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ORIGINAL PAPER

Different interaction among *Glomus* and *Rhizobium* species on *Phaseolus vulgaris* and *Zea mays* plant growth, physiology and symbiotic development under moderate drought stress conditions

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Abstract Even though the positive interactions between arbuscular mycorrhizal (AM) fungi and rhizobial bacteria in legume plants are well documented, their interactions under drought conditions could be negative in some species. In the present study, we examined six different strains of *Rhizobium* in combination with two AM fungi (*Glomus mosseae* and *Glomus intraradices*) on the responses of *Phaseolus vulgaris* plants to moderate drought conditions. Moreover, to discriminate between direct competition for carbon resources from direct inhibition processes, a non-legume plant (*Zea mays*) was also used. Although all inoculants (single or double) increased *P. vulgaris* growth, only one double combination further increased total or pod dry weights. On the other hand, three double combinations decreased pod dry weight compared to plants inoculated with a single AM fungus. In *Z. mays* plants, one double inoculation treatment further increased shoot dry weight, but another double inoculation treatment decreased root dry weight in plants inoculated with *G. mosseae*. In addition, in both plant species, a higher percentage of decrease in AM root colonization by some rhizobial strains was observed. This was most likely caused by a direct inhibition of AM fungal growth by the rhizobial strains and also depended on the host plant involved. Further research is needed to elucidate on the mechanisms behind this inhibition.

Keywords Drought · *Glomus* sp. · *Rhizobium* sp. · *Phaseolus vulgaris* · Symbiosis · *Zea mays*

Introduction

Improving the nutritional status of plants using soil symbiotic microorganisms which are known to enhance crop productivity has received a lot of interst (Bowen and Rovira 1999). Thus, arbuscular mycorrhizal (AM) fungi have been investigated as growth and quality enhancers in agricultural plants. At the same time, it has been demonstrated that double symbiosis with the nitrogen-fixing bacteria including *Rhizobium* and AM fungi enhances both growth and yield of many legumes (Barea et al. 1992; Azcón and Barea 2010).

Phaseolus vulgaris is a major food resource, particularly in drytropical and subtropical regions (Fageria and Santos 2008). The growth and survival of plants depends on their ability to cope with adverse environmental conditions including water and nutrient deficiencies. The decline of plant growth caused by water deficiency is an important ecological factor limiting legumes establishment and production (Franzini et al. 2010). Factors associated with *Rhizobium leguminosarum* and AM fungal colonization may affect plant resistance to drought. There is evidence that AM fungi help plants to grow under arid and semiarid conditions by reducing water stress symptoms and by increasing the supply of nutrients, particularly phosphorous (Aroca and Ruíz-Lozano 2009; Ruíz-Lozano and Azcón 1996).

A previous study using *P. vulgaris* cultivars demonstrated a wide diversity between different *P. vulgaris* cultivars in their response to dual colonization with *R.*

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leguminosarum and AM (Franzini et al. 2010). Some of the applied biological treatmentsinvolving mixtures of various AM fungi and some *R. leguminosarum* strains caused a deleterious effect on certain *P. vulgaris* cultivars depending on the particular symbionts involved (Franzini et al. 2010). The AM fungi and *R. leguminosarum* bacteria do not seem to compete for infection sites, and when host photosynthesis is limited by any detrimental environmental factors, AM fungi usually show a competitive advantage for carbohydrates over the rhizobia (Ruíz-Lozano and Azcón 1994). Under non-stress conditions, the photosynthetic capacity of plants exceeds the C demand of the dual symbiosis (Ha and Gray 2008), however, in tripartite symbiosis, CO_2 fixation increases to compensate for the C cost of both symbionts (Mortimer et al. 2008).

Based on the results found in the previous study (Franzini et al. 2010), we have planned the present investigation in which we selected the *P. vulgaris* variety "Contender" that showed the strongest negative effect of AM symbiosis on nodules development and N_2 fixation causing the diminution of plant growth (Franzini et al. 2010). In the present study, we examined six different *R. leguminosarum* strains individually and applied to roots in a mixture with *Glomus intraradices* and *Glomus mosseae* (Franzini et al. 2010).

