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Relevance of mycorrhizal fungal origin and host plant genotype to inducing growth and nutrient uptake in *Medicago* species

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Abstract

This study reports the effects of three selected arbuscular mycorrhizal- (AM-) forming species (*Glomus mosseae*, *Glomus fasciculatum* or *Glomus caledonium*) in comparison with autoctone endophytes by determining changes in plant growth, nutritional and symbiotic parameters in four species of *Medicago*: *M. trunculata*, *M. rigidula*, *M. polimorpha* and *M. rotata*. The relative susceptibility to and dependence upon AM fungi of *Medicago* species was also evaluated. Results showed a high functional compatibility between *Medicago* species and autoctone endophytes, and a specific plant response to individual *Glomus* species. Differences among the *Medicago* species in their reaction to *Glomus* species ranged from negative to highly positive. A combination of host and AM fungal species indicates that a specific compatibility exists among symbionts. Nutrient uptake as a result of AM colonization also indicated a wide degree of responsiveness in each species according to the associated fungal symbiont to the plant. A positive effect on N, P and K, and negative on Ca and Mg nutrition was found in all mycorrhizal treatments, especially with the autoctone endophytes. Mycorrhizal infectivity could not be related to endophyte effectiveness, with generally fewer and larger *Rhizobium* nodules found on the most effective mycorrhizal treatments. Results support the role of indigenous AM endophytes in the efficacy of mycorrhizal symbiosis and the importance of host–endophyte selection to maximize growth and nutrition of *Medicago*. This study shows that AM symbiotic efficiency attributed to *Medicago* is dependent on endophyte association and plant species. The importance of selecting suitable AM fungi is of practical interest for improving the effectiveness of the tripartite symbiosis.

Keywords: Arbuscular mycorrhiza; *Medicago* species; Autoctone endophytes; *Glomus* species

1. Introduction

Medicago species, like all legume plants, are, in general, highly dependent on mycorrhiza to achieve maximum growth (Barea and Azcón-Aguilar, 1983). The P demand for nodulation and effectiveness in the symbiotic system is well known. The dual symbiosis often results in enhanced N_2-fixation and plant growth depending on the degree of compatibility of intersymbionts. Alfalfa plants are responsive to arbuscular mycorrhizal (AM) fungi, but exhibit a range of capabilities of association with a particular *Rhizobium* strain in order to increase N_2 fixation and plant nutrition (Azcón et al., 1991; Ruiz-Lozano and Azcón, 1993, 1994). Little information exists about

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the relevance of plant species on additive or synergistic relationships between the two symbiotic microorganisms. Previous studies on host plants other than *Medicago* showed a significant plant genotype effect relative to the AM fungal susceptibility and compatibility (Azcón and Ocampo, 1981; Krishna et al., 1985; Heckman and Angle, 1987; Estaum et al., 1987; Boyetchko and Tewari, 1995).

Attempts were also made to specify the effect of native endophytes compared with non-autoctone ones. The importance of selecting suitable AM fungi for inoculation studies has been recently highlighted (Khalil et al., 1994). The selection of a proper AM isolate was based on experimental estimation of host response in terms of growth and nutrition. The influence of soil characteristics on the AM symbiotic relationship is well documented. In the present work several species of AM fungi (belonging to the *Glomus* genus) isolated from soils with similar chemical characteristics were used. Considering the problems associated with *Medicago* spp. production in the semi-arid alkaline soils of the Mediterranean area, information regarding the susceptibility of different *Medicago* spp. to AM fungi is very limited but highly important.

This paper reports results of an experiment designed to determine the efficacy of mycorrhiza formed by native AM inoculum compared with different isolates of *Glomus* species, using four *Medicago* species as host plants. The study examines not only the relevance of plant species to the effectiveness of the symbiotic relationship, but also shows the variation in response of *Medicago* spp. to inoculation with *Rhizobium meliloti* and AM fungi from different origins, with regard to plant growth and nutrition, and the extent of symbiotic parameters.

2. Materials and methods

Four *Medicago* species were selected. Treatments used were either non-mycorrhizal or inoculation with each of the fungal species *Glomus mosseae*, *G. fasciculatum*, *G. caledonium* and a native mycorrhizal inoculum giving 20 treatments in all. There were five replications.

