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# Selection of appropriate host plants used in trap culture of arbuscular mycorrhizal fungi

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**Abstract** Arbuscular mycorrhizal (AM) fungi in coalmine spoil, island forest and saline soils were enriched in pot culture with maize (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), white clover (*Trifolium repens* Linn.) and silverweed cinquefoil (*Potentilla anserina* L.). Based on spores, there were more species of AM fungi in the coalmine spoil (15 species, 3 genera), than in the forest soil (11 species, 4 genera) and the saline soil (5 species, 2 genera). In the trap cultures, the total of 28 species in *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis* detected in the original soils were all recovered with at least one of the four trap plants. The highest spore and species numbers were recovered in trap cultures of *T. repens* inoculated with coalmine spoil. *Glomus constrictum* and *Glomus multicaule* were the dominant species associated with *N. tabacum* grown in saline soil and forest soil. The dominant species of AM fungi on the four hosts was *Acaulospora mellea*, which had over 90% of the spore incidence in pot trap culture in coalmine spoil. It is suggested that there be selectivity between host plants and AM fungi. The number of species of AM fungi detected was influenced by host plants under certain conditions and white clover was generally the optimal host plant to detect diversity of AM fungi.

**Keywords** Arbuscular mycorrhizal fungi · *Zea mays* L. · *Nicotiana tabacum* L. · *Trifolium repens* L. · *Potentilla anserina* L.

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## Introduction

Arbuscular mycorrhizal (AM) fungi inhabit various ecosystems with a wide range of host plant species. However, as these fungi are obligate symbionts with living roots, the hosts play an important role in mycorrhizal development, spore formation and distribution of AM fungi. The community of AM fungus species in the rhizosphere may vary with host species (McGonigle and Fitter 1990). Hetrick and Bloom (1986) investigated the influence of five host plants, including red clover, sudan grass and tomato, on colonization and spore production of AM fungi. They showed that spore development of *Glomus fasciculatum* was influenced by the host plant, while *Glomus mosseae* and *Glomus macrocarpum* were not, and suggested that there were differences in plant-fungus compatibility. The findings on *G. mosseae* have been confirmed recently by Helgason et al. (1998). It has been suggested that species diversity of AM fungi may be determined by the variety of plant species in natural ecosystems (Al-Raddad 1993; Sieverding 1989).

The diversity of AM fungi has been investigated in many ecosystems, including arable sites (Franke-Snyder et al. 2001; Helgason et al. 1998), conservation and forest lands (Helgason et al. 1998; Zhao et al. 2001), and saline and spoil soils (Wang and Liu 2001). Usually, AM fungal diversity in farm or degraded soils has been shown to be lower than in soils supporting a diverse flora of native plants (Helgason et al. 1998; Liu and Li 2000). In addition, Genney et al. (2001) noted that the degree of AM colonization was related to host density in the field. Whether the host can influence diversity of mycorrhizal fungi under controlled conditions is an important issue as different researchers often use different test plants for studies on AM fungal diversity (Frank-Snyder et al. 2001; Helgason et al. 1998). AM fungal trap cultures can be very helpful in unveiling fungal community members that are undetected in initial extraction of spores from field soil (Morton et al. 1995). Although many AM fungi are thought to have a broad host range, the appropriate test plants for trap cultures should be evaluated to ensure

maximum detection of fungal species in specific soils or site types. The primary purpose of this investigation was to evaluate maize, tobacco, white clover and silverweed cinquefoil as trap plants under pot-culture conditions for detecting the presence of species of AM fungi.

## Materials and methods

Samples were collected from three diverse types of soil: coalmine spoil from Zhaozhuang City, forest soil under *Pinus thunbergii* Parl. on Changshan Islands, and saline soil from the Yellow River Delta in Shandong Province, China. About 2 kg soil (2–20 cm) was collected from five randomly selected locations at each site. Soils at each site were bulked, mixed and five replicate subsamples taken. Spores of AM fungi of each soil were extracted by wet-sieving from aliquots (50 ml) and identified to species (Schenck and Perez 1988).

