

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^{1,3} · Timothy R. Cavagnaro²

Received: 17 November 2014 / Accepted: 18 March 2015 / Published online: 11 April 2015
© Springer-Verlag Berlin Heidelberg 2015

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 disinfection or sterilisation, 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, 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, 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, b; 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

✉ Stephanie J. Watts-Williams
sjw266@cornell.edu

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

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

³ Present address: Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, USA

(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 orthologues 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 for, 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. 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 include soil disinfection or sterilisation, 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, 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 disinfection or sterilisation to inactivate the AM fungal propagules (Endlweber and Scheu 2006; Koide and Li 1989; Smith and Smith 1981a, b). 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 methanesulphonate (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

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
<i>rmc</i>	Tomato (<i>Solanum lycopersicum</i> L. cv. 76R)	Dependent on AM fungal isolate, see text	Fast neutron mutagenesis	Barker et al. (1998)
<i>M161</i>	Tomato (<i>Solanum lycopersicum</i> L. cv. Micro-Tom)	Pre-symbiotic	Fast neutron mutagenesis	David-Schwartz et al. (2001)
<i>M20</i>	Tomato (<i>Solanum lycopersicum</i> L. cv. Micro-Tom)	Pre-symbiotic	Fast neutron mutagenesis	David-Schwartz et al. (2003)
<i>BC1</i>	Tomato (<i>Solanum lycopersicum</i> L. cv. Micro-Tom)	Pre-symbiotic	F2 segregate of wild-type and M161	Kapulnik and Bonifante (unpublished) cited in Rilling et al. (2008)
<i>nope1</i>	Maize (<i>Zea mays</i>) W64A	Pre-symbiotic	Transposon mutagenesis (Mutator)	Paszkowski et al. (2006)
<i>tact1</i>	Maize (<i>Zea mays</i>) W64A	Intra-radical colonisation	Transposon mutagenesis (Mutator)	Paszkowski et al. (2006)
<i>Pram1</i>	Maize (<i>Zea mays</i>) W64A	Intra-radical colonisation (enhanced)	Transposon mutagenesis (Mutator)	Paszkowski et al. (2006)
<i>pam1, pam1S1, pam1S2</i>	Petunia (<i>Petunia hybrida</i>) W138	Intra-radical colonisation	Transposon mutagenesis	Reddy et al. (2007)
<i>OsDMI3</i> (NF8513)	Rice (<i>Oryza sativa</i> L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion line	Chen et al. (2007)
<i>OsIPD3</i> (NC0263, NC2713, NC2794)	Rice (<i>Oryza sativa</i> L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	Chen et al. (2008)
<i>CASTOR</i> (1B-08643)	Rice (<i>Oryza sativa</i> L. ssp. <i>japonica</i> cv. Dongjin)	Pre-symbiotic	T-DNA insertion	Gutjahr et al. (2008)
<i>POLLUX</i> (1C-03411)	Rice (<i>Oryza sativa</i> L. ssp. <i>japonica</i> cv. Hwayoung)	Pre-symbiotic	T-DNA insertion	Gutjahr et al. (2008)
<i>POLLUX</i> (NC6423, ND5050)	Rice (<i>Oryza sativa</i> L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	Gutjahr et al. (2008)
<i>CCAMK</i> (NE1115, NF8513)	Rice (<i>Oryza sativa</i> L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	Gutjahr et al. (2008)
<i>CYCLOPS</i> (<i>IPD3</i>) (NG0782, NC2415, NC2713)	Rice (<i>Oryza sativa</i> L. cv. Nipponbare)	Pre-symbiotic	Retrotransposon Tos17 insertion lines	Gutjahr et al. (2008)

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 *Glomus 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 (*Medicago sativa*) (Bradbury et al. 1991), barrel medic (*Medicago truncatula*) (Sagan et al. 1995), bean (*Phaseolus vulgaris*) (Shirliffe 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, 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 *M. 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 (Grönlund et al. 2013; Javot et al. 2011, Watts-Williams et al. 2015a). In rice (*O. sativa*) and Chinese milk vetch (*As-tragalus 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. 2015a). 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* (reduced mycorrhizal colonisation) 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 USA, and glasshouse studies have used a range of AM fungal species and soils containing native AM fungal communities (from Europe, Australia and the USA).