Soil for the pot experiment was from the province of Granada, Spain. It was low in organic matter and P content but with high pH and Ca. The main characteristics of this calcareous soil are: pH (H_2O), 7.45; clay (%), 28.00; loam (%), 49.00; sand (%), 32.50; organic matter (%), 1.16; total N (%), 0.10; extractable P (mg kg^{-1}) 6.3; extractable K (mg g^{-1}), 132; extractable Ca (mEq $100g^{-1}$), 43.3; extractable Mg (mEq $100g^{-1}$), 3.82. Soils were sieved (2 mm), diluted with sand (5:2, v:v), steam-sterilized (100°C for 1 h on 3 consecutive days) and then re-inoculated with a soil filtrate containing the specific soil microbial population except for AM propagules. The soil filtrates were obtained by suspending 100 g of the experimental soil in 500 ml sterile water. After shaking and decanting, the suspension was filtered twice (Whatman No. 1). Each pot was given 2 ml of the filtrate.

Medicago rigidula, *M. rotata*, *M. polimorpha* and *M. trunculata* plants were used in the experiments. Five-day-old seedlings were transplanted (4 plants per pot) into pots containing 500 g of the experimental soil–sand mixture. At transplanting, a standard inoculum of *Rhizobium meliloti* (GrBr 4 strain; 1 ml per pot) was applied to all plants.

Medicago species seed obtained from ICARDA (Syria) were either non-inoculated or inoculated with equal amounts of native inoculum or each of the three AM fungal species: *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, *G. fasciculatum* (Taxter sensu Gerd.) Gerd. and Trappe and *G. caledonium* (Nicol. and Gerd.) Trappe and Gerd. This was achieved by placing 5 g per pot of each mycorrhizal inoculum, obtained from a stock culture raised with *Allium cepa*. Mycorrhizal inoculum from each endophyte was multiplied in an open pot culture of *Allium cepa* and after 6 months of plant growth the shoots were eliminated and the undergrown part (mycorrhizal roots plus soil possessing fungal spores and mycelium) maintained by storage for 3–6 months in polyethylene bags at 5°C. Inocula consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and mycorrhizal root fragments. The level of application was saturating and, in a preliminary test, all of them produced a similar level of infectivity with onion plants (*Allium cepa* L.). Native inoculum obtained from roots and rhizospheric soil were taken from the root zone of each *Medicago* species cultivated under natural conditions in an ICARDA field. Samples were screened by the method

Table 1
Shoot dry weights (mg per plant) of *Medicago* species non-inoculated or inoculated with AM fungi (*G. mosseae*, *G. fasciculatum*, *G. caledonium* or native inoculum)

	M. rigidula	*M. rotata*	*M. polimorpha*	*M. trunculata*
Control	600 b	600 b	330 c	550 bc
G. mosseae	450 c	580 b	567 b	540 c
G. fasciculatum	490 c	680 a	640 b	740 a
G. caledonium	450 c	650 b	390 c	620 b
Native AM inoc.	790 a	830 a	900 a	730 a

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range.

of Gerdemann and Nicolson (1963) for spore quantification and staining of roots. The extent of natural colonization and spore population was assessed. Most of the extracted spores from the natural soil samples apparently belonged to the genus *Glomus*. The natural root infection was nearly 50% and 2–4 spores g^{-1} soil were evaluated. Rhizospheric soil and mycorrhizal roots were used to establish pot cultures in sterile soil with *Allium cepa*. Inoculum from native mycorrhizal fungus was obtained, maintained and applied in the same way.

The plants were grown in a controlled greenhouse under a 16-h light (21°C) and 8-h dark (15°C) cycle, with 50% relative humidity and a photosynthetic photon flux density of 700 μmol m^{-2} s^{-1} for the compensating photophase. During the assay the plants were fertilized (10 ml $week^{-1}$ per pot) with a micronutrient (macronutrient-free) solution (Hewitt, 1952). Throughout the experiment, pots were weighed every day and water loss was replaced by top watering to maintain soil moisture close to 100% field capacity during the period of plant growth.

