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Compatibility of a wild type and its genetically modified *Sinorhizobium* strain with two mycorrhizal fungi on *Medicago* species as affected by drought stress

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Abstract

The effect of double inoculation with two strains of *Sinorhizobium meliloti* [the wild type (WT) strain GR4 and its genetically modified (GM) derivative GR4(pCK3)], and two species of arbuscular mycorrhizal (AM) fungi (*Glomus deserticola* and *Glomus intraradices*) was examined in a microcosm system on three species of *Medicago* (*M. nolana*, *M. rigidula*, *M. rotata*). Two water regimes (80 and 100% water holding capacity, WHC) were assayed. The efficiency of each AM fungus increasing plant growth, nutrient content, nodulation and water-stress tolerance was related to the *Sinorhizobium* strains and *Medicago* species. This indicates selective and specific compatibilities between microsymbionts and the common host plant. Differential effects of the mycorrhizal isolates were not associated with their colonizing ability. Nodulation and mycorrhizal dependency (MD) changed in each plant genotype in accordance with the *Sinorhizobium* strain and AM fungi involved. Generally, *Medicago* sp. MD decreased under water-stress conditions even when these conditions did not affect AM colonization (%). Proline accumulation in non-mycorrhizal plant leaves was increased by water stress, except in *M. rotata* plants. Differences in proline accumulation in AM-colonized plants suggest that both the AM fungus and the *Sinorhizobium* strain were able to induce different degrees of osmotic adjustment. Mycorrhizal plants nodulated by the WT strain accumulated more proline in *M. rigidula* and *M. rotata* under water stress than non-mycorrhizal plants. Conversely, mycorrhizal plants nodulated by the GM strain accumulated less proline in response to both AM colonization and drought. These results indicated changes in the synthesis of this nitrogenous osmoregulator product associated with microbial inoculation and drought tolerance. Mycorrhizal plants nodulated by the GM *Sinorhizobium* strain seem to suffer less from the detrimental effect of water stress, since under water limitation relative plant growth, percentage of AM colonization, root dry weight and the highest R/S ratio remained the same. The fact that GM nodulated plants are better adapted to drought stress could be of practical interest and the management of GM microorganism inoculation may be crucial for biotechnological approaches to improving crop yield in dry environments.

Keywords: Arbuscular mycorrhizal fungi; *Sinorhizobium*; Genetically modified organisms; Drought stress

1. Introduction

Legumes provide an important source of protein for people and livestock throughout the world. In Mediterranean areas legume plants are often exposed to drought stress, which decreases the efficiency of nodule formation and/or function, as well as plant productivity. It has been demonstrated that *Rhizobium* and arbuscular mycorrhizal (AM) fungi double symbiosis enhances the growth and yield of many legumes [1]. Mycorrhizal hyphae have access to a greater volume of soil and can absorb and translocate fairly large amounts of low-diffusing nutrients to their host plants. One of the benefits of AM fungi is

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that they improve the ability of legumes to obtain P from soil, especially under P-limiting conditions, since P is an important limiting factor for nodulation, N_2 fixation and legume production. Certain ecological factors such as water stress reduce nutrient diffusion, uptake by roots and transport from roots to shoots, because of restricted transpiration rates, impaired active transport and membrane permeability. This leads to a reduced root absorbing power in crop plants [2]. It has been proposed that mycorrhizal associations increase drought resistance by means of several mechanisms. Previous studies have reported that mycorrhizal symbiosis not only influences plant P nutrition [3] in response to drought stress, but also improves physiological values and increases proline accumulation [4]. It also has been suggested that free proline acts as an osmoregulator [5].

Plant growth responses to AM fungi are influenced by factors such as soil, endophyte and host plant as well as by the compatibility of interactions between them [6]. This relationship is regulated tightly at both the structural and physiological levels. In spite of the lack of specificity, particular associations can occur, thus resulting in considerable variations in symbiotic responses [7–9]. Previous studies [10–12] have demonstrated that specific functional compatibilities between endophytes change when partners are genetically modified. Although improved genetically modified (GM) *Sinorhizobium* greatly enhances mycorrhiza performance [10–12], less information exits concerning how the strain behaves under stress conditions and how it interacts with AM symbiosis under such conditions.

The objective of this study was to compare the effect of *Sinorhizobium meliloti* strains (WT vs GM) in interaction with AM fungi on the development of three *Medicago* species, under both well-watered and drought stress conditions. Two AM fungal species well adapted to drought [4] were selected. Previously, responses of these rhizobial strains were determined in *Medicago sativa* [10,11] and *Medicago arborea* [13] under well-watered conditions. Thus, this study is of interest because it uses three other *Medicago* species to evaluate responses linked to the host plant genotype under both well-watered and drought stress conditions.

2. Materials and methods

2.1. Experimental design

Two parallel experiments were carried out in a microcosm system. In each one of them, the wild type (WT) *S. meliloti* GR4 strain or its GM derivative GR4(pCK3) strain were tested. The experiments were set up as a complete randomized block design with two factors. One factor, AM treatments, contained three levels, two AM fungal inoculated treatments (*Glomus deserticola* or *Glomus intraradices*) and one non-mycorrhizal treatment. The second factor, water stress, contained two levels, i.e. 80 or 100% of the water holding capacity (WHC) of the test soil. The 12 treatments were replicated five times to give a total of 60 completely randomly arranged pots per *Medicago* species assayed (*M. nolana*, *M. rigidula*, *M. rotata*).