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 colonisation 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]), *Glomus fasciculatum* [Thaxter] Gerd. & Trappe emend. Walker & Koske [LPA7] and *Glomus 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, *Glomus coronatum* Giovannetti [WUM16], formerly known as *G. 'City Beach'*, and *G. mosseae* [Nicolson & 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. 2008, 2010; 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 trend was recorded for tissue copper and sulphur concentrations, with concentrations in the 76R plants higher than those in the *rmc* plants. Furthermore, the meta-analysis confirmed that the colonisation phenotype displayed by the AM fungi had a significant influence on the extent to which roots were colonised. The results of the meta-analysis also highlighted that there was no substantial mycorrhizal growth response in the 76R

genotype relative to the *rmc* genotype. It is important to note that, with respect to the criteria for assessing suitable mycorrhiza-defective mutant and wild-type pairs by Rillig et al. (2008), the 76R/*rmc* pair are matched in terms of growth in the absence of AM fungi in all studies (Cavagnaro et al. 2004a; Facelli et al. 2010; Poulsen et al. 2005) except one (Marschner and Timonen 2005).

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

The 76R and *rmc* genotypes continue to be valuable for numerous studies focusing on different aspects of plant nutrition (Cavagnaro et al. 2007b, 2010; Poulsen et al. 2005; Watts-Williams and Cavagnaro 2012; Watts-Williams et al. 2013, 2014, 2015b), 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. 2008, 2009, 2010), soil ecology (Cavagnaro et al. 2006, 2007a, 2012; Hallett et al. 2009; Marschner and Timonen 2005), soil greenhouse gas emissions (Cavagnaro et al. 2008, 2012; Lazcano et al. 2014) and plant genetics (Barker et al. 2005; Gao et al. 2006; Larkan et al. 2007; Ruzicka et al. 2012, 2013).

Micro-Tom mutants

Micro-Tom, 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, 2003). The mutants M20 and M161 were obtained by fast neutron bombardment mutagenesis, while 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

In maize, mutator-mutagenised F2 families of the normal line W64A were screened by Paszkowski et al. (2006) for alteration in yellow root pigmentation compared to wild-type roots (see above for detail on screening). From this screen, the authors described seven mycorrhiza-defective mutants in maize and categorised them into three colonisation phenotype classes: *nope1* (no perception 1) mutants, which showed a marked reduction in intra-radical colonisation by *G. mosseae*, but displayed occasional root sectors containing normal mycorrhizal structures (appressoria and arbuscules); *taci1* (taciturn 1) mutants, which had lower colonisation levels than the wild-type genotype (45 % compared to 86 % root length colonised)

and slightly modified fungal structures; and *Pram1* (Precocious arbuscular mycorrhiza 1) mutants, which are in fact colonised more rapidly and intensely than the wild-type, becoming saturated with intra-radical fungal structures (arbuscules and vesicles) much earlier. These maize mutants represent the first mycorrhiza-defective mutant plants to be characterised in an agronomically important cereal crop. Future research using these mutants will be very useful for research into nutrient uptake in cereal crops, especially in field trials. However, to our knowledge, no such studies have yet been reported in the literature.

Reddy et al. (2007) used a transposon-mutagenised population of petunia (W138, Gerats et al. 1990) in order to identify and characterise a mycorrhiza-defective genotype in this line. The resulting mycorrhiza-defective petunia genotype, *pam1* (penetration and arbuscule morphogenesis1), displayed a strong decrease in AM fungal colonisation compared to its wild-type progenitor. *G. intraradices* formed complex appressoria on *pam1* roots but could not easily penetrate the epidermal cells. Where the fungus did penetrate epidermal cells, the resulting hyphae were distorted 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, intra-radical 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 Ca²⁺ spiking (see Pamiske (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* (does not make infections 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 cells, without any arbuscule formation. However, most observed fungal growth (appressoria and external hyphae) was restricted to the root surface.