After growth periods of 10 weeks plants were harvested. Shoot and root dry weights were recorded after drying the plant material at about 70°C. Concentrations of P, N, K, Ca and Mg were determined in plant tissues (Lachica et al., 1973).

Nodulation was checked visually, and the percentage of mycorrhizal root length was estimated by a microscopic examination of stained samples (Phillips

Table 2
Nutrient contents (mg per plant) in shoots of *Medicago* species non-inoculated or inoculated with AM fungi (*G. mosseae*, *G. fasciculatum*, *G. caledonium* or native inoculum)

Treatments		Nutrient contents				
		N	P	K	Ca	Mg
Medicago rigidula	Control	6.5 ± 0.5	1.20 ± 0.10	10.5 ± 0.9	27.1 ± 2.0	3.9 ± 0.4
	Glomus mosseae	4.8 ± 0.5	1.26 ± 0.10	7.4 ± 0.8	14.1 ± 0.8	2.3 ± 0.2
	Glomus fasciculatum	5.5 ± 0.3	1.62 ± 0.10	8.5 ± 0.6	13.3 ± 1.0	2.1 ± 0.2
	Glomus caledonium	5.0 ± 0.1	1.19 ± 0.10	7.7 ± 0.3	15.2 ± 0.8	2.1 ± 0.1
	AM native	23.5 ± 1.5	2.37 ± 0.10	17.1 ± 0.8	21.4 ± 1.5	3.4 ± 0.2
Medicago rotata	Control	10.7 ± 1.6	1.02 ± 0.10	12.9 ± 1.3	26.5 ± 2.5	2.2 ± 2.2
	Glomus mosseae	13.1 ± 1.3	1.34 ± 0.10	11.3 ± 1.3	16.1 ± 1.5	1.6 ± 1.8
	Glomus fasciculatum	16.3 ± 1.4	1.22 ± 0.05	14.5 ± 1.0	17.5 ± 1.1	1.4 ± 1.3
	Glomus caledonium	14.3 ± 0.9	1.17 ± 0.10	12.0 ± 0.9	17.2 ± 1.1	1.8 ± 0.6
	AM native	20.3 ± 0.8	1.83 ± 0.10	13.9 ± 1.1	20.3 ± 1.2	1.5 ± 0.1
Medicago polimorpha	Control	5.0 ± 0.4	0.66 ± 0.06	8.2 ± 1.1	10.9 ± 0.7	1.6 ± 1.8
	Glomus mosseae	15.8 ± 1.5	1.98 ± 0.07	16.5 ± 1.2	15.1 ± 1.4	2.0 ± 0.2
	Glomus fasciculatum	16.5 ± 1.4	1.73 ± 0.05	16.7 ± 1.1	12.1 ± 1.0	2.0 ± 0.1
	Glomus caledonium	9.5 ± 1.2	1.09 ± 0.05	11.3 ± 1.7	9.0 ± 1.0	1.4 ± 0.1
	AM native	24.7 ± 2.2	2.25 ± 0.24	18.0 ± 1.4	14.76 ± 1.2	2.2 ± 0.1
Medicago trunculata	Control	8.0 ± 0.9	1.10 ± 0.07	15.1 ± 1.3	15.9 ± 1.1	2.4 ± 0.6
	Glomus mosseae	11.8 ± 0.8	1.94 ± 0.11	16.2 ± 0.8	12.4 ± 0.9	1.9 ± 0.1
	Glomus fasciculatum	19.2 ± 0.12	1.92 ± 0.20	19.7 ± 1.7	16.5 ± 0.8	2.4 ± 0.1
	Glomus caledonium	16.0 ± 1.6	1.30 ± 0.08	17.0 ± 1.1	15.5 ± 1.1	2.3 ± 0.1
	AM native	19.1 ± 1.5	2.12 ± 0.08	17.2 ± 1.3	17.2 ± 1.2	2.3 ± 0.1

Standard errors of the mean are given ($P = 0.05$).

and Hayman, 1970), using the grid-line intersect method of Giovannetti and Mosse (1980).

Data were subjected to an analysis of variance. When the main effects were significant ($P < 0.05$) differences between means were evaluated for significance using Duncan's multiple range test.