2.2. Test soil

The test soil collected from Granada province (Spain) was a 'reddish-brown calcareous' type (42.0% clay, 39.8% loam, 18.2% sand) at pH 7.4; 1.23% organic matter; 4.5 mg P kg^{-1} soil extractable with 0.5 M $NaHCO_3^-$ (Olsen P). The soil was sieved (4 mm pore size), diluted with quartz sand (5/2, v/v) and steam sterilized (100°C for 1 h on three consecutive days) and then reinoculated with a soil filtrate containing the normal microbiota without AM propagules. Pots were filled with 500 g of sterilized soil/sand mixture.

2.3. Host plant and inoculation treatments

Three species of *Medicago* (*M. nolana*, *M. rigidula*, *M. rotata*) were used as test plants. Surface sterilized seeds were sown in the pasteurized soil/sand mixture. The mycorrhizal strains used were: *G. deserticola* (Trappe, Bloss and Menge) and *G. intraradices* (Schenk and Smith). Mycorrhizal inoculation was carried out by addition of 10 g per pot of a mycorrhizal inoculum obtained from our stock culture collection and maintained for 3–6 months in polyethylene bags at 4°C. This consisted of thoroughly mixed rhizosphere

samples containing spores, hyphae and mycorrhizal root fragments. The *S. meliloti* strains used were GR4 (WT strain) and its GM derivative (GM strain) GR4(pCK3) containing the plasmid pCK3, which carries the *Klebsiella pneumoniae nif*A gene, constitutively expressed from a kanamycin gene promoter [14]. This genetic modification has been shown to enhance *S. meliloti* nodulation competitiveness [14]. The inocula of *Sinorhizobium* were grown in TY medium (tryptone 0.5%, $CaCl_2$ 0.05%, yeast extract 0.3%) and applied 1 ml per pot (containing 10^8 cfu ml^{-1}) on the roots of both mycorrhizal and non-mycorrhizal plants.

2.4. Growth conditions

Plants were grown for 60 days under greenhouse conditions with temperature ranging from 19 to 25°C, a 16/8 light/dark photoperiod and a relative humidity of 70–90%. A photosynthetic flux density of 400–700 μmol m^{-1} s^{-1} was applied as supplementary light when necessary.

The water regimes were applied by weighing each pot and adding water to the weight calculated for the desired water regime, i.e. 80 and 100% WHC. Throughout the experiment, the pots were weighed once a day and water loss replaced by top watering.

2.5. Measurements

After harvest, the weights of shoots and roots were recorded and the shoot tissues were analyzed for N and P [15]. The extent of root colonization by the mycorrhizal fungus was assessed by the staining method of Phillips and Hayman [16]. The percentage of mycorrhizal root length was calculated by a gridline intersect technique [17]. Mycorrhizal dependency (MD), or response to mycorrhizal colonization, was calculated by using the following formula [18]:

$$\mathrm{MD} = \frac{(\text{dry wt}_{\mathrm{AM}}) - (\text{dry wt}_{\text{non-AM}})}{\text{dry wt}_{\mathrm{AM}}} \times 100$$

where dry wt_{AM} and dry $wt_{non\text{-}AM}$ are the dry weights of mycorrhizal (AM) or non-mycorrhizal plants (non-AM).

The number of nodules was determined visually on carefully washed roots. Proline content was determined by colorimetry [19].

The results were analyzed according to a two-way analysis of variance (ANOVA). Significance was detected according to least-significance difference (LSD) and Fisher's protected LSD tests. In the case of percentage values these are represented after arcsin square-root (%/100) transformation.

3. Results

3.1. Plant growth, mycorrhizal dependency and nutrient content

Colonization by the AM fungi *G. deserticola* or *G. intraradices* highly increased shoot and root growth and plant nutrition under both non-stress and water-stress conditions (Figs. 1 and 2).

The effect of water limitation was greater in *M. rigidula* inoculated with the WT *Sinorhizobium* strain (Fig. 1E and F). Mycorrhizal *Medicago* plants grown under drought stress had equal [inoculated with the WT strain (Fig. 1A, B, E, F, I and J)] or higher weights [when the GM strain was inoculated (Fig. 2A, B, E, F, I and J)] than those corresponding to non-mycorrhizal plants grown under well-watered conditions. Such growth responses were related more closely to the rhizobial strain than to the colonizing AM fungi.

Although it can be established that the effectiveness of both AM fungi in increasing plant growth was generally quite similar, there were two exceptions. For *M. nolana*, *G. intraradices* was more effective than *G. deserticola* when coinoculated with the GM strain under drought stress conditions (Fig. 2A and B). In *M. rotata*, *G. deserticola* was more effective than *G. intraradices* when coinoculated with the WT strain under well-watered conditions (Fig. 1I and J). These results indicate a specific influence of the *Sinorhizobium* strain which depends on both plant species and water regime.