Similarly, Chen et al. (2008) characterised three knockout mutants defective in AM fungal colonisation (NC0263, NC2713, NC2794), by searching for putative *Tos17* insertion lines available for *OsIPD3*, another gene required for mycorrhiza formation in rice. When inoculated with *G. intraradices*, the root epidermal cells of the three *OsIPD3* mutants could not be penetrated, and there was no intra-radical colonisation of the roots by the AM fungi (i.e. no hyphae, arbuscules or vesicles) except in one root segment of a NC2713 mutant that displayed aborted intracellular fungal hyphae. There is no explanation given for this observation in NC2713, but it is assumed that the observed aborted hyphae did not confer functionality of the symbiosis. Chen et al. (2008) noted that the colonisation phenotype displayed by the *OsIPD3* mutants was comparable to that of the previously identified *OsDMI3* mutant genotype (Chen et al. 2007).

Future directions

Mycorrhiza-defective plant mutant genotypes have the potential to be used in a broad range of studies. Future uses of both legume and non-legume mutant genotypes may be extended to areas of study where mycorrhiza have previously been shown to improve plant or soil health, but hypotheses have not yet been tested using a mycorrhiza-defective mutant, such as soil nutrient cycling (Jeffries and Barea 1994; Read and Perez-Moreno 2003) and interactions with foliar-feeding insects (Gange and West 1994; Gehring and Whitham 1994; Wamberg et al. 2003) and foliar pathogens (Campos-Soriano et al. 2012; Nair et al. 2015; West 1997). Research that directly 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. 2015b). 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 mycorrhiza 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.

