mycorrhizal soil ecology.
693 Plant Soil 308:267-275 doi:10.1007/s11104-008-9629-x

694 Rinaudo V, Barberi P, Giovannetti M, van der Heijden MGA (2010) Mycorrhizal
695 fungi suppress aggressive agricultural weeds. Plant Soil 333:7-20
696 doi:10.1007/s11104-009-0202-z

697 Ruzicka D, Chamala S, Barrios-Masias FH, Martin F, Smith S, Jackson LE, Barbazuk
698 WB, Schachtman DP (2013) Inside arbuscular mycorrhizal roots – molecular probes
699 to understand the symbiosis. Plant Gen 6:- doi:10.3835/plantgenome2012.06.0007

700 Ruzicka DR, Hausmann NT, Barrios-Masias FH, Jackson LE, Schachtman DP (2012)
701 Transcriptomic and metabolic responses of mycorrhizal roots to nitrogen patches
702 under field conditions. Plant Soil 350:145-162 doi:10.1007/s11104-011-0890-z

703 Sagan M, Morandi D, Tarenghi E, Duc G (1995) Selection of nodulation and
704 mycorrhizal mutants in the model-plant *Medicago-truncatula* (Gaertn) after gamma-
705 ray mutagenesis. Plant Sci 111:63-71 doi:10.1016/0168-9452(95)04229-n

706 Senoo K, Solaiman MZ, Kawaguchi M, Imaizumi-Anraku H, Akao S, Tanaka A,
707 Obata H (2000) Isolation of two different phenotypes of mycorrhizal mutants in the
708 model legume plant *Lotus japonicus* after EMS-treatment. Plant and Cell Physiology
709 41:726-732

710 Shirtliffe SJ, Vessey JK (1996) A nodulation (Nod(+)/Fix-) mutant of *Phaseolus*
711 *vulgaris* L has nodule-like structures lacking peripheral vascular bundles (Pvb(-)) and
712 is resistant to mycorrhizal infection (Myc(-)). Plant Sci 118:209-220
713 doi:10.1016/0168-9452(96)04427-5

714 Shtark OY, Borisov AY, Zhukov VA, Provorov NA, Tikhonovich IA (2010) Intimate
715 associations of beneficial soil microbes with host plants. Soil Microbiology and
716 Sustainable Crop Production. doi:10.1007/978-90-481-9479-7_5

717 Smith FA, Smith SE (1981a) Mycorrhizal infection and growth of *Trifolium*
718 *subterraneum*: comparison of natural and artificial inocula. New Phytol 88:311-325
719 doi:10.2307/2431807

720 Smith FA, Smith SE (1981b) Mycorrhizal infection and growth of *Trifolium*
721 *subterraneum*: use of sterilized soil as a control treatment. New Phytol 88:299-309
722 doi:10.1111/j.1469-8137.1981.tb01726.x

723 Smith SE, Facelli E, Pope S, Smith FA (2010) Plant performance in stressful
724 environments: interpreting new and established knowledge of the roles of arbuscular
725 mycorrhizas. Plant Soil 326:3-20 doi:10.1007/s11104-009-9981-5

726 Smith SE, Read DJ (2008) Mycorrhizal Symbiosis. Third edn. Academic Press, New
727 York,

728 Stacey G, Libault M, Brechenmacher L, Wan J, May G (2006) Genetics and
729 functional genomics of legume nodulation. Curr Opin Plant Biol 9:110-121

730 Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N,
731 Stougaard J, Szczyglowski K, Parniske M (2002) A plant receptor-like kinase
732 required for both bacterial and fungal symbiosis. Nature 417:959-962

733 Sun SB, Wang JJ, Zhu LL, Liao DH, Gu MA, Ren LX, Kapulnik Y, Xu GH (2012)
734 An active factor from tomato root exudates plays an important role in efficient
735 establishment of mycorrhizal symbiosis. Plos One 7 doi:e43385
736 10.1371/journal.pone.0043385

737 Tisdall JM (1991) Fungal hyphae and structural stability of soil. Soil Research
738 29:729-743 doi:10.1071/SR9910729

739 Tisdall JM, Oades JM (1980) The management of ryegrass to stabilise aggregates of a
740 red brown earth. Soil Research 18:415-422 doi:10.1071/SR9800415

741 van der Heijden MGA (2010) Mycorrhizal fungi reduce nutrient loss from model
742 grassland ecosystems. Ecology 91:1163-1171 doi:10.1890/09-0336.1

743 van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998a) Different
744 arbuscular mycorrhizal fungal species are potential determinants of plant community
745 structure. Ecology 79:2082-2091 doi:10.1890/0012-
746 9658(1998)079[2082:damfsa]2.0.co;2

