Characterization of the life cycle and cellular interactions of AM fungi with the reduced mycorrhizal colonization (r*mc*) mutant of tomato (*Solanum lycopersicum* L.)

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Summary

The broad aim of the work described in this thesis was to use the arbuscular mycorrhizal (AM) defective *rmc* tomato to explore the development and function of different types of fungus-plant interfaces (phenotypes) and to characterize the cellular modifications preceding colonization of *rmc* by a range of different AM fungi. Three main patterns of colonization with *rmc* have been described: 1) Pen- phenotype in which the AM fungus is restricted to the root surface with several attempts to penetrate the epidermal cells without success; 2) Coi- phenotype where AM fungi penetrate the epidermis but cannot develop cortical colonization; and 3) Myc+ phenotype (with *G. intraradices* WFVAM23), where the AM fungus penetrates the cortex and forms a "normal" colonization after a delayed penetration of the epidermal cells (Review of literature). Little is known about cellular interactions, nutrient transfer or the ability of the fungi to complete their life cycles in the different phenotypes. These aspects were the main foci of this work. In addition further fungal isolates were screened to asses their ability to colonize *rmc*.

The first experiments involved compartmented pots to follow the fungal life cycle, production of external mycelium and spores in the different *rmc* phenotypes (Chapter 3). The results showed that in the Pen- and Coi- phenotypes, AM fungi are unable to form spores to complete the life cycle. However, in the Coi- phenotype, the fungus remained alive up to week 18, suggesting that some C transfer occurred. The fungus forming the Myc+ phenotype, *G. intraradices* WFVAM23, was able to produce spores, although they were significantly smaller than those produced with the wild-type tomato. The results suggested that arbuscules are essential for completion of the fungal

life cycle. Labeled ^{32}P was used to determine whether arbuscules are also essential for P transfer (Chapter 4). A compartmented pot system was used in which only fungal hyphae but not roots could obtain ^{32}P. ^{32}P was found in the shoots of *rmc* inoculated with *S*. *calospora* (Coi- phenotype), indicating that interfaces other than arbuscules can be involved in transfer of P. A nurse pot system was used to obtain synchronized colonization to determine how long AM fungi stay alive during the interactions with *rmc* and to elucidate the cellular modifications preceding colonization of *rmc* by a range of different AM fungi (Chapter 5). The results showed that *rmc* did attract the AM fungi, that the plant nucleus moved to the middle of the plant cell only after fungal penetration of plant roots and that callose deposition in *rmc* was not involved in blocking the AM fungi. Fourteen AM fungi with different taxonomic affiliations and fourteen different *G*. *intraradices* isolates were screened to try to relate phylogeny of AM fungi with phenotypes in *rmc* (Chapter 6). There were a large number of interactions, depending on the inoculated AM fungi, and although there were some similarities in the *rmc* phenotypes within phylogenetic groups, there was no clear relationship between phylogeny and development of interactions with *rmc***.**

This study showed the following. 1) Arbuscules/arbusculate coils are necessary for the completion of the AM fungal cycle. However, intraradical hyphae also participate in transfer of both P and C as demonstrated with the Coi- phenotype. 2) r*mc* clearly attracted AM fungi and the fungi stay alive and induce plant cellular responses such as nuclear movement only after penetrating *rmc* roots. 3) Plant defense responses such as callose deposition are not involved in blocking AM fungi in *rmc*; and 4) there was no relationship between the phenotypes described in *rmc* and phylogeny of the *Glomeromycota*.

Publications from this thesis

Journal Paper

Manjarrez Maria, F. Andrew Smith, Petra Marschner and Sally E. Smith (2007) Completion of the life cycle of arbuscular mycorrhizal (AM) fungi showing different colonization phenotypes in the *r*educed *m*ycorrhizal *c*olonization (*rmc*) mutant of tomato: implications for C transfer. New Phytologist. Submitted.

Conference posters and oral presentations

Manjarrez, Maria, Smith, S.E., Marschner, P., Dickson, S. and Wallwork, M. (2005) Completion of the life cycle of arbuscular mycorrhizal fungi in a reduced mycorrhizal colonisation (*rmc*) tomato mutant. COMBIO 2005. Adelaide Convention Centre, Adelaide, South Australia. 25-29 September 2005. Poster

Manjarrez, Maria, Smith, S.E., Marschner, P., Smith, A., and Wallwork, M. (2006) Can AM fungi transfer C and P in a mutant tomato lacking arbuscules? Centre for Plant Root Symbiosis. The University of Adelaide. Adelaide, South Australia. Oral presentation.

Manjarrez, Maria, Smith, S.E., Marschner, P., Dickson, S. and Wallwork, M. (2005) Outcomes of cellular interactions between AM fungi and the rmc tomato mutant. Centre for Plant Root Symbiosis. The University of Adelaide. Adelaide, South Australia. 15-17 February 2005. Oral presentation.

Manjarrez, Maria, Smith, S.E. and Smith, F. A. (2006) Arbuscules are not an absolute requirement for P transfer in AM symbiosis: evidence from ^{32}P transfer. 5th International Conference on Mycorrhiza. Granada, Spain. 23-27 July 2006. Hosen for oral presentation.

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To my Mum, brothers and sisters.

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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