Acknowledgments 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 *rnc* 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, Penmetza 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: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 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.1365-3113X.1998.00252.x
- 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, Dordrecht, 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 (*rnc*) mutant of tomato reveals genetic interaction between symbiosis and parasitism. *Physiol Mol Plant Pathol* 67:277–283. doi:10.1016/j.pmp.2006.03.003
- 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
- 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
- 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* 7:18. 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* 78:34–38. doi:10.1016/j.soilbio.2014.07.007
- Cavagnaro TR (2015) Biologically regulated nutrient supply systems: compost and arbuscular mycorrhizas—a review. *Adv Agron* 129:291–321. doi:10.1016/bs.agron.2014.09.005
- 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
- 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, 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, Sokolow SK, Jackson LE (2007b) Mycorrhizal effects on growth and nutrition of tomato under elevated atmospheric carbon dioxide. *Funct Plant Biol* 34:730–736. doi:10.1071/fp06340
- 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, 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, 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
- 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 Physiol* 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 Phytol* 180:311–315. doi:10.1111/j.1469-8137.2008.02612.x
- Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. *J Plant Nutr* 23:867–902. doi:10.1080/01904160009382068
- 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 J* 27:561–569. doi:10.1046/j.1365-3113X.2001.01113.x
- David-Schwartz R, Gadkar V, Winer 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. *Mol Plant-Microbe Interact* 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 Plant Sci* 18:298–304. doi:10.1016/j.tplants.2013.01.008
- Doyle JJ (1998) Phylogenetic perspectives on nodulation: evolving views of plants and symbiotic bacteria. *Trends Plant Sci* 3:473–478. doi:10.1016/S1360-1385(98)01340-5
- Duc G, Trouvelot A, Gianinazzi-Pearson V, Gianinazzi S (1989) 1st report of non-mycorrhizal plant mutants (myc-) obtained in pea (*Pisum sativum* L) and fababeans (*Vicia faba* L). *Plant Sci* 60:215–222. doi:10.1016/0168-9452(89)90169-6
- Endlweber K, Scheu S (2006) Establishing arbuscular mycorrhiza-free soil: a comparison of six methods and their effects on nutrient mobilization. *Appl Soil Ecol* 34:276–279. doi:10.1016/j.apsoil.2006.04.001
- Endre G, Kereszt A, Kevei Z, Mihacea S, Kalo P, Kiss GB (2002) A receptor kinase gene regulating symbiotic nodule development. *Nature* 417:962–966. doi:10.1038/nature00842
- Engvild KC (1987) Nodulation and nitrogen fixation mutants of pea, *Pisum sativum*. *Theor Appl Genet* 74:711–713. doi:10.1007/BF00247546
- Facelli E, Smith SE, Facelli JM, Christophersen HM, Smith FA (2010) Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. *New Phytol* 185:1050–1061. doi:10.1111/j.1469-8137.2009.03162.x
- Froese-Gertzen EE, Konzak C, Foster R, Nilan R (1963) Correlation between some chemical and biological reactions of ethyl methanesulphonate. *Nature* 198:447–448
- Gadkar V, David-Schwartz R, Nagahashi G, Douds DD, Winer S, Kapulnik Y (2003) Root exudate of pmi tomato mutant M161 reduces AM fungal proliferation in vitro. *FEMS Microbiol Lett* 223:193–198. doi:10.1016/s0378-1097(03)00357-4
- Gange AC, West HM (1994) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytol* 128:79–87. doi:10.1111/j.1469-8137.1994.tb03989.x
- Gao LL, Delp G, Smith SE (2001) Colonization patterns in a mycorrhiza-defective mutant tomato vary with different arbuscular-mycorrhizal fungi. *New Phytol* 151:477–491. doi:10.1046/j.0028-646x.2001.00193.x
- Gao LL, Smith FA, Smith SE (2006) The rmc locus does not affect plant interactions or defence-related gene expression when tomato (*Solanum lycopersicum*) is infected with the root fungal parasite, *Rhizoctonia*. *Funct Plant Biol* 33:289–296. doi:10.1071/fp05153
- Gehring CA, Whitham TG (1994) Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. *Trends Ecol Evol* 9:251–255. doi:10.1016/0169-5347(94)90290-9
- Gerats AG, Huits H, Vrijlandt E, Marañón C, Souer E, Beld M (1990) Molecular characterization of a nonautonomous transposable element (dTph1) of petunia. *Plant Cell* 2:1121–1128. doi:10.1105/tpc.2.11.1121
- Grønlund M, Albrechtsen M, Johansen IE, Hammer EC, Nielsen TH, Jakobsen I (2013) The interplay between P uptake pathways in mycorrhizal peas: a combined physiological and gene-silencing approach. *Physiol Plant* 149:234–248. doi:10.1111/ppl.12030
- Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-Anraku H, Paszkowski U (2008) Arbuscular mycorrhiza-specific signaling in rice transcends the common symbiosis signaling pathway. *Plant Cell* 20:2989–3005. doi:10.1105/tpc.108.062414

- Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* 245: 83–93. doi:10.1023/a:1020663916259
- Hallett PD, Feeney DS, Bengough AG, Rillig MC, Scrimgeour CM, Young IM (2009) Disentangling the impact of AM fungi versus roots on soil structure and water transport. *Plant Soil* 314:183–196. doi:10.1007/s11104-008-9717-y
- Hartnett DC, Wilson GWT (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* 80:1187–1195. doi:10.1890/0012-9658(1999)080[1187:mipcsa]2.0.co;2
- Hirsch AM, Kapulnik Y (1998) Signal transduction pathways in mycorrhizal associations: comparisons with the *Rhizobium*–legume symbiosis. *Fungal Genet Biol* 23:205–212. doi:10.1006/fgbi.1998.1046
- Horváth B, Yeun LH, Domonkos Á, Halász G, Gobatto E, Ayaydin F, Miró K, Hirsch S, Sun J, Tadege M, Ratet P, Mysore KS, Ané J-M, Oldroyd GED, Kaló P (2011) *Medicago truncatula* IPD3 is a member of the common symbiotic signaling pathway required for rhizobial and mycorrhizal symbioses. *Mol Plant-Microbe Interact* 24: 1345–1358. doi:10.1094/MPMI-01-11-0015
- Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi H, Murakami Y, Mulder L, Vickers K, Pike J, Allan Downie J, Wang T, Sato S, Asamizu E, Tabata S, Yoshikawa M, Murooka Y, Wu G-J, Kawaguchi M, Kawasaki S, Parniske M, Hayashi M (2005) Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. *Nature* 433:527–531. doi:10.1038/nature03237
- Javot H, Penmetza RV, Terzaghi N, Cook DR, Harrison MJ (2007) A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci U S A* 104: 1720–1725. doi:10.1073/pnas.0608136104
- Javot H, Penmetza RV, Breuille F, Bhattarai KK, Noar RD, Gomez SK, Zhang Q, Cook DR, Harrison MJ (2011) *Medicago truncatula* mtp4 mutants reveal a role for nitrogen in the regulation of arbuscule degeneration in arbuscular mycorrhizal symbiosis. *Plant J* 68:954–965. doi:10.1111/j.1365-313X.2011.04746.x
- Jeffries P, Barea JM (1994) Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil systems. In: Gianinazzi S, Schüepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser, Basel, pp 101–115. doi:10.1007/978-3-0348-8504-1_9
- Klingner A, Bothe H, Wray V, Marner FJ (1995) Identification of a yellow pigment formed in maize roots upon mycorrhizal colonization. *Phytochemistry* 38:53–55. doi:10.1016/0031-9422(94)00538-5
- Koide RT, Li MG (1989) Appropriate controls for vesicular arbuscular mycorrhiza research. *New Phytol* 111:35–44. doi:10.1111/j.1469-8137.1989.tb04215.x
- Koornneeff M, Dellaert LWM, van der Veen JH (1982) EMS- and relation-induced mutation frequencies at individual loci in *Arabidopsis thaliana* (L.). *Heynh Mutat Res* 93:109–123
- Krüger M, Krüger C, Walker C, Stockinger H, Schüßler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytol* 193:970–984. doi:10.1111/j.1469-8137.2011.03962.x
- Larkan NJ, Smith SE, Barker SJ (2007) Position of the reduced mycorrhizal colonisation (Rmc) locus on the tomato genome map. *Mycorrhiza* 17:311–318. doi:10.1007/s00572-007-0106-9
- Larkan N, Ruzicka D, Edmonds-Tibbett T, Durkin JH, Jackson L, Smith FA, Schachtman D, Smith S, Barker S (2013) The reduced mycorrhizal colonisation (rnc) mutation of tomato disrupts five gene sequences including the CYCLOPS/IPD3 homologue. *Mycorrhiza* 23(7):573–584. doi:10.1007/s00572-013-0498-7
- Lazcano C, Barrios-Masias FH, Jackson LE (2014) Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes. *Soil Biol Biochem* 74:184–192. doi:10.1016/j.soilbio.2014.03.010