The main objective of this study was to evaluate physiological responses, growth and yield as well as N, P and K nutrition linked to the microbial symbionts compatibility. We also carried out the second experiment to evaluate the plant growth promoting abilities of *R. leguminosarum* strains and their interactions with AM fungi apart from the symbiotic relationship. For this experiment, we selected a non-legume plant *Zea mays* L. to determine if the effects of dual symbiosis previously reported in *P. vulgaris* were also present in a *Z. mays* plants. We expected the plants to have lower energy requirements since the host root carbohydrates are only required by the AM symbiont as nutrition and energy source. In the second experiment we only assayed the AM fungus *G. mosseae* applied individually or in combination with each one of the six *R. leguminosarum* strains, since positive interactions between AM fungi and *R. leguminosarum* strains have also seen in non-legume species (Galleguillos et al. 2000).

Materials and methods

Biological material and experimental design

Seeds of *Phaseolus vulgaris* L. cv Contender and *Zea mays* L. were washed for 3 min in ethanol and rinsed three times with distilled water. Seeds were then germinated in wet sepiolite (a clay mineral) and after 7 days, seedlings were transferred to 1 L pots. The pots were filled with a sterilized mixture of soil/sand (1:1, v/v).

Treatments used in the *P. vulagaris* experiment were non-AM inoculated control or AM inoculated with *G. mosseae* or *G. intraradices* and each of these fungal treatments was assayed with one of the six *R. leguminosarum* strains: 912 (R1), 997 (R2), 999 (R3), 8002 (R4), CIAT 899 (R5), CCN Etli (R6), giving 21 treatments with five replications each for the total of 105 experimental units.

In the *Z. mays* experiment, only the mycorrhizal fungus *G. mosseae* was assayed singly or dually inoculated with each of the six *R. leguminosarum* strains giving 14 treatments with five replications each for the total of 70 experimental units. Soil, microbial inocula and the experimental environmental conditions were similar in both experiments.

The soil was collected from the field area in the Estación Experimental del Zaidín (Granada, Spain) sieved (2 mm), and autoclaved (100 °C for 1 h daily on three consecutive days). The soil had a pH of 8.1, 1.81 % organic matter, nutrient concentrations (mg Kg^{-1}): N, 2.5; P, 6.2 ($NaHCO_3$-extractable P); K, 132.0. The soil was made up of 35.8 % sand, 43.6 % silt and 20.5 % clay. Once a week, plants received 10 mL of the nutrient solution containing 6 mM $Ca(NO_3)_2$, 6 mM $CaCl_2$, 3 mM KNO_3, 2.3 mM K_2SO_4, 1.5 mM $MgSO_4$, 1.3 mM NaH_2PO_4, 68 μM EDTA-Fe, 13 μM $MnSO_4$, 9 μM H_3BO_4, 1 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.2 μM Na_2MoO_4.

Mycorrhizal inoculum from each endophyte was multiplied in an open pot culture of *Z. mays* and consisted of soil, spores, hyphae and AM root fragments. The AM fungal species used, belonging to the collection of Estación Experimental del Zaidín, were *G. mosseae* (Nicol. And Gerd) (strain 121) or *G. intraradices* (Schenck and Smith) (strain 119) for Experiment 1 (*P. vulgaris*) and *G. mosseae* for Experiment 2 (*Z. mays*). Ten grams of each mycorrhizal inoculum, having similar characteristics (an average of 40 spores g^{-1} soil and root fragments with 85 % of colonized roots length), were placed below the seedlings.

The strains of *R. leguminosarum* which were used in the study were grown in TY medium (5 g L^{-1} tryptone, 3 g L^{-1} yeast extract, 9 mM $CaCl_2$) and applied (1 mL containing 10^8 cells per pot) at transplanting time. Plants without *Rhizobium* strains received the same amount of autoclaved culture medium.