3. Results

Responses of *Medicago* species to each AM endophyte were different. The three *Glomus* spp. or native inoculum clearly caused significant differences in shoot weight within each *Medicago* species (Table 1). *M. trunculata* plus *G. mosseae* did not enhance the yield but *G. fasciculatum* increased plant growth by 34% compared with non-inoculated plants. In contrast, all three isolates of *Glomus* reduced the yield of *M. rigidula*. *M. rotata* was unaffected by mycorrhizal colonization except for the native endophytes. *M. polimorpha* biomass was much increased when the root system was infected by *G. mosseae* or *G. fasciculatum*. The remarkable result is the high plant–fungus compatibility between all four *Medicago* species and the native endophytes, especially with *M. polimorpha* where there was a 273% increase in plant growth compared with non-mycorrhizal plants (Table 1).

Uptake of N, P and K by *Medicago* spp. was increased with AM symbiosis and was maximized with native fungal inoculation (Table 2). *Medicago polimorpha* responded most with N, P and K contents increased by 495%, 341% and 219%, respectively, in comparison with non-mycorrhizal plants. N was increased to a much lesser extent by isolates of *Glomus* species, except in *M. rigidula*; N and P shoot content was enhanced in AM plants even when there was no increase in growth.

In contrast Ca and Mg shoot contents diminished in all mycorrhizal plants (Table 2) and this was especially the case with the native inoculum.

Although the effectiveness of mycorrhizal inoculation for improving plant growth and nutrition varied with host plant species and/or fungal isolates, the level of infectivity of AM fungi tested in this experiment remained similar irrespective of the plant species or endophyte involved (Table 3). Thus the enhancement of plant growth was not correlated with the level of mycorrhizal infection. AM colonization generally did not affect root growth (Table 4).

The number of nodules formed was influenced by the fungal symbiont. Non-inoculated plants formed abundant but small nodules. Generally, fewer and larger nodules were formed in the bigger mycorrhizal colonizations than with the lesser ones.

Table 4
Root fresh weights (g per plant) of *Medicago* species non-inoculated or inoculated with AM fungi (*G. mosseae*, *G. fasciculatum*, *G. caledonium* or native inoculum)

	M. rigidula	*M. rotata*	*M. polimorpha*	*M. trunculata*
Control	3.91 a	3.16 a	2.60 a	3.47 a
G. mosseae	2.80 b	2.50 b	2.27 a	2.85 b
G. fasciculatum	2.91 b	2.52 b	2.26 a	2.94 b
G. caledonium	2.88 b	2.27 b	2.71 a	2.64 b
Native AM inoc.	3.51 a	3.04 ab	2.64 a	2.65 b

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple-range.

Table 3
Formation of symbiotic structures (nodule number and mycorrhizal infection %) in *Medicago* species non-inoculated or inoculated with AM fungi (*G. mosseae*, *G. fasciculatum*, *G. caledonium* or native inoculum)

Medicago var.	Control		*G. mosseae*		*G. fasciculatum*		*G. caledonium*		Native AM inoculum	
	Nod. no.	AM%	Nod. no.	AM%	Nod. no.	AM%	Nod. no.	AM%	Nod. no.	AM%
M. rigidula	70_S	–	65_M	45	40_S	50	55_M	55	30_{M-L}	60
M. rotata	80_S	–	40_{M-S}	55	20_{M-S}	50	30_S	45	70_L	56
M. polimorpha	–	–	5_L	77	10_L	87	3_L	36	30_L	64
M. trunculata	85_M	–	50_M	87	20_L	80	10_L	75	5_L	80

Subscripts S, M and L indicate small, medium and large size of nodules, respectively.

Tables 3 and 4 show a lack of relationship between nodule formation and plant nutrient uptake: the best nodulated *Medicago* host–fungal combination did not achieve the highest N content.