- Lévy J, Bres C, Geurts R, Chalhoub B, Kulikova O, Duc G, Journet E-P, Ané J-M, Lauber E, Bisseling T, Dénarié J, Rosenberg C, Debelle F (2004) A putative Ca²⁺ and calmodulin-dependent protein kinase required for bacterial and fungal symbioses. *Science* 303:1361–1364. doi:10.1126/science.1093038
- Li X, Song YJ, Century K, Straight S, Ronald P, Dong XN, Lassner M, Zhang YL (2001) A fast neutron deletion mutagenesis-based reverse genetics system for plants. *Plant J* 27:235–242. doi:10.1046/j.1365-313x.2001.01084.x
- Manjarrez M, Smith FA, Marschner P, Smith SE (2008) Is cortical root colonization required for carbon transfer to arbuscular mycorrhizal fungi? Evidence from colonization phenotypes and spore production in the reduced mycorrhizal colonization (rnc) mutant of tomato. *Botany* 86:1009–1019. doi:10.1139/b08-043
- Manjarrez M, Wallwork M, Smith SE, Smith FA, Dickson S (2009) Different arbuscular mycorrhizal fungi induce differences in cellular responses and fungal activity in a mycorrhiza-defective mutant of tomato (rnc). *Funct Plant Biol* 36:86–96. doi:10.1071/fp08032
- Manjarrez M, Christophersen HM, Smith SE, Smith FA (2010) Cortical colonisation is not an absolute requirement for phosphorus transfer to plants in arbuscular mycorrhizas formed by *Scutellospora calospora* in a tomato mutant: evidence from physiology and gene expression. *Funct Plant Biol* 37:1132–1142. doi:10.1071/FP09248
- Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* 159:89–102. doi:10.1007/BF00000098
- Marschner P, Timonen S (2005) Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. *Appl Soil Ecol* 28:23–36. doi:10.1016/j.apsoil.2004.06.007
- Marsh JF, Schultze M (2001) Analysis of arbuscular mycorrhizas using symbiosis-defective plant mutants. *New Phytol* 150:525–532. doi:10.1046/j.1469-8137.2001.00140.x
- Meissner R, Jacobson Y, Melamed S, Levyatov S, Shalev G, Ashri A, Elkind Y, Levy A (1997) A new model system for tomato genetics. *Plant J* 12:1465–1472. doi:10.1046/j.1365-313x.1997.12061465.x
- Merrild MP, Ambus P, Rosendahl S, Jakobsen I (2013) Common arbuscular mycorrhizal networks amplify competition for phosphorus between seedlings and established plants. *New Phytol* 200:229–240. doi:10.1111/nph.12351
- Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biol Biochem* 22:579–584. doi:10.1016/0038-0717(90)90001-g
- Nair A, Kolet SP, Thulasiram HV, Bhargava S (2015) Systemic jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against *Alternaria alternata*. *Plant Biol*. doi:10.1111/plb.12277
- Neumann E, George E (2005) Does the presence of arbuscular mycorrhizal fungi influence growth and nutrient uptake of a wild-type tomato cultivar and a mycorrhiza-defective mutant, cultivated with roots sharing the same soil volume? *New Phytol* 166:601–609. doi:10.1111/j.1469-8137.2005.01351.x
- O'Connor PJ, Smith SE, Smith FA (2002) Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland. *New Phytol* 154:209–218. doi:10.1046/j.1469-8137.2002.00364.x
- Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6:763–775. doi:10.1038/nrmicro1987
- Paszkowski U (2006) A journey through signaling in arbuscular mycorrhizal symbioses 2006. *New Phytol* 172:35–46. doi:10.1111/j.1469-8137.2006.01840.x
- Paszkowski U, Jakovleva L, Boller T (2006) Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis. *Plant J* 47: 165–173. doi:10.1111/j.1365-313X.2006.02785.x
- Poulsen KH, Nagy R, Gao LL, Smith SE, Bucher M, Smith FA, Jakobsen I (2005) Physiological and molecular evidence for Pi uptake via the

- symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. *New Phytol* 168:445–453. doi:10.1111/j.1469-8137.2005.01523.x
- Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? *New Phytol* 157:475–492. doi:10.1046/j.1469-8137.2003.00704.x
- Reddy SDMR, Schorderet M, Feller U, Reinhardt D (2007) A petunia mutant affected in intracellular accommodation and morphogenesis of arbuscular mycorrhizal fungi. *Plant J* 51:739–750. doi:10.1111/j.1365-3113X.2007.03175.x
- Redecker D, Schüßler A, Stockinger H, Stürmer SL, Morton JB, Walker C (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 23(7): 515–531. doi:10.1007/s00572-013-0486-y
- Rillig MC (2004a) Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecol Lett* 7:740–754. doi:10.1111/j.1461-0248.2004.00620.x
- Rillig MC (2004b) Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can J Soil Sci* 84:355–363. doi:10.4141/S04-003
- Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. *New Phytol* 171:41–53. doi:10.1111/j.1469-8137.2006.01750.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 Soil* 308:267–275. doi:10.1007/s11104-008-9629-x
- Rinaudo V, Barberi P, Giovannetti M, van der Heijden MGA (2010) Mycorrhizal fungi suppress aggressive agricultural weeds. *Plant Soil* 333:7–20. doi:10.1007/s11104-009-0202-z
- Ruzicka DR, Hausmann NT, Barrios-Masias FH, Jackson LE, Schachtman DP (2012) Transcriptomic and metabolic responses of mycorrhizal roots to nitrogen patches under field conditions. *Plant Soil* 350:145–162. doi:10.1007/s11104-011-0890-z
- Ruzicka D, Chamala S, Barrios-Masias FH, Martin F, Smith S, Jackson LE, Barbazuk WB, Schachtman DP (2013) Inside arbuscular mycorrhizal roots—molecular probes to understand the symbiosis. *Plant Gen* 6. doi:10.3835/plantgenome2012.06.0007
- Sagan M, Morandi D, Tarengi E, Duc G (1995) Selection of nodulation and mycorrhizal mutants in the model-plant *Medicago truncatula* (Gaertn) after gamma-ray mutagenesis. *Plant Sci* 111:63–71. doi:10.1016/0168-9452(95)04229-n
- Senoo K, Solaiman MZ, Kawaguchi M, Imaizumi-Anraku H, Akao S, Tanaka A, Obata H (2000) Isolation of two different phenotypes of mycorrhizal mutants in the model legume plant *Lotus japonicus* after EMS-treatment. *Plant Cell Physiol* 41:726–732. doi:10.1093/pcp/41.6.726
- Shirliffe SJ, Vessey JK (1996) A nodulation (Nod(+)/Fix(-) mutant of *Phaseolus vulgaris* L has nodule-like structures lacking peripheral vascular bundles (Pvb(-)) and is resistant to mycorrhizal infection (Myc(-)). *Plant Sci* 118:209–220. doi:10.1016/0168-9452(96)04427-5
- Shtark OY, Borisov AY, Zhukov VA, Provorov NA, Tikhonovich IA (2010) Intimate associations of beneficial soil microbes with host plants. *Soil Microbiol Sustain Crop Prod*. doi:10.1007/978-90-481-9479-7_5
- Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, New York
- Smith FA, Smith SE (1981a) Mycorrhizal infection and growth of *Trifolium subterraneum*: comparison of natural and artificial inocula. *New Phytol* 88:311–325. doi:10.2307/2431807
- Smith FA, Smith SE (1981b) Mycorrhizal infection and growth of *Trifolium subterraneum*: use of sterilized soil as a control treatment. *New Phytol* 88:299–309. doi:10.1111/j.1469-8137.1981.tb01726.x
- Smith SE, Facelli E, Pope S, Smith FA (2010) Plant performance in stressful environments: interpreting new and established knowledge of the roles of arbuscular mycorrhizas. *Plant Soil* 326:3–20. doi:10.1007/s11104-009-9981-5
- Stacey G, Libault M, Brechenmacher L, Wan J, May G (2006) Genetics and functional genomics of legume nodulation. *Curr Opin Plant Biol* 9:110–121. doi:10.1016/j.pbi.2006.01.005
- Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K, Parniske M (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* 417:959–962. doi:10.1038/nature00841
- Sun SB, Wang JJ, Zhu LL, Liao DH, Gu MA, Ren LX, Kapulnik Y, Xu GH (2012) An active factor from tomato root exudates plays an important role in efficient establishment of mycorrhizal symbiosis. *PLoS One* 7:e43385. doi:10.1371/journal.pone.0043385
- Tisdall JM (1991) Fungal hyphae and structural stability of soil. *Soil Res* 29:729–743. doi:10.1071/SR9910729
- Tisdall JM, Oades JM (1980) The management of ryegrass to stabilise aggregates of a red brown earth. *Soil Res* 18:415–422. doi:10.1071/SR9800415
- van der Heijden MGA (2010) Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. *Ecology* 91:1163–1171. doi:10.1890/09-0336.1
- van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79:2082–2091. doi:10.1890/0012-9658(1998)079[2082:damsfa]2.0.co;2
- van der Heijden MGA, Klironomos JN, Ursic M, Moutoglou P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72. doi:10.1038/23932
- Veiga RSL, Jansa J, Frossard E, van der Heijden MGA (2011) Can arbuscular mycorrhizal fungi reduce the growth of agricultural weeds? *PLoS One* 6:e27825. doi:10.1371/journal.pone.0027825
- Wagg C, Jansa J, Schmid B, van der Heijden MGA (2011) Belowground biodiversity effects of plant symbionts support aboveground productivity. *Ecol Lett* 14:1001–1009. doi:10.1111/j.1461-0248.2011.01666.x
- Wamberg C, Christensen S, Jakobsen I (2003) Interaction between foliar-feeding insects, mycorrhizal fungi, and rhizosphere protozoa on pea plants. *Pedobiologia* 47:281–287. doi:10.1078/0031-4056-00191
- Wang B, Yeun LH, Xue JY, Liu Y, Ané JM, Qiu YL (2010) Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytol* 186:514–525. doi:10.1111/j.1469-8137.2009.03137.x
- Watts-Williams SJ, Cavagnaro TR (2012) Arbuscular mycorrhizas modify tomato responses to soil zinc and phosphorus addition. *Biol Fertil Soils* 48:285–294. doi:10.1007/s00374-011-0621-x
- Watts-Williams SJ, Cavagnaro TR (2014) Nutrient interactions and arbuscular mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair. *Plant Soil* 384:79–92. doi:10.1007/s11104-014-2140-7
- Watts-Williams SJ, Jakobsen I, Cavagnaro TR, Grønlund M (2015a) Local and distal effects of arbuscular mycorrhizal colonisation on direct pathway Pi uptake and root growth in *Medicago truncatula*. *J Exp Bot* (in press)
- Watts-Williams SJ, Patti AF, Cavagnaro TR (2013) Arbuscular mycorrhizas are beneficial under both deficient and toxic soil zinc conditions. *Plant Soil* 371:299–312. doi:10.1007/s11104-013-1670-8
- Watts-Williams SJ, Turney TW, Patti AF, Cavagnaro TR (2014) Uptake of zinc and phosphorus by plants is affected by zinc fertiliser material and arbuscular mycorrhizas. *Plant Soil* 376:165–175. doi:10.1007/s11104-013-1967-7
- Watts-Williams SJ, Smith FA, McLaughlin MJ, Patti AF, Cavagnaro TR (2015b) How important is the mycorrhizal pathway for plant Zn uptake? *Plant Soil*. doi:10.1007/s11104-014-2374-4
- West H (1997) Interactions between arbuscular mycorrhizal fungi and foliar pathogens: consequences for host and pathogen. In: Gange