R/S (root/shoot) ratio was higher in mycorrhizal plants nodulated by the GM strain under drought stress. However, the opposite effect was found for the WT strain under the same stress conditions (Tables 1 and 2).

MD was lower under drought stress than under well-watered conditions (Tables 1 and 2). In some instances (*M. rigidula* and *M. rotata*), it was higher when plants were inoculated with the GM strain (Tables 1 and 2).

Shoot N and P content in *Medicago* plants displayed similar trends to those described for

Table 1
Effect of *Sinorhizobium meliloti* GR4 (WT) and mycorrhizal inoculation on root/shoot ratio, shoot N/P ratio and mycorrhizal dependency (MD) in three species of *Medicago* (*M. nolana*, *M. rigidula* and *M. rotata*), under non-limited (100% WHC) and limited (80% WHC) water supply[a]

Water regime	AMF[b]	Root/shoot ratio			N/P ratio		Mycorrhizal dependency			
		M. nolana	*M. rigidula*	*M. rotata*	*M. nolana*	*M. rigidula*	*M. rotata*	*M. nolana*	*M. rigidula*	*M. rotata*
100% WHC	C	1.07b	0.42a	0.41a	31.96b	20.06b	44.74b			
	D	0.58a	0.42a	0.38a	13.93a	6.63a	9.05a	59.60a	40.51a	58.26b
	I	0.58a	0.42a	0.37a	14.14a	9.33a	11.76a	59.69a	36.81a	50.89a
80% WHC	C	1.09z	0.34x	0.68y	43.80y	30.52y	57.51y			
	D	0.59y	0.50x	0.44x	23.96x	25.13x	22.75x	52.71x	43.19x	39.99x
	I	0.48x	0.52x	0.47x	22.47x	24.16x	26.05x	55.94x	45.76x	39.60x
	LSD	0.13	0.13	0.18	5.50	3.00	11.90	7.68	8.07	7.61

[a] Data are given on a per plant (i.e. on a per pot unit) basis. Different letters within a column indicate significant differences ($P \leq 0.05$; Fisher's protected LSD test) at one water level.

[b] C, non-mycorrhizal control; D, *G. deserticola*; I, *G. intraradices*; LSD, least-significance difference ($P \leq 0.05$).

Table 2
Effect of *Sinorhizobium meliloti* GR4(pCK3) (GM) and mycorrhizal inoculation on root/shoot ratio, shoot N/P ratio and mycorrhizal dependency (MD) in three species of *Medicago* (*M. nolana*, *M. rigidula* and *M. rotata*), under non-limited (100% WHC) and limited (80% WHC) water supply[a]

Water regime	AMF[b]	Root/shoot ratio			N/P ratio			Mycorrhizal Dependency		
		M. nolana	*M. rigidula*	*M. rotata*	*M. nolana*	*M. rigidula*	*M. rotata*	*M. nolana*	*M. rigidula*	*M. rotata*
100% WHC	C	0.53a	0.46a	0.36a	85.06b	25.52b	95.16b			
	D	0.67b	0.55a	0.52b	9.83a	7.43a	13.24a	60.26a	64.26a	59.65a
	I	0.63b	0.55a	0.60b	11.88a	10.20a	14.48a	62.16b	63.20a	60.70a
80% WHC	C	0.55x	0.42x	0.42x	99.57z	59.29y	82.35y			
	D	0.90y	0.94y	0.68y	14.48x	21.68x	16.44x	58.73x	54.04x	56.62x
	I	0.83y	0.82y	0.63y	34.04y	13.22x	16.35x	64.16y	56.43x	52.94x
	LSD	0.09	0.13	0.13	13.66	15.30	6.67	2.97	3.77	7.77

[a] Data are given on a per plant (i.e. on a per pot unit) basis. Different letters within a column indicate significant differences ($P \leq 0.05$; Fisher's protected LSD test) at one water level.

[b] C, non-mycorrhizal control; D, *G. deserticola*; I, *G. intraradices*;LSD, least-significance difference ($P \leq 0.05$).

plant growth (Figs. 1C, G, K, 2C, G, K and 1D, H, L, 2D, H, L). Nitrogen uptake was increased by mycorrhizal colonization for all soil-water conditions and *Medicago* species; similar effects were found for both AM fungi, except in *M. nolana* under stress conditions, where *G. intraradices* was most effective when coinoculated with the GM strain (Fig. 2C). The mycorrhizal effect on P acquisition was greatest at 100% WHC for all plants regardless of which *Sinorhizobium* strain was inoculated.

Shoot N/P ratios greatly decreased as a result of mycorrhizal colonization (Tables 1 and 2). *G. deserticola* and *G. intraradices* similarly decreased the N/P ratio under both well-watered and drought stress conditions. The highest N/P ratios were found under stress conditions (Tables 1 and 2).

3.2. Mycorrhizal colonization

Significant differences were found between the length of roots colonized by *G. deserticola* or *G. intraradices* depending on water level, host plant and the nodulating *Sinorhizobium* strain.