Pots were filled with 1,000 ml sterilized (121°C, 1 h) sandy loam and 50 ml soil inoculum was mixed throughout each pot. Seeds of maize (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.) and white clover (*Trifolium repens* Linn.) were surface-sterilized, germinated and sown directly into the pot. Silverweed cinquefoil (*Potentilla anserina* L.) was grown from cuttings in sterilized sand before transplanting. There were 12 treatments (4 hosts ×3 soil inocula) with 6 replicates (pots). Pots were randomized in a greenhouse, watered once every 2 days, with addition of 30% strength Hoagland's nutrient solution without phosphorus every 2 weeks. Ninety days after inoculation, the soil was separated from the roots by hand, and the roots were washed free of soil. AM fungal spores were separated from the soil, identified to species, and spore numbers were counted (Schenck and Perez 1988). Roots were washed with tap water, cut into 0.5–1.0 cm segments, cleared in 5% KOH and stained with acid fuchsin. Mycorrhizal colonization percentage was determined (Biermann and Linderman 1981). Analysis of variance (ANOVA) was applied to spore and species numbers, and the colonization rate and means were compared by Duncan's Multiple Range Test, $P \leq 0.05$.

## Results

In total, 4 genera and 28 species of AM fungi were isolated from the different ecosystem sites (Table 1), and all of these species were recovered in at least one of the host plant cultures (Table 2). There were significant differences in the genera, species and spore numbers isolated from under each host. More genera, species and spores of AM fungi were isolated using *T. repens* than *Z. mays* and *P. anserina* (Table 2). Similar numbers of species were formed under each of the four plants grown in saline soil inoculum. The highest spore number and the most species were obtained under *T. repens* grown in coalmine spoil (Table 3). *Glomus constrictum* and *Glomus multicaule* were the dominant species on *N. tabacum* grown in pots inoculated with saline soil and forest soil, with a spore incidence of 55% and 51% of total spores, respectively, however, there were no obvious dominant species of AM fungi on *T. repens*, *Z. mays* and *P. anserina*. The dominant species of AM fungus on all four hosts was *Acaulospora mellea* in pot trap culture with coalmine spoil, with over 90% of the spore incidence (Table 4). *Glomus claroideum* and *G. mosseae* appeared

**Table 1** Numbers of genera and species of arbuscular mycorrhizal (AM) fungi in the soil inocula

| Soil inocula | Plant vegetation | Genera | Species |
|---|---|---|---|
| Coalmine spoil soil | Moderate | *Acaulospora* | *A. dilatata*, *A. elegans*, *A. mellea*, *A. rehmii*, *A. rugosa*, *A. scrobiculata* |
| | | *Glomus* | *G. aggregatum*, *G. caledonieum*, *G. clarum*, *G. fecundisporum*, *G. geosporum*, *G. manihotis*, *G. mosseae*, *G. versiforme* |
| | | *Sclerocystis* | *Scl. Liquidambaris* |
| Island forest soil | Rich | *Acaulospora* | *A. denticulata* *A. lacunosa* |
| | | *Glomus* | *G. constrictum*, *G. multicaule*, *G. etunicatum*, *G. fasciculatum*, *G. mosseae*, *G. reticulatum* |
| | | *Gigaspora* | *Gi. gigantea*, *Gi. margarita* |
| | | *Sclerocystis* | *Scl. liquidambaris* |
| Saline soil | Poor | *Acaulospora* | *A. foveata*, *A. laevis* |
| | | *Glomus* | *G. albidum*, *G. claroideum*, *G. mosseae* |

**Table 2** Genera and species of AM fungi recovered in cultures with four species of host plants

| Genera and species | Soil[a] | Hosts[b] |
|---|---|---|
| *Acaulospora* | | |
| *A. denticulata* | B | 2, 3, 4 |
| *A. dilatata* | A | 1, 2 |
| *A. elegans* | A | 1, 2, 3, 4 |
| *A. foveata* | C | 1, 2, 3, 4 |
| *A. laevis* | C | 1, 2, 3 |
| *A. lacunosa* | B | 1, 2, 3, 4 |
| *A. mellea* | A | 1, 2, 3, 4 |
| *A. rehmii* | A | 1, 2, 3, 4 |
| *A. rugosa* | A | 1, 3 |
| *A. scrobiculata* | A | 1, 2 |
| *Glomus* | | |
| *G. aggregatum* | A | 1, 2, 3, 4 |
| *G. albidum* | C | 1, 2, 3 |
| *G. caledonium* | A | 1, 2, 3, 4 |
| *G. claroideum* | C | 1, 4 |
| *G. clarum* | A | 1, 2, 3, 4 |
| *G. constrictum* | B | 1, 2, 3 |
| *G. etunicatum* | B | 1, 3 |
| *G. fasciculatum* | B | 1, 2, 3 |
| *G. fecundisporum* | A | 1, 2 |
| *G. geosporum* | A | 1, 2, 3 |
| *G. manihotis* | A | 1 |
| *G. mosseae* | A, B, C | 1, 2, 3, 4 |
| *G. multicaule* | B | 1, 2, 3 |
| *G. reticulatum* | B | 1 |
| *G. versiforme* | A | 1, 2, 3, 4 |
| *Gigaspora* | | |
| *Gi. gigantea* | B | 1, 4 |
| *Gi. margarita* | B | 1, 2 |
| *Sclerocystis* | | |
| *Scl. liquidambaris* | A, B | 1, 2 |