- A, Brown V (eds) Multitrophic interactions in terrestrial systems, vol 36. Blackwell Science, Oxford, p 79
- West HM, Fitter AH, Watkinson AR (1993) The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata* ssp. *Ambigua* under natural conditions. *J Ecol* 81:345–350. doi:10.2307/2261504
- Xie X, Huang W, Liu F, Tang N, Liu Y, Lin H, Zhao B (2013) Functional analysis of the novel mycorrhiza-specific phosphate transporter AsPT1 and PHT1 family from *Astragalus sinicus* during the arbuscular mycorrhizal symbiosis. *New Phytol* 198:836–852. doi:10.1111/nph.12188
- Yang S-Y, Grønlund M, Jakobsen I, Grotemeyer MS, Rentsch D, Miyao A, Hirochika H, Kumar CS, Sundaresan V, Salamin N, Catausan S, Mattes N, Heuer S, Paszkowski U (2012) Nonredundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the PHOSPHATE TRANSPORTER1 gene family. *Plant Cell* 24:4236–4251. doi:10.1105/tpc.112.104901
- Zhu HY, Riely BK, Burns NJ, Ané JM (2006) Tracing nonlegume orthologs of legume genes required for nodulation and arbuscular mycorrhizal symbioses. *Genetics* 172:2491–2499. doi:10.1534/genetics.105.051185