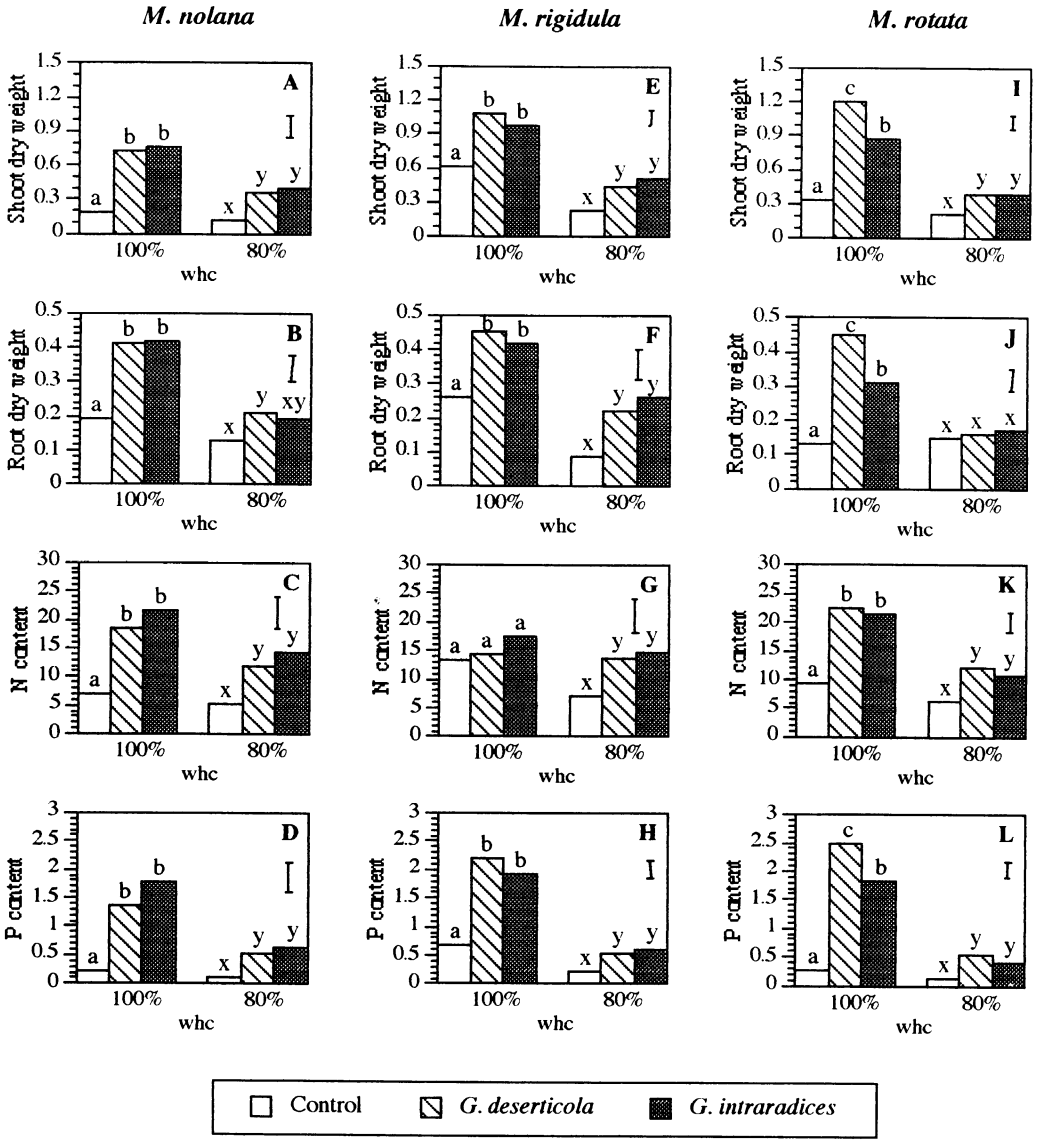

Fig. 1. Effect of *Sinorhizobium meliloti* GR4 (WT strain) and mycorrhizal inoculation on plant growth: (A, E, I) shoot dry weight (g plant^{-1}), (B, F, J) root dry weight (g plant^{-1}); and plant nutrition: (C, G, K) N content (mg plant^{-1}), (D, H, L) P content (mg plant^{-1}), in three species of *Medicago* (*M. nolana*, *M. rigidula* and *M. rotata)*, under non-limited (100% WHC) and limited (80% WHC) water supply. Vertical bar represents LSD ($P \leq 0.05$). Different letters indicate significant differences ($P \leq 0.05$; Fisher's protected LSD test) at one water level.