Phaseolus vulgaris and *Z. mays* plants were grown for 60 days under greenhouse conditions with temperature ranging from 19 to 25 °C, 16/8 light/dark photoperiod and a relative humidity of 50–70 %. A photosynthetic photon flux density of 400–700 μmol m^{-2} s^{-1} was applied as supplementary light. All plants were grown under moderately drought conditions by keeping soil water capacity to

75 % each day after water application. Water level decreased along 24 h to 50 % of soil water capacity before the next water application. Soil moisture was measured daily with a ML2 ThetaProbe (AT Delta-T Devices Ltd. Cambridge, UK), and the water needed to reach 75 % soil water capacity was daily added (Marulanda et al. 2003).

Plant analyses

Once the plants were harvested the dry weight of shoot, pods (only in *P. vulgaris* plants) and roots was recorded after 2 days at 75 °C.

Leaf relative water content (RWC) was calculated in *P. vulgaris* plants using the following equation: (Fresh weight − Dry weight)/(Turgid weight − Dry weight) × 100 (Aroca et al. 2003). Stomatal conductance was measured in five plants of each treatment using a porometer system (Porometer AP4, Delta-T Devices Ltd, Cambridge, UK) following the user manual instructions. Stomatal conductance measurements were taken in one leaflet of each plant at midday on the day before harvest.

Nodules formed in *P. vulgaris* root were visually determined and weighted. The percentage of mycorrhizal root length infected was estimated by visual observation of fungal colonization after clearing washed roots in 10 % KOH and staining with 0.05 % trypan blue in lactic acid (v/v) (Phillips and Hayman 1970). Quantification was carried out using the grid-line intersect method (Giovannetti and Mosse 1980).

After the analyses of plant growth data, samples of shoot plus pods (*P. vulgaris*) or shoots alone (*Z. mays*) in all of treatments were analysed for N, P and K contents in the Ionomic Service of Centro de Edafología y Biología Aplicada del Segura (CSIC, Murcia, Spain).

Statistical analysis

Five replicates were made per treatment and data were subjected to the analysis of variance. Differences between the means were analyzed by ANOVA and LSD tests ($P < 0.05$). For the percentage values, the data were arcsin square transformed before the statistical analysis was applied.

Results

Phaseolus vulgaris experiment

All inoculation treatments increased total plant dry weight of *P. vulgaris* plants, except that of R2 alone or R4 plus *G. mosseae* (Fig. 1a). At the same time, none of the double inoculation treatments had any effect in total plant dry weight, except a positive interaction between R2 and *G. mosseae*, and negative interaction between R4 and *G. mosseae* (Fig. 1a; Table 1S). These differences were not caused by different shoot biomass (without pods; data not shown), but by different pod dry weights (Fig. 1b). Thus, all the inoculant treatments increased pod dry weight (Fig. 1b). Double inoculation had negative effects on pod dry weight in the combinations of R4, R5 and R6 with *G. mosseae* when compared to plants only inoculated with *G. mosseae* (Fig. 1b). Also, a positive interaction in terms of pod dry weight increments was observed when adding treatment R3 to the *G. intraradices* inoculation (Fig. 1b; Table 1S). Finally, the positive interaction in terms of total dry weight observed between *G. mosseae* and R2 was caused by an increase in root dry weight (Fig. 1c).

Most of the inoculation treatments improved leaf relative water content (RWC) except those of R1 or R6 alone, *G. mosseae* plus R1 or R2 and *G. intraradices* plus R2 or R6 (Fig. 2a). Also, the treatments involving R2 plus any of