747 van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R,
748 Boller T, Wiemken A, Sanders IR (1998b) Mycorrhizal fungal diversity determines
749 plant biodiversity, ecosystem variability and productivity. Nature 396:69-72

750 Veiga RSL, Jansa J, Frossard E, van der Heijden MGA (2011) Can arbuscular
751 mycorrhizal fungi reduce the growth of agricultural weeds? Plos One 6 doi:e27825
752 10.1371/journal.pone.0027825

753 Wagg C, Jansa J, Schmid B, van der Heijden MGA (2011) Belowground biodiversity
754 effects of plant symbionts support aboveground productivity. Ecol Lett 14:1001-1009
755 doi:10.1111/j.1461-0248.2011.01666.x

756 Wamberg C, Christensen S, Jakobsen I (2003) Interaction between foliar-feeding
757 insects, mycorrhizal fungi, and rhizosphere protozoa on pea plants. Pedobiologia
758 47:281-287 doi:10.1078/0031-4056-00191

759 Wang B, Yeun LH, Xue JY, Liu Y, Ané JM, Qiu YL (2010) Presence of three
760 mycorrhizal genes in the common ancestor of land plants suggests a key role of
761 mycorrhizas in the colonization of land by plants. New Phytol 186:514-525
762 doi:10.1111/j.1469-8137.2009.03137.x

763 Watts-Williams SJ, Cavagnaro TR (2012) Arbuscular mycorrhizas modify tomato
764 responses to soil zinc and phosphorus addition. Biol Fertility Soils 48:285-294
765 doi:10.1007/s00374-011-0621-x

766 Watts-Williams SJ, Cavagnaro TR (2014) Nutrient interactions and arbuscular
767 mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato
768 genotype pair. Plant Soil 384:79-92 doi:10.1007/s11104-014-2140-7

769 Watts-Williams SJ, Patti AF, Cavagnaro TR (2013) Arbuscular mycorrhizas are
770 beneficial under both deficient and toxic soil zinc conditions. Plant Soil 371:299-312
771 doi:10.1007/s11104-013-1670-8

772 Watts-Williams SJ, Smith FA, McLaughlin MJ, Patti AF, Cavagnaro TR (2015) How
773 important is the mycorrhizal pathway for plant Zn uptake? Plant Soil
774 doi:10.1007/s11104-014-2374-4

775 Watts-Williams SJ, Turney TW, Patti AF, Cavagnaro TR (2014) Uptake of zinc and
776 phosphorus by plants is affected by zinc fertiliser material and arbuscular
777 mycorrhizas. Plant Soil 376:165-175 doi:10.1007/s11104-013-1967-7

778 West H (1997) Interactions between arbuscular mycorrhizal fungi and foliar
779 pathogens: consequences for host and pathogen. In: Gange A, Brown V (eds)
780 Multitrophic Interactions in Terrestrial Systems, vol 36. Blackwell Science, p 79

781 West HM, Fitter AH, Watkinson AR (1993) The influence of three biocides on the
782 fungal associates of the roots of *Vulpia ciliata* ssp. Ambigua under natural conditions.
783 J Ecol 81:345-350 doi:10.2307/2261504

784 Xie X, Huang W, Liu F, Tang N, Liu Y, Lin H, Zhao B (2013) Functional analysis of
785 the novel mycorrhiza-specific phosphate transporter AsPT1 and PHT1 family from
786 Astragalus sinicus during the arbuscular mycorrhizal symbiosis. New Phytol 198:836-
787 852 doi:10.1111/nph.12188

788 Yang S-Y, Grønlund M, Jakobsen I, Grotemeyer MS, Rentsch D, Miyao A, Hirochika
789 H, Kumar CS, Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, Paszkowski
790 U (2012) Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two
791 members of the PHOSPHATE TRANSPORTER1 gene family. The Plant Cell Online
792 24:4236-4251 doi:10.1105/tpc.112.104901

793 Zhu HY, Riely BK, Burns NJ, Ané JM (2006) Tracing nonlegume orthologs of
794 legume genes required for nodulation and arbuscular mycorrhizal symbioses. Genetics
795 172:2491-2499 doi:10.1534/genetics.105.051185
796

Table 1. List of non-legume mycorrhiza-defective mutant genotypes and their properties. ‘Stage affected’ category follows the steps in AM symbiosis proposed by Delaux et al. (2013).