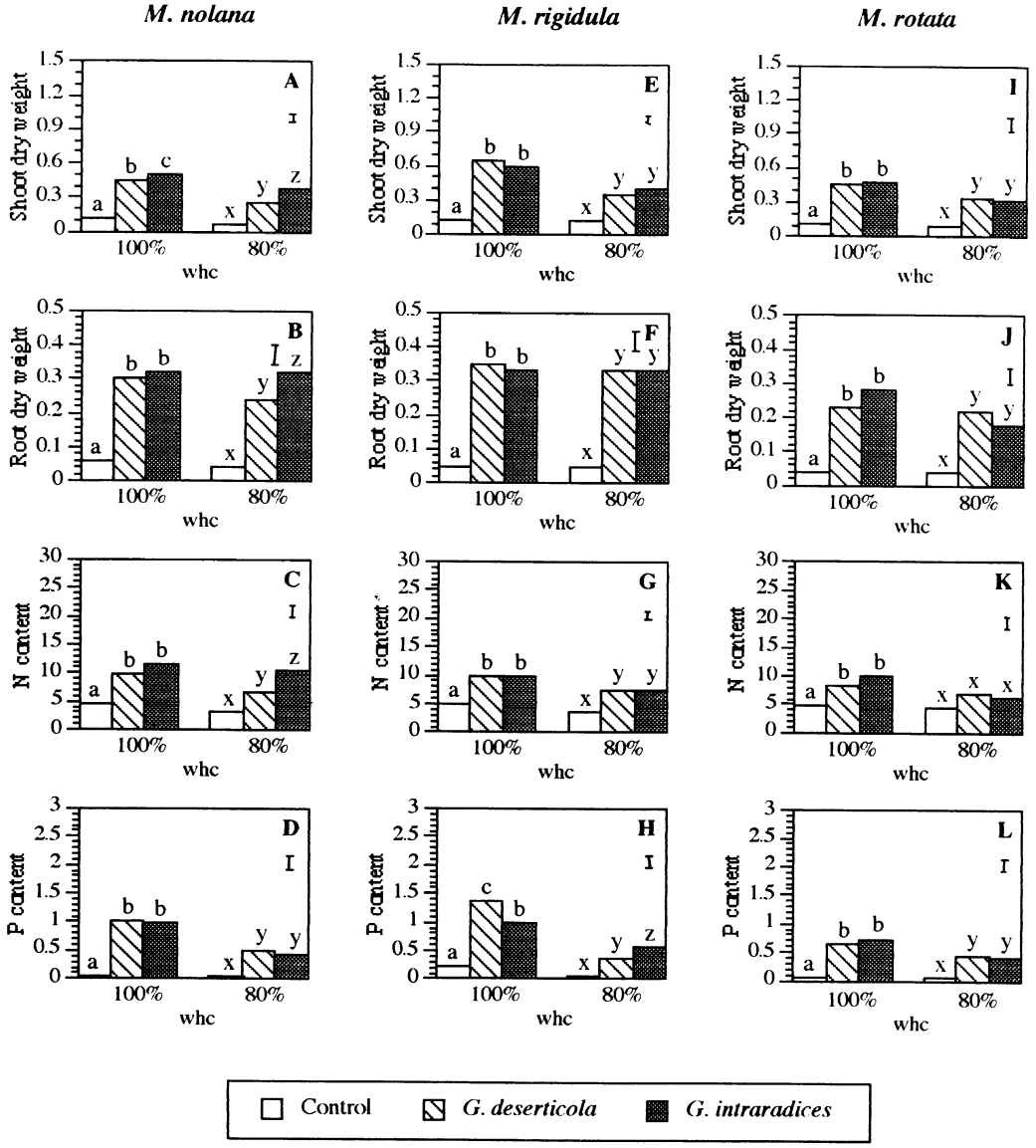

Fig. 2. Effect of *Sinorhizobium meliloti* GR4(pCK3) (GM strain) and mycorrhizal inoculation on plant growth: (A, E, I) shoot dry weight (g plant^{-1}), (B, F, J) root dry weight (g plant^{-1}); and plant nutrition: (C, G, K) N content (mg plant^{-1}), (D, H, L) P content (mg plant^{-1}), in three species of *Medicago* (*M. nolana*, *M. rigidula* and *M. rotata*), under non-limited (100% WHC) and limited (80% WHC) water supply. Vertical bar represents LSD ($P \leq 0.05$). Different letters indicate significant differences ($P \leq 0.05$; Fisher's protected LSD test) at one water level.

In general, the percentage of mycorrhizal colonization in plants nodulated by the WT strain decreased under drought stress (Fig. 3A, D and G). This effect was more significant for *G. deserticola* colonizing *M. nolana* roots (Fig. 3A) and for *G. intraradices* colonizing both *M. rigidula* and *M. rotata* roots (Fig. 3D and G). In contrast, when mycorrhizal plants were nodulated by the GM strain, the percentage of mycorrhization by both AM fungi increased under drought stress in all three *Medicago* species (Fig. 4A, D and G). This effect was most significant for *G. deserticola* colonizing both *M. nolana* and *M. rotata* roots (Fig. 4A and G).

3.3. Nodulation

The number of nodules formed on roots was largely increased by mycorrhizal colonization under both water conditions. This effect was particularly relevant for mycorrhizal plants grown under drought stress where nodulation was even higher than it was in non-mycorrhizal control plants grown under well-watered conditions (Figs. 3B, E,

H and 4B, E, H). In fact, drought significantly reduced nodulation falling to zero in most non-mycorrhizal plants. In mycorrhizal plants, reduction of nodule formation under water stress was more striking in inoculation with the WT strain (Fig. 3B, E and H); maximum reduction was found in *M. rotata* colonized by *G. deserticola* where no nodules were formed under water limitation (Fig. 3H).

