Arbuscular mycorrhizal community in a permanent pasture and development of species-specific primers for detection and quantification of two AM fungi

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SUMMARY

Arbuscular mycorrhizal (AM) fungi are a group of symbionts that occupy different niches during their life cycle in roots and in the rhizosphere. They occur in soil as spores, hyphae and other propagules such as colonised root fragments. During the interaction with the plant roots a bidirectional transfer of mineral nutrients and carbon occurs, frequently ensuring a positive benefit to both partners. The 152 species of mycorrhizal fungi can be difficult to identify and quantify because the taxonomy of these fungi is based on the description of spores, which are probably produced asexually. Because identification is based on spore morphology few attempts have been made to identify the species which are present in roots. Identification of AM fungal spores from field samples of spores is time consuming, requires considerable expertise and only provides information on those fungi that produce spores. It cannot be assumed that the spore population reflects the situation within the root, and therefore it is important to develop tools for identification of AM fungi during the vegetative stages. Several approaches have been tested in previous work and the development of sensitive molecular methods for identification and quantification of two species of AM fungi is described in this study.

Mycorrhizal fungal communities were sampled in both natural and agricultural ecosystems at two sites in Southern Australia, to provide a comparison between different location and cultural practices. Presence of mycorrhizal spores were used as indicators of fungal occurrence, while species composition was determined by identification of the spores. It was shown that mycorrhizal associations were common and that there was considerable fungal diversity in agro-ecosystems within direct drill and permanent pasture systems. However, in the adjacent natural or semi-natural vegetation, 80% of plant species appeared to be non-mycorrhizal and the fungal diversity and numbers of spores were very low. Based on the diversity of AM fungal populations the permanent rotation trial at the Waite Campus, South Australia was chosen for a more thorough investigation of the mycorrhizal spore population. Using the permanent pasture sampling in July and December 1996 and July and December 1997 recovered spores of thirteen species of AM fungi were recovered. Trap cultures were set up at each collection time using *Lolium perenne*, *Plantago lanceolata*,

Sorghum sp. and *Trifolium subterraneum* which also recovered thirteen species. Nine species were recovered by both methods; *Acaulospora* sp., *Glomus aggregatum, G. macrocarpum* were only recovered in field-collected soil and *Entrophospora sp., G. clarum, G. coronatum* and *G. etunicatum* only in trap cultures. In both field-collected soil and trap cultures the dominant species was *G. mosseae*. The community diversity was not significantly different in field-collected soil at different sampling times, but it was significantly different between sampling times in trap cultures. Single spore pot cultures of *G. mosseae, G. constrictum, Glomus* sp. and *Gigaspora margarita* were established. The combination of spore identification from trap culture and field-collected soil promises to be an effective means to study diversity of AM fungi in a particular system, but more study is necessary to obtain clear picture of the activity of the AM fungal population particularly during vegetative growth.

The internal transcribed spacer (ITS) region was chosen for designing species-specific PCR primers. This required a knowledge of the genetic variability in this region in *G. mosseae* and *Gi. margarita*. To study the genetic variability in *G. mosseae* and *Gi. margarita,* sequence similarity of the ITS regions of ribosomal DNA was analysed in spores collected from the permanent pasture and from pot cultures. PCR amplification with the primers ITS1 and ITS4 was performed and products were cloned and sequenced. The sequences from single spores of *G. mosseae* and *Gi. margarita* confirmed that there is variation in the ITS region in single spores. Phenetic analysis of sequences from both species supported the morphological identification, and placed the species into two separate groups as expected. Through the analysis of these sequences an estimate of genetic diversity was derived which clearly showed that the three field spores of *G. mosseae* were at least 2 - 5 times more genetically diverse than that one single spore (field) and a pool of spores (pot culture) of *Gi. margarita.* This demonstrates that a high degree of variation exists in this natural population of *G. mosseae*.

PCR primers for *G. mosseae* and *Gi. margarita* were designed from the ITS sequences of field-collected spores, with the aim of providing tools well suited to field diagnostics. The specificity of the primers was assessed by PCR amplification of genomic DNA extracted from spores of 12 species of Glomalean (AM) fungi, from mycorrhizal roots of *Allium porrum, L. perenne, P. lanceolata, Sorghum* sp., and *T.*

subterraneum and from several non-mycorrhizal, root inhabiting fungi. Primers designed from *G. mosseae* were highly specific for spores of *G. mosseae* and roots colonised by this fungus. The primers designed form *Gi. margarita* were specific with respect to other AM fungi tested, but they amplified a fragment of a different size from *Rhizoctonia*, which is a commonly occurring root pathogen. Quantification using a DNA slot-blot hybridisation assay with synthetic oligonucleotides showed that 100 ng of total genomic DNA from 95% colonised roots contained 0.78 ng DNA of *G. mosseae*. This DNA-based quantification method could be used to estimate the amount of DNA of particular fungal species in colonised roots. The ability to differentiate between species and isolates of AM fungal symbionts using synthetic oligonucleotides has great potential for investigating fungal ecology, studying competition between species, and in field experiments where plants are inoculated with specific fungal species.