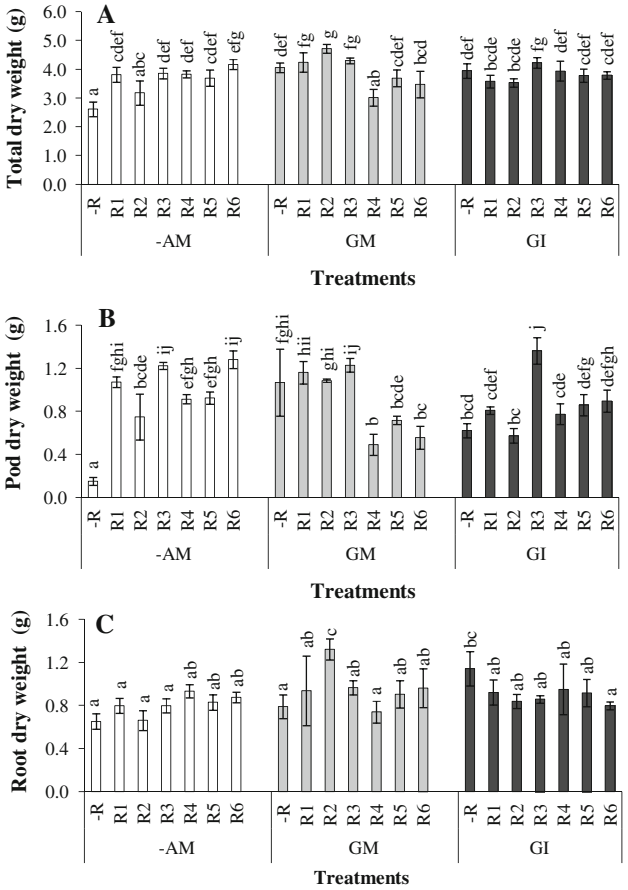

Fig. 1 Total (**a**), pod (**b**) and root (**c**) dry weights of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*), or *G. intraradices* (GI, *dark grey bars*) inoculated *P. vulgaris* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. Different *letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

the two fungi had a negative effect on RWC when compared with single fungal inoculation (Fig. 2a).

Stomatal conductance increased in almost all of the inoculation treatments except for those involving R2 strain, R1 plus *G. intraradices*, and R5 alone (Fig. 2b). On the other hand, interaction of *G. intraradices* with R1 or R2 had a negative effect in stomatal conductance, while the same fungus co-inoculated with R4 and R5 caused an increase in stomatal conductance compared to plants inoculated with *G. intraradices* alone (Fig. 2b). Also, a negative interaction in terms of stomatal conductance was observed between *G. mosseae* and R2 or R4 strains (Fig. 2b; Table 1S).

Percentage of mycorrhizal root length was decreased by all rhizobial treatments except for R2 plus *G. mosseae* (Fig. 3). Nodule formation was differentially regulated depending on the rhizobia and fungi species involved (Fig. 4). Inoculation with *G. mosseae* increased the number of nodules in R1 strain and decreased it in R4 and R6 (Fig. 4a). *G. intraradices* inoculation decreased the number of nodules in R6 strain, since no nodules could be detected (Fig. 4a). Total nodule weight by plant was increased by inoculation with *G. mosseae* when interacting with R1 and R2 strains and decreased it when interacting with R6 strain (Fig. 4b).

The following inoculation treatments decreased shoot (stem plus leaves and pods) N concentration: *G. mosseae*

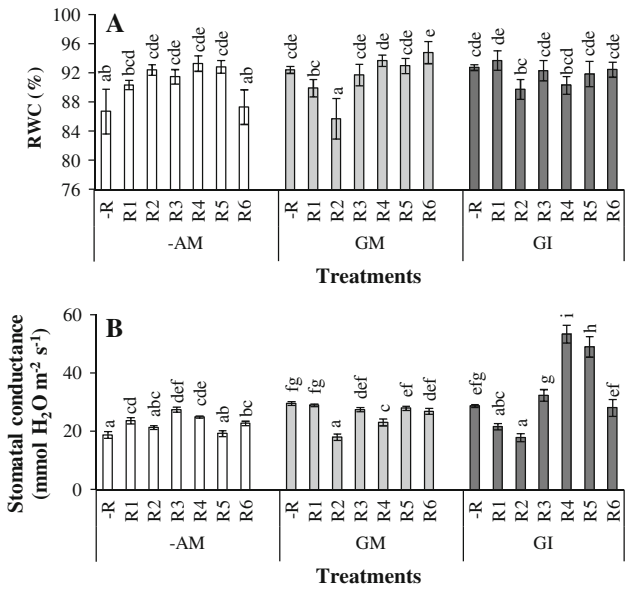

Fig. 2 Relative water content (RWC, **a**) and stomatal conductance (**b**) of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*), or *G. intraradices* (GI, *dark grey bars*) inoculated *P. vulgaris* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