In general, *G. deserticola* increased nodulation to the same or to a greater extent than *G. intraradices* under well-watered conditions (Figs. 3 and 4). Under drought stress conditions, however, *G. deserticola* enhanced only the number of nodules formed in *M. rigidula* and *M. rotata* nodulated by the GM strain (Fig. 4E and H). The GM strain induced fewer nodules per plant (Fig. 4) than the WT strain (Fig. 3) on all three of the *Medicago* species.

3.4. Shoot proline content

In general, shoot proline content increased during drought stress. The effect of mycorrhizal fungi on proline accumulation varied according to the *Sinorhizobium* strain involved. Under drought stress conditions WT *Sinorhizobium*-nodulated mycorrhizal plants accumulated a similar or higher proline content in shoot than non-mycorrhizal control plants (Fig. 3C, F, and I). In *M. rigidula* nodulated by the WT strain under drought stress, *G. intraradices* colonization maximized proline content (Fig. 3F). However, in *M. rotata* less proline was synthesized in *G. intraradices* than in *G. deserticola* colonized plants (Fig. 3I). Mycorrhizal plants nodulated by the GM strain accumulated less proline in shoot than non-mycorrhizal control plants under both well-watered and drought stress situations. No differences between colonizing fungal species were

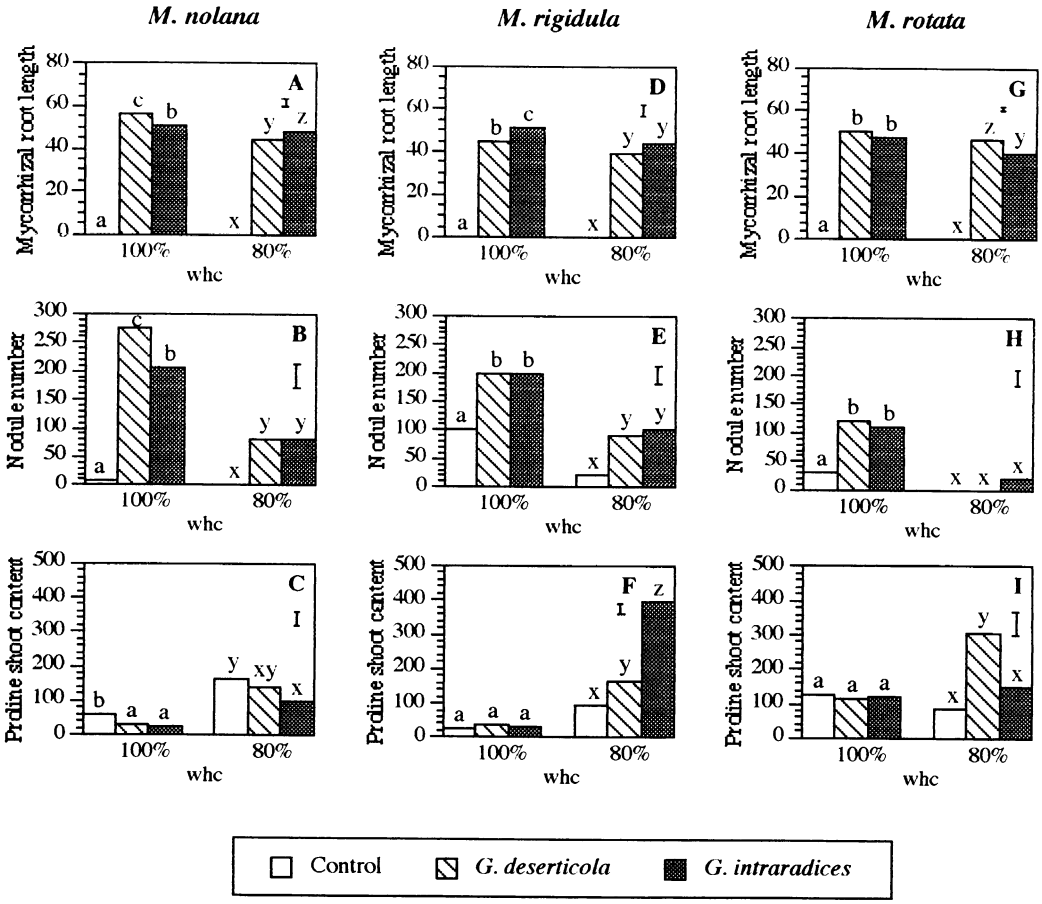

Fig. 3. Effect of *Sinorthizobium meliloti* GR4 (WT strain) and mycorrhizal inoculation on: (A, D, G) mycorrhizal root length ($\arcsin(\sqrt{x/100})$), (B, E, H) nodule number per plant, and (C, F, I) proline shoot content (nmol g^{-1}) in three species of *Medicago* (*M. nolana*, *M. rigidula and M. rotata*), under non-limited (100% WHC) water supply. Vertical bar represents LSD ($P \leq 0.05$). Different letters indicate significant differences ($P \leq 0.05$; Fisher's protected LSD test) at one water level.