[a] *A* Coalmine spoil soil, *B* Island forest soil, *C* Saline soil
[b] *1* White clover, *2* tobacco, *3* maize, *4* silverweed cinquefoil

in most of the treatments, but were not the dominant species.

The abundance of *Glomus* spores on *Z. mays* in coalmine spoil was significantly higher than on *T. repens* or *P. anserina* (Table 4). The spore incidence of *Acaulospora* on *P. anserina* in pot culture with the saline soil was significantly higher than that with the other plants, while with plants cultured in forest soil the highest incidence (65%) was on *T. repens* (Table 4). The differences in selection between AM fungi and host may be due to different inocula collected from natural ecosystems.

There were significant differences in mycorrhizal colonization status of the host plants inoculated with different soil inocula. The treatment with forest soil inocula showed the highest colonization percentage. The percentage of colonization of corn grown in the three soils was highest, followed by white clover, while tobacco and silverweed cinquefoil showed the lowest levels of colonization (Table 5).

## Discussion

Trap cultures, using host plants grown in soil diluted with sterile sand, are most commonly used to isolate AM fungi (Brundrett et al. 1999a, b; Menge 1984; Morton et al. 1993). This pot culturing method usually results in the isolation of more species than other methods (An et al. 1990; Watson and Milner 1996). It provides additional information on fungal diversity that complements spore occurrence data obtained using the same soil samples and may provide valuable new information about the biology of AM fungi (Brundrett et al. 1999a). In our present investigation, all the species found in soil from ecosystems as measured from spore types were recovered in trap cultures with at least one of the four trap plants. However, Brundrett et al. (1999a) considered that the number of species (especially *Glomus*) isolated in pot cultures always exceeded the number identified from field-collected spores, suggesting that fungal surveys based solely on spore observations are inaccurate since some species may not produce their spores in the soil (Liu and Li 2000; Smith and Read 1997). Therefore, other

**Table 3** Species and spore numbers of AM fungi in pot cultures with four host plants inoculated with three soil inocula

| Host plant | Coalmine spoil soil | | Island forest soil | | Saline soil | | Totals | |
|---|---|---|---|---|---|---|---|---|
| | Species | Spores | Species | Spores | Species | Spores | Species | Spores |
| *Zea mays* | 9 ab* | 378 b | 6 ab | 81 c | 4 a | 30 c | 19 bc | 489 c |
| *Nicotiana tabacum* | 12 a | 360 b | 8 a | 155 a | 4 a | 63 bc | 22 ab | 568 b |
| *Trifolium repens* | 15 a | 410 a | 10 a | 130 b | 5 a | 126 a | 27 a | 666 a |
| *Potentilla anserina* | 8 b | 350 b | 4 b | 72 c | 3 a | 45 c | 12 c | 467 c |

* Data in the same column with same letter are not significantly different at $P$=0.05

**Table 4** Spore incidences (%) of *Acaulospora* and *Glomus* in enrichment pot cultures with different plant species

| Host plant | Coalmine spoil soil | | Island forest soil | | Saline soil | |
|---|---|---|---|---|---|---|
| | *Acaulospora* | *Glomus* | *Acaulospora* | *Glomus* | *Acaulospora* | *Glomus* |
| *Z. mays* | 83.0 a* | 14.5 a | 45.0 b | 52.0 a | 54.0 b | 46.0 a |
| *N. tabacum* | 90.0 a | 8.5 ab | 52.0 ab | 40.0 ab | 56.0 b | 44.0 a |
| *T. repens* | 92.0 a | 7.5 b | 65.0 a | 30.0 b | 55.0 b | 45.0 a |
| *P. anserina* | 80.0 a | 6.0 b | 55.0 a | 43.0 ab | 74.0 a | 26.0 b |

* Data in the same column with same letter are not significantly different at $P$=0.05

**Table 5** Percentage of mycorrhizal colonization of host plants

| Host plants | Coalmine spoil soil | Island forest soil | Saline soil |
|---|---|---|---|
| *Z. mays* | 77.5 a*A | 75.0 a A | 35.0 a B |
| *N. tabacum* | 6.5 c B | 15.5 b A | 5.5 b B |
| *T. repens* | 28.0 b B | 65.0 a A | 12.5 b C |
| *P. anserina* | 7.5 c B | 13.5 b A | 6.5 b B |

* Data in the same column with same letter and in the same line with same capital letter are not significantly different at $P$=0.05

identification methods, such as root colonization pattern, biomass, and morphological characteristics (Brundrett et al. 1996), and molecular techniques (Liu and Li 2000; Smith and Read 1997) should be employed to obtain more complete information about AM fungal diversity.