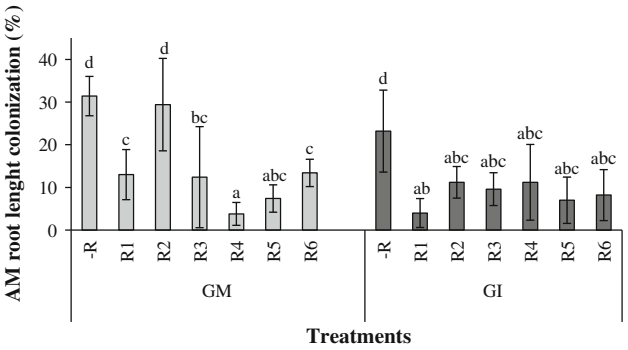

Fig. 3 Arbuscular mycorrhyzal root length colonization of *G. mosseae* (GM, *light grey bars*), or *G. intraradices* (GI, *dark grey bars*) inoculated *P. vulgaris* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

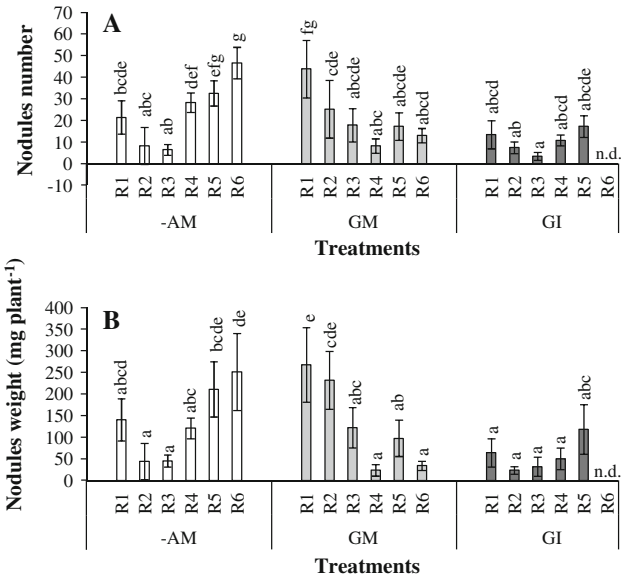

Fig. 4 Number of nodules per plant (**a**) and weight of nodules per plant (**b**) of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*), or *G. intraradices* (GI, *dark grey bars*) inoculated *P. vulgaris* plants co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

alone or plus R2 or R3 strains, *G. intraradices* plus R3 strain, and R4 and R6 strains alone (Fig. 5a). Inoculation of *G. mosseae* together with R4, R5 and R6 strains increased N concentration when comparing with *G. mosseae* single inoculated plants (Fig. 5a). When *G. intraradices* was co-inoculated with R1, R5 and R6 strains also got increased N concentrations (Fig. 5a). Shoot P concentration decreased in plants when *G. intraradices* was co-inoculated with strains R2, R3 and R6 (Fig. 5b). Shoot P concentration also decreased compared to *G. mosseae* single inoculated plants if strains R4 and R5 were involved (Fig. 5b).

Shoot K concentration was decreased by all inoculation treatments (Fig. 5c). Shoot K concentration decreased when strain R1 was co-inoculated with *G. mosseae* or when strain R3 was co-inoculated with *G. intraradices* when compared to single fungus inoculated plants (Fig. 5c). Also, when *G. mosseae* was co-inoculated with strains R4 and R6 a positive interaction was found regarding shoot K concentration (Fig. 5c; Table 1S).

Zea mays experiment

To understand the contrasting interactions described above between the AM fungi and particular rhizobial strains, we designed an experiment employing a non-legume species (*Zea mays* L., maize) in order to discriminate between competition for carbon resources and direct negative interaction between a particular rhizobial strain and AM fungi. For this experiment, we selected *G. mosseae* which had a wider response to different rhizobial strains in *P. vulgaris*. None of the rhizobial strains caused changes in the growth of the maize pants (Fig. 6). On the contrary, all of the inoculation treatments involving *G. mosseae* increased total plant dry weight of maize plants (Fig. 6a). A negative interaction between *G. mosseae* and R6 strain was observed in terms of root dry weight, causing a reduction in total plant dry weight (Fig. 6; Table 2S). Contrary, the co-inoculation of *G. mosseae* and R4 strain increased shoot dry weight of maize plants (Fig. 6b).