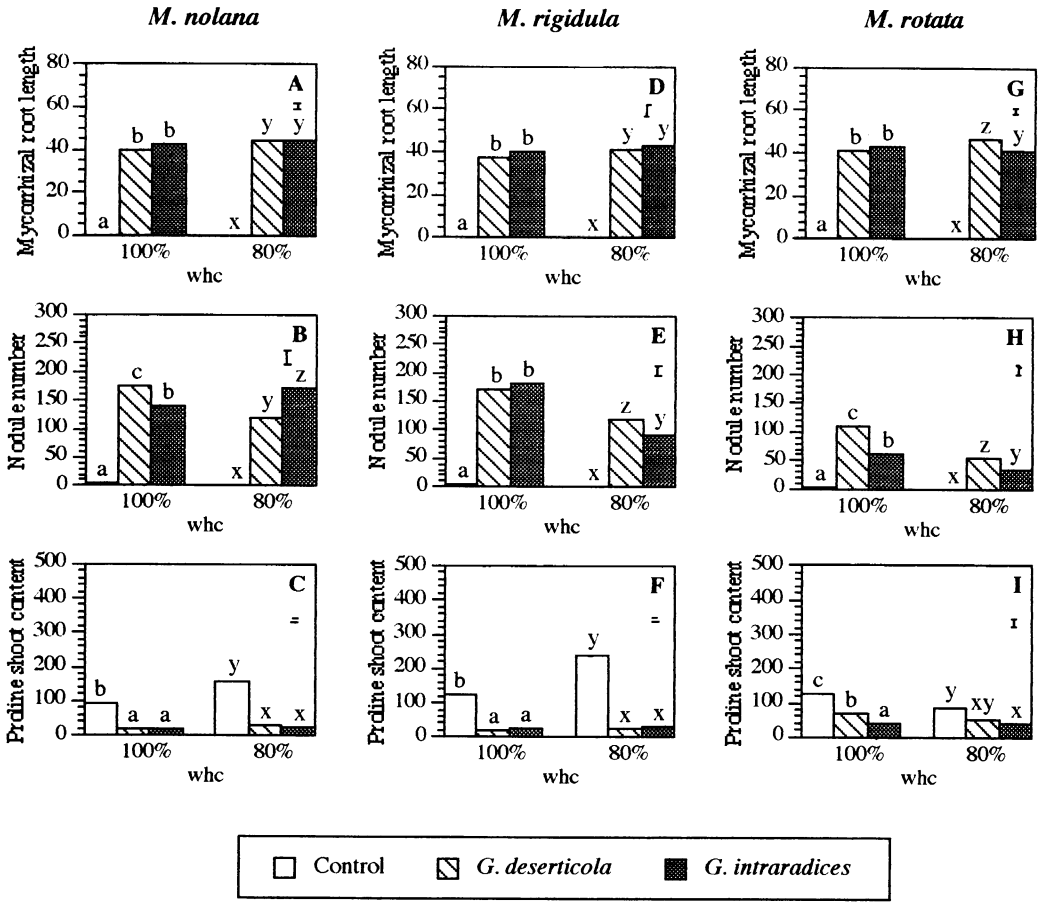

Fig. 4. Effect of *Sinorhizobium meliloti* GR4(pCK3) (GM strain) and mycorrhizal inoculation on: (A, D, G) mycorrhizal root length (arcsin($\sqrt{x/100}$)), (B, E, H) nodule number per plant, and (C, F, I) proline shoot content (nmol g^{-1}), in three species of *Medicago* (*M. nolana*, *M. rigidula* and *M. rotata*), under non-limited (100% WHC) and limited (80% WHC) water supply. Vertical bar represents LSD ($P \leq 0.05$). Different letters indicate significant differences ($P \leq 0.05$; Fisher's protected LSD test) at one water level.

found when plants were nodulated by the GM strain (Fig. 4C, F and I).

4. Discussion

In this study it is shown that *S. meliloti* (WT vs GM) influenced AM symbiosis, thus ameliorating drought stress tolerance in three different *Medicago* species. Since the two experiments for testing the abilities of both *Sinorhizobium* strains were not carried out simultaneously, the results are not comparable from a quantitative point of view, but they do allow us to make some interesting suggestions.

As the results indicate, water limitation affects both growth and nutrient uptake. The decline in soil moisture results in a decrease in the diffusion rate of nutrients (particularly those slowly diffusing such as P) from the soil matrix to the absorbing root surface [20]. Effects of AM fungi on plant water status have been associated with improved host nutrition, particularly P nutrition. Extraradical AM fungal mycelia extend root surface area and enhance the acquisition of nutrients and water by the roots [21,22]. However, this AM activity does not seem to be the principal cause of water-stress tolerance in *Medicago* plants. It has also been reported that the effect of AM fungi on drought resistance may not depend only on P uptake [21,23,24]. The present results show that mycorrhizal plant response to water stress is not unilateral and that there are different strategies for preventing possible drought-produced injuries, which depend on the microbial group (*Sinorhizobium* strain) involved.