4. Discussion

The results show large differences in the degree of functional compatibility between the *Medicago* species and the *Glomus* isolates involved in the symbiosis. The preference of the *Medicago* species for native endophytes indicates a degree of compatibility between host species and fungi, the native fungi being the most adapted to survive and function in the root environment of these plant species (Roldan-Fajardo, 1994). The physiological compatibility is manifested by the ability of both symbionts to contribute to the nutrition of the association (Smith and Gianinazzi-Pearson, 1988). The functional compatibility was tested as ability to improve plant nutrition and growth.

The effectiveness of AM infection appears to be improved P status of the plants as a consequence of the hydrocarbon requirement of the fungal activity. These related mechanisms would be a possible basis for differences observed in response to mycorrhizal infection.

In the present experiment the colonization rate was not sufficient to indicate a specific symbiotic interaction, because no relationship seemed to exist between colonization and growth response. Specific mechanisms conferring functional differences could be expected from variations in the characteristics of external hyphae like length, distribution and nutrient translocation. The study of Graham et al. (1982) demonstrated that the spread of external hyphae from roots colonized by AM fungi varies greatly with the fungal isolate and Sylvia (1988) showed that hyphal spread of arbuscular fungi is an important factor influencing the fungal nutrient supply to the host plant. Differences found can also be attributed to functional variation at the level of the plant species–fungus interface.

Graham et al. (1982) and Azcón et al. (1991) showed that mycorrhizal fungi differ in their ability to enhance nutrient uptake and growth of the host species even when the extent of the AM root colonization was similar. The functional compatibility found between symbiont partners is less well documented than that between AM fungi and soil fertility or agrochemical amendments.

The reported experiment shows that it is not possible to generalize on interactions between symbionts because each partner needs a particular study. The results highlight the different effects found between isolates of AM fungi on host plants at the species level. Mycorrhizal responses in the four *Medicago* species were always higher in the presence of autoctone endophytes, which indicated a host–native fungus adaptation. The symbiont adaptations may result in an early mycorrhizal formation which is critical for the benefit of the association. Nevertheless, native mycosymbionts adapted to survive under given environmental conditions normally exhibit low efficiencies in terms of host growth and nutrition increases (Owusu-Benneah and Mosse, 1979; Powell et al., 1980; Powell, 1981). Hence, frequent studies are conducted with regard to the success of particular host–fungi inoculations.

Alterations to the balance of nutrients (Nielsen and Jensen, 1983) can affect biomass production. In the present study mycorrhizal fungi increased N, P and K, but decreased Ca and Mg in *Medicago* shoots. It is noteworthy that the mycorrhizal fungi lowers the presence of cations in plants (Hale and Orcutt, 1987; Azcón et al., 1991; Azcón and Barea, 1992; Khalil et al., 1994). The number of nodules did not correlate well with the plant nitrogen content because the few nodules which are produced in *M. rigidula* and *M. trunculata* induced the highest N acquisition by the plants. With *M. polimorpha*, the high extent of nodulation improved the total N content. Size of nodules seems to be correlated more to the degree of effectiveness. *Glomus* isolates–*Rhizobium* combinations did not always result in improved nitrogen fixation as with *M. rigidula*. Mycorrhizal fungi already present in a specific locality are obvious candidates for reintroduction because of their adaptation to local edaphic and climatic conditions. Intraspecific variations in the ability of AM fungi to induce growth and nutrient uptake are related to local adaptation. Boerner (1990) also found that efficiency of N and P uptake were the greatest in plants given inocula from low fertility soil. Results from this study also show that efficacy of mycor-

rhizal association is closely related to host plant and the associated fungal isolate.

According to Smith et al. (1994) and Khalil et al. (1994) the identification of efficient species of both fungus and host and understanding the way they function is crucial in processes involved in uptake and use of nutrients.

In agreement with these considerations, the selection of a suitable fungus for inoculation purposes needs to be based on aspects such as competitive ability and adaptation to survive in the particular plant and soil environment. Populations of AM fungi from dissimilar environments are of different ecotypes with high physiological diversity. Considering the results from this study, future attempts to predict the behaviour of AM symbiosis under specific soil conditions should include study of fungal–host interaction to determine the symbiotic efficiency.

In conclusion, *Medicago* species must posses compatible AM fungi to maximize growth and nutrition. In this study the most effective inocula were formed by native endophytes. Compatibility of the plant–fungus components is critical.

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