None of the rhizobial strains increased fungal colonization and even R1, R2, R4 and R5 strains diminished fungal colonization (Fig. 7). None of the rhizobial treatments modified leaf stomatal conductance (Fig. 8). On the contrary, all of the biological treatments including *G. mosseae* increased leaf stomatal conductance, however stomatal conductance decreased slightly by co-inoculation with rhizobial strains R2 and R4, and strongly decreased by co-inoculation of strains R5 and R6 (Fig. 8).

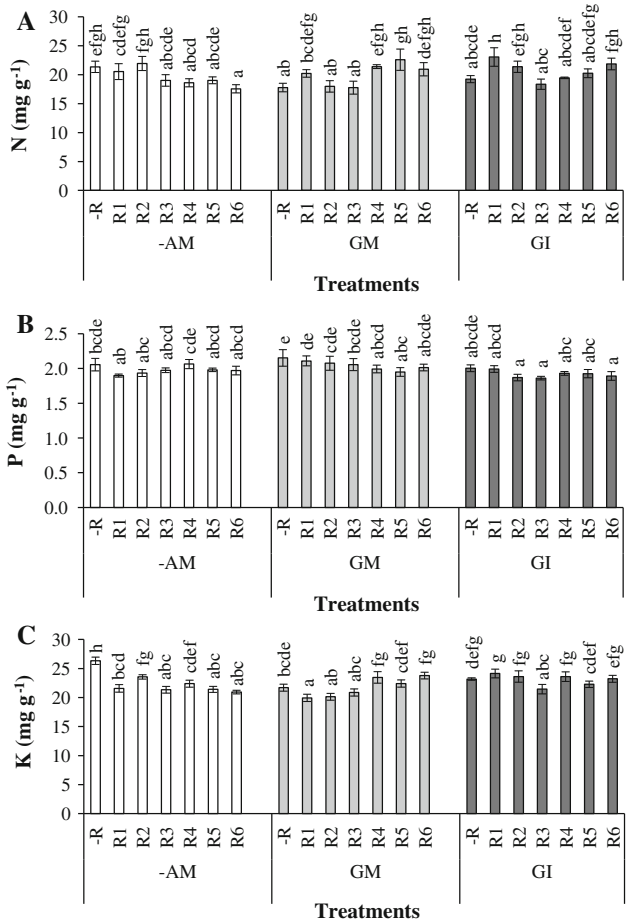

Fig. 5 Shoot N (**a**), P (**b**), and K (**c**) concentrations of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*), or *G. intraradices* (GI, *dark grey bars*) inoculated *P. vulgaris* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