Mutant	Plant species	Stage affected	Method of mutagenesis	Reference
rmc	Tomato (*Solanum lycopersicum* L. cv 76R)	Dependent on AM fungal isolate, see text	Fast neutron mutagenesis	Barker et al. (1998)
M161	Tomato (*Solanum lycopersicum* L. cv. Micro-Tom)	Pre-symbiotic	Fast neutron mutagenesis	David-Schwartz et al. (2001)
M20	Tomato (*Solanum lycopersicum* L. cv. Micro-Tom)	Pre-symbiotic	Fast neutron mutagenesis	David-Schwartz et al. (2003)
BC1	Tomato (*Solanum lycopersicum* L. cv. Micro-Tom)	Pre-symbiotic	F2 segregate of wild-type and M161	Kapulnik and Bonfante (unpublished) cited in Rillig et al. (2008)
nope1	Maize (*Zea mays*) W64A	Pre-symbiotic	Transposon mutagenesis (Mutator)	Paszkowski et al. (2006)
taci1	Maize (*Zea mays*) W64A	Intraradical colonisation	Transposon mutagenesis (Mutator)	“”
Pram1	Maize (*Zea mays*) W64A	Intraradical colonisation (enhanced)	Transposon mutagenesis (Mutator)	“”
pam1, pam1S1, pam1S2	Petunia (*Petunia hybrida*) W138	Intraradical colonisation	Transposon mutagenesis	Reddy et al. (2007)
OsDMI3 (NF8513)	Rice (*Oryza sativa* L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion line	Chen et al. (2007)
OsIPD3 (NC0263, NC2713, NC2794)	Rice (*Oryza sativa* L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	Chen et al. (2008)
CASTOR (1B-08643)	Rice (*Oryza sativa* L. ssp. *japonica* cv. Dongjin)	Pre-symbiotic	T-DNA insertion	Gutjahr et al. (2008)
POLLUX (1C-03411)	Rice (*Oryza sativa* L. ssp. *japonica* cv. Hwayoung)	Pre-symbiotic	T-DNA insertion	“”
POLLUX (NC6423, ND5050)	Rice (*Oryza sativa* L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	“”
CCAMK (NE1115, NF8513)	Rice (*Oryza sativa* L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	“”
CYCLOPS (IPD3) (NG0782, NC2415, NC2713)	Rice (*Oryza sativa* L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	“”

References

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998) A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. Plant Journal 15:791-797 doi:10.1046/j.1365-313X.1998.00252.x

Chen C, Gao M, Liu J, Zhu H (2007) Fungal symbiosis in rice requires an ortholog of a legume common symbiosis gene encoding a Ca2+/calmodulin-dependent protein kinase. Plant Physiology 145:1619-1628 doi:10.1104/pp.107.109876

Chen CY, Ané JM, Zhu HY (2008) OsIPD3, an ortholog of the *Medicago truncatula* DMI3 interacting protein IPD3, is required for mycorrhizal symbiosis in rice. New Phytologist 180:311-315 doi:10.1111/j.1469-8137.2008.02612.x

David-Schwartz R, Badani H, Smadar W, Levy AA, Galili G, Kapulnik Y (2001) Identification of a novel genetically controlled step in mycorrhizal colonization: plant resistance to infection by fungal spores but not extra-radical hyphae. Plant Journal 27:561-569 doi:10.1046/j.1365-313X.2001.01113.x

David-Schwartz R, Gadkar V, Wininger S, Bendov R, Galili G, Levy AA, Kapulnik Y (2003) Isolation of a premycorrhizal infection (pmi2) mutant of tomato, resistant to arbuscular mycorrhizal fungal colonization. Molecular Plant-Microbe Interactions 16:382-388 doi:10.1094/mpmi.2003.16.5.382

Delaux P-M, Séjalon-Delmas N, Bécard G, Ané J-M (2013) Evolution of the plant–microbe symbiotic 'toolkit'. Trends in Plant Science 18:298-304 doi:10.1016/j.tplants.2013.01.008

Gutjahr C et al. (2008) Arbuscular mycorrhiza–specific signaling in rice transcends the common symbiosis signaling pathway. The Plant Cell 20:2989-3005 doi:10.1105/tpc.108.062414

Paszkowski U, Jakovleva L, Boller T (2006) Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis. Plant Journal 47:165-173 doi:10.1111/j.1365-313X.2006.02785.x

Reddy SDMR, Schorderet M, Feller U, Reinhardt D (2007) A petunia mutant affected in intracellular accommodation and morphogenesis of arbuscular mycorrhizal fungi. The Plant journal : for cell and molecular biology 51:739-750 doi:10.1111/j.1365-313X.2007.03175.x

Rillig MC, Ramsey PW, Gannon JE, Mummey DL, Gadkar V, Kapulnik Y (2008) Suitability of mycorrhiza-defective mutant/wildtype plant pairs (*Solanum lycopersicum* L. cv Micro-Tom) to address questions in mycorrhizal soil ecology. Plant and Soil 308:267-275 doi:10.1007/s11104-008-9629-x