In general, solute accumulation induced by water deficit, such as proline, should act to lower

osmotic potential, thus allowing higher water retention during drought [25,26]. A high proline accumulation, as occurs in mycorrhizal plants nodulated by the WT strain, could therefore provide the leaf with an osmotic mechanism for preventing excessive water loss. High proline concentrations can also protect cell metabolism by avoiding protein denaturalization and/or by controlling the cell pH [27]. On the other hand, it has been proposed that a lower proline content indicates a greater tolerance to drought [24]. Lower proline accumulation in leaves and therefore greater tolerance to drought in plants coinoculated with the GM strain could be related to the higher R/S ratio observed in these treatments. The fact that water stress produced no root biomass reduction could mean that a higher water uptake is possible, and therefore these plants would not need to synthesize high amounts of proline in leaves in order to avoid excessive water loss. Moreover, mycorrhizal root length was not reduced under drought stress conditions in plants nodulated by the GM strain. This could be relevant for improving the water status in the plant via hyphal water uptake [22].

Although it has been reported that soil-water content affects mycorrhizal formation [28], the final colonization levels in the present study were almost unaffected, as previously determined [29]. In spite of the low soil-water content, the high infectivity levels of *G. deserticola* and *G. intraradices* suggest that these species are well adapted to drought and are very aggressive colonizers under drought conditions, as previously reported [4]. Both AM fungi were effective in increasing the drought tolerance of the host plant, thus maintaining growth under stress conditions. This provides evidence of a more efficient water use. Variations in the extent of AM infection among plant species have been linked to the host plant genotype [30]. In this study, however, the *Sinorhizobium* strain also influenced this value.

It is assumed that AM fungi colonize roots before rhizobia and thereby alter the quantity and quality of root and root exudates available to *Rhizobium*. Among the constituents of exudates are isoflavonoid compounds that could chemotactically attract rhizobia [31]. This could explain the significant differences in nodulation that we observed between the two *Sinorhizobium* strains according to plant genotype. On the other hand, changes produced by AMF in root exudation patterns could alter the composition of the rhizosphere microflora which may in turn interact with AM fungi, rhizobia and the host.

Our results on nodule formation do not agree with those reported by Kirda et al. [32], who found that the adverse effect of water stress was greater on nodule weight than on the number of nodules developed. This suggests that successful root infections must have been less affected by drought than the growth of nodules. However, a small number of nodules is expected under drought since soil moisture affects the movement of rhizobia in soil and, therefore, also nodule formation and function [33]. Previous studies have reported that the GM strain produces less, but larger nodules than the WT strain, and that the nodules of the GM strain are more effective [10,11].

Improvements in growth and in N and P acquisition due to the interaction between AM fungi and *Sinorhizobium* were found in this study. This is a well-documented effect [1] which requires no additional discussion. Nevertheless, the plant response depended on the particular combination of *Medicago* genotype, *Sinorhizobium* strain and *Glomus* species or isolate involved. This suggests specific compatibilities between the host plant and the microorganisms associated in the tripartite symbiosis [7,34].

The increase in N uptake usually determined in AM-colonized and nodulated legumes has been attributed to an enhancement of N_2 fixation since nodule formation and function is stimulated by AM fungal infection [35]. Our results add nothing new in this respect, being that the number of nodules was increased by AM colonization and decreased by lowering the soil water potential.

The effect of AM inoculation on dry matter yield (shoots) was not related to the colonization level of the AM fungi involved. In some instances, both *G. deserticola* and *G. intraradices* displayed different colonizing abilities, although no differential effect on plant growth was observed.

The dependency of plants on mycorrhizal associations has been related often to physical characteristics of the roots; plants with few short root hairs, such as many legumes, display a high degree of MD, whereas those with extensive, fine root systems, such as grasses, have a low degree of dependency [36]. MD has been reported to vary

between plant species and even within cultivars of the same host [37–39]. In this study, a varying degree of MD was observed in some cases. The highest MD in *Medicago* plants nodulated by the GM strain appears to be related to the highest R/S ratio. One unexpected result was a lower degree of MD under water-stress conditions. This could be related to the effectiveness of AM fungi in increasing plant N and P uptake under certain limiting ion diffusing conditions, such as water stress. Nevertheless, the benefits that plants obtain from the AM symbiosis under water-stress conditions are less relevant than those obtained under well-watered conditions when compared to non-mycorrhizal control plants.

Amelioration of drought stress tolerance by different AM fungal species may be due to specific physiological and nutritional mechanisms depending on the fungus/host plant involved in the symbiotic association. Our results support the hypothesis that there are differences in the symbiotic physiology of various host–AM fungi associations [4,40]; however, it can be added that these differences are also modified by the *Sinorhizobium* strain involved.

It has been demonstrated that the genetically improved *Rhizobium meliloti* strain improves plant growth and does not interact negatively with the AMF [10,11]. We found that plants inoculated with both the AM fungi and the GM *S. meliloti* are better adapted to drought stress. This is due to the fact that they accumulate less proline in shoots and show higher relative growth compared to plants inoculated with the WT strain under drought stress conditions. These results could lead to practical applications since GM microorganism inoculation may be crucial for biotechnological approaches to improving crop yield in dry environments.

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