In ecosystem studies and glasshouse experiments, host plants and soil factors can influence both diversity and overall levels of mycorrhizal formation and sporulation (Brundrett 1991; Brundrett et al. 1999b; Hendrix et al. 1995; Johnson et al. 1992; Kaushal 2000; Loth 1997). For instance, high levels of phosphorus in soil and plant are able to inhibit mycorrhiza formation (Douds and Schenck 1990; Menge et al. 1978) and influence the diversity of AM fungi in field soil (Cuenca and Meneses 1996; Thomson et al. 1992). Due to more suitable conditions for the development of AM fungi in the forest soil used in this investigation (pH 6.3, available phosphorus 19.5 μg $g^{-1}$, organic matter 2.6%) compared to the coalmine spoil (pH 3.9, available phosphorus 2.6 μg $g^{-1}$, organic matter 3.3%) and saline soil (pH 8.5, available phosphorus 3.7 μg $g^{-1}$, organic matter 1.3%), mycorrhizal formation in forest soil was better than in the latter soils.

Variation in spore production could not be explained by mycorrhizal colonization level (Brundrett et al. 1999a). The root length colonized by a single species of AM fungus on a host plant is not necessarily correlated with the spore number produced on the same plant (Gazey et al. 1992). Sporulation may have been further influenced by the presence of other species or by the different soil characteristics. This may explain why there was no correlation between root length colonized and the spore numbers formed in this investigation (data not shown).

The differences in spore numbers produced with different trap plants might contribute to the variation in host plant root type and morphology, carbon biomass, nutrient and endogenous hormone level. These factors might be expected to influence the richness of AM fungi isolated from soil in trap cultures (Brundrett et al. 1999b; Cuenca and Meneses 1996; Stutz and Morton 1996). The different species and spore numbers of AM fungi found on different species of trap plants supports findings elsewhere (Bever et al. 1996; Johnson et al. 1992; Montanes and Monge 1997; Schenck and Kinloch 1980). White clover was the optimal host plant to detect species diversity of AM fungi compared to the other hosts tested. *N. tabacum* was a favorable host for *G. constrictum* and *G. multicaule*. The wide range of effective host plants for detecting different species of AM fungi in field soils needs further testing. The best trap plants may vary in different ecosystems. The host-dependence of the relative growth rates of AM fungal communities may be important in the maintenance of their diversity (Bever et al. 1996).

There is an increasing interest in the importance of diversity in the functioning of mycorrhiza and plant communities. Some experiments have shown that mycorrhizal diversity may play an important role in the origin, evolution, distribution, survival, growth and development of plants. Van der Heijden et al. (1998) concluded that mycorrhizal fungal diversity determined plant biodiversity, ecosystem variability and productivity, while we suggest on the other hand, that mycorrhizal diversity may be dependent on plant diversity in modern natural ecosystems (Wang and Liu 2001; Zhao et al. 2001). So we hypothesize that mycorrhiza and plant diversity both stimulate and retard each other and this needs further research.

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## References

Al-Raddad AM (1993) Distribution of different *Glomus* species in rainfed areas in Jordan. Dirasat-Series B Pure Appl Sci 20:165–182

An Z-Q, Hendrix JW, Hershman DE, Henson GT (1990) Evaluation of the "most probable number (MPN)" and wet-sieving methods for determining soil-borne populations of endogonaceous mycorrhizal fungi. Mycologia 82:576–581

Bever JD, Morton JB, Antonovics J, Schultz PA (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71–82

Biermann B, Linderman RG (1981) Quantifying vesicular arbuscular mycorrhizae: a proposed method towards standardization. New Phytol 87:63–67

Brundrett MC(1991) Mycorrhizas in natural ecosystems. In: Macfayden A, Begon M, Fitter AH (eds) Advances in ecological research, vol 21. Academic Press, London, pp 171–313

Brundrett MC, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra

Brundrett MC, Abbott LK, Jasper DA (1999a) Glomalean mycorrhizal fungi from tropical Australia I. Comparison of the effectiveness and specificity of different isolation procedures. Mycorrhiza 8:305–314

Brundrett MC, Jasper DA, Ashwath N (1999b) Glomalean mycorrhizal fungi from tropical Australia II. The effect of nutrient

levels and host species on the isolation of fungi. Mycorrhiza 8:315–321
Cuenca G, Meneses E (1996) Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. Plant Soil 183:315–322
Douds DD Jr, Schenck NC (1990) Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. New Phytol 116:621–627
Franke-Snyder M, Douds DD Jr, Galvez L, Phillips JG, Wagoner P, Drinkwater L, Morton B (2001) Diversity of communities of arbuscular mycorrhizal (AM) fungi present in conventional versus low-input agricultural sites in eastern Pennsylvania, USA. Appl Soil Ecol 16:35–48
Gazey C, Abbott LK, Robson AD (1992) The rate of development of mycorrhizas affects the onset of sporulation and production of external hyphae by two species of *Acaulospora*. Mycol Res 96:643–650
Genney DR, Hartley SH, Alexander IJ (2001) Arbuscular mycorrhizal colonization increases with host density in a heathland community. New Phytol 152:355–363
Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? Nature 394:431
Hendrix JW, Guo BZ, An ZQ (1995) Divergence of mycorrhizal fungal communities in crop production systems. Plant Soil 170:131–140
Hetrick BAD, Bloom J (1986) The influence of host plant on production ability of vesicular-arbuscular mycorrhizal spores. Mycologia 78:32–36
Johnson NC, Tilman D, Wedin D (1992) Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034–2042
Kaushal S (2000) Influence of edaphic factors on VAMF spore population and root colonization in *Acacia nilotica* in Rajasthan. J Mycol Plant Pathol 30:386–388
Liu RJ, Li XL (2000) Arbuscular mycorrhiza and application (in Chinese). Science Press, Beijing, pp 1–224
Loth FG (1997) Abundance of arbuscular mycorrhizal fungi spores at different native sites as affected by sewage sludge applications (in German). Bodenkultur 47:89–96
McGonigle TP, Fitter AH (1990) Ecological specificity of vesicular-arbuscular mycorrhizal associations. Mycol Res 94:120–122
Menge JA (1984) Inoculum production. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC Press, Boca Raton, Fla. pp 187–203
Menge JA, Steirle DJ, Bagyaraj DJ, Johnson ELV, Leonard RT (1978) Phosphorus concentration in plants responsible for inhibition of mycorrhizal infection. New Phytol 80:575–578
Montanes VJ, Monge LE (1997) Influence of different soil conditions and nitrogen application on arbuscular mycorrhizas with apple trees. Acta Hortic 448:119–124
Morton JB, Bentivenga SP, Wheeler WW (1993) Germplasm in the international collection of vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. Mycotaxon 48:491–528
Morton JB, Bentivenga SP, Bever JD (1995) Discovery measurement and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). Can J Bot 73:25–32
Schenck NC, Kinloch RA (1980) Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia 72:445–456
Schenck NC, Perez Y (1988) Manual for identification of vesicular arbuscular mycorrhizal fungi, 2nd edn. INVAM. University of Florida, Gainesville, Fla.
Sieverding E (1989) Ecology of VAM fungi in tropical agrosystems. Agric Ecosyst Environ 29:369–390
Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, London
Stutz JC, Morton JB (1996) Successive pot cultures reveal high species richness of arbuscular mycorrhizal fungi in arid ecosystems. Can J Bot 74:1883–1889
Thomson BD, Roberson AD, Abbott LK (1992) The effect of long-term application of phosphorus fertilizer on populations of vesicular-arbuscular mycorrhizal fungi in pastures. Aust J Agric Res 43:1131–1142
Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72
Wang FY, Liu RJ (2001) A preliminary survey of arbuscular mycorrhizal fungi in saline-alkaline soil of Yellow River Delta (in Chinese). Biodivers Sci 9:389–392
Watson DMH, Milner PD (1996) Assessment of glomalean species biodiversity as influenced by trapping methods. In: Szaro TM, Bruns TD (eds) Programs and Abstracts of the First International Conference on Mycorrhizae. University of California, Berkeley, Calif. p 125
Zhao ZW, Xia YM, Qin XZ, Li XW, Cheng LZ, Sha T, Wang GH (2001) Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rainforest of Xishuangbanna, Southwest China. Mycorrhiza 11:159–162