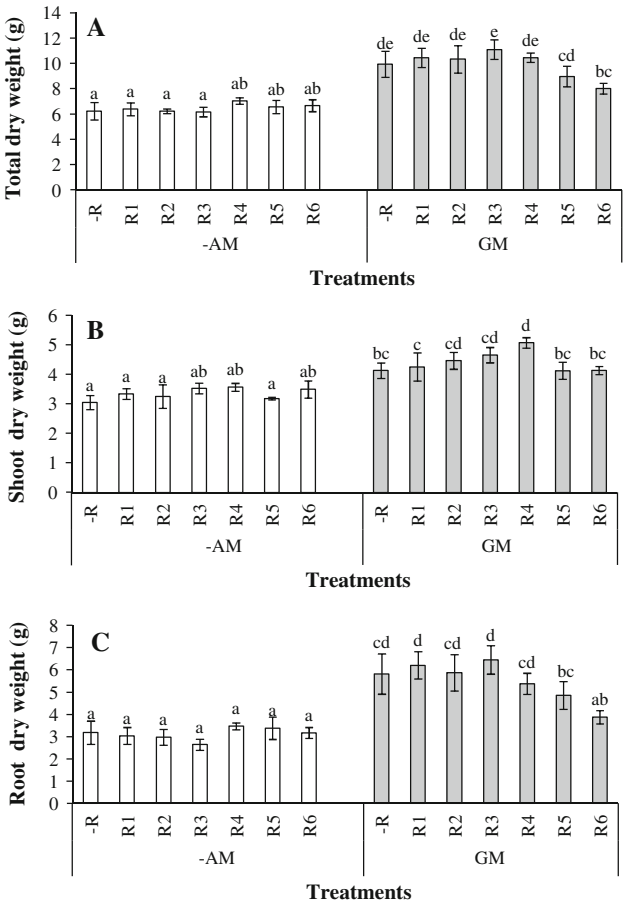

Fig. 6 Total (**a**), shoot (**b**), and root (**c**) dry weights of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*) inoculated *Z. mays* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

The application of rhizobial strains alone did not cause any changes in shoot N concentrations in maize plants (Fig. 9a). When *G. mosseae* was applied alone maize plants reached its highest shoot N concentration, which was slightly diminished when co-inoculated with rhizobial strain R4 and strongly decreased when co-inoculated with strains R1, R5 and R6 (Fig. 9a). All the treatments involving the AM fungus increased shoot P concentration independently of the rhizobial strain co-inoculated (Fig. 9b). Application of R5 strain alone also increased shoot P concentration in maize plants (Fig. 9b). Shoot K concentration was increased by all the fungal treatments except when those plants were co-inoculated with strain R1 (Fig. 9c). None of the rhizobial treatments caused any effect in shoot K concentration of maize plants (Fig. 9c).

Discussion

In a previous study, Franzini et al. (2010) found that the interaction between two different rhizobial strains (here R1 and R5) and two different AM fungi (*G. mosseae* and *G. intraradices*) depended on the *P. vulgaris* cultivar involved under moderate drought conditions. Here, we intended to extend these findings by using other four rhizobial strains (for a total of six), but selecting only one *P. vulgaris* cultivar (Contender). Moreover, to understand if this different interaction was caused by competition for C resources or by direct interaction between rhizobial strains and AM fungus under moderate drought conditions, we used a non-legume plant (*Z. mays*) as a target plant.

We have found a positive interaction between AM fungus *G. mosseae* and rhizobial strain R2 in terms of total and root dry weights. Similar effects on the total plant growth and root growth by the combination of AM fungi and *Rhizobium* were previously observed under optimal water conditions (Ibijbijen et al. 1996; Mortimer et al. 2008). This higher root growth of plants double-inoculated with *G. mosseae* and R2 strain was not correlated with a

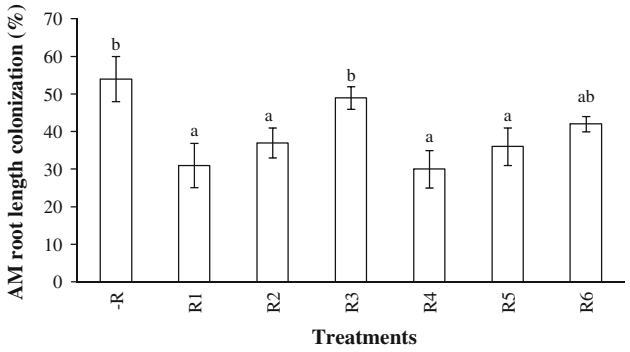

Fig. 7 Arbuscular mycorrhizal root length colonization of *G. mosseae* (GM, *light grey bars*) inoculated *Z. mays* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

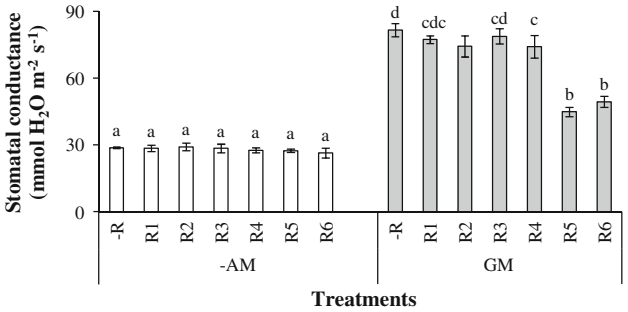

Fig. 8 Stomatal conductance of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*) inoculated *Z. mays* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

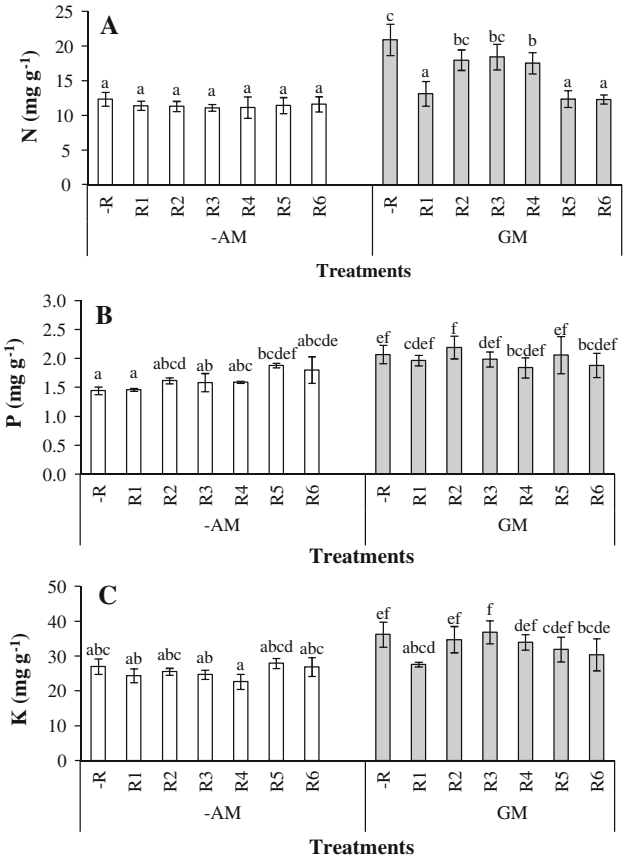

Fig. 9 Shoot N (**a**), P (**b**), and K (**c**) of non AM (-AM, *white bars*), or *G. mosseae* (GM, *light grey bars*) inoculated *Z. mays* plants non co-inoculated (-R) or co-inoculated with R1 to R6 rhizobial strains. *Different letters* means significant differences ($p < 0.05$) among different treatments after ANOVA and LSD tests. *Bars* represents mean ± SE (n = 5)

higher stomatal conductance or RWC, so it is possible that these roots were less efficient in absorbing water. Root water uptake is partially regulated by aquaporins function which is affected by the rhizobial symbiosis (Porcel et al. 2006). However the direct effect of rhizobial symbiosis on water uptake properties of the roots have yet not been tested. On the other hand, the combination of R2 and *G. mosseae* was the only inoculation treatment that did not decrease AM root length colonization, while presenting the biggest nodules (total nodule dry weight/nodule number). Ibijbijen et al. (1996) also found a higher AM colonization when a rhizobium strain was co-inoculated depending on the *P. vulgaris* cultivar. Also, Mortimer et al. (2008) found higher nodule dry weight and more efficient nodules when the two symbionts were present in *P. vulgaris* plants but, again, under optimal water conditions.

A negative interaction between both symbionts in terms of the total plant growth was observed in the combination of *G. mosseae* and R4. This negative interaction was caused by a reduction in the pod dry weight. Similar pod dry weight reduction was found in the combinations of *G. mosseae* plus R5 or R6. Therefore, three out of six possible combinations between *G. mosseae* and the rhizobial strains caused a reduction in pod dry weight when compared to *G. mosseae* alone inoculated plants. Under optimal water conditions it has been found that the dual inoculation of AM fungi and rhizobial strains increased pod dry weight in several legume plants (Aryal et al. 2003; Vejsadová et al. 1992; Thiagarajan et al. 1992). However, under the drought conditions in the present study, this positive effect was found only in the combination of R3 with *G. intraradices*. The negative interaction found here could be caused by a diminution of the root mycorrhizal length colonization and of the nodule number and weight. For the positive interaction in terms of pod dry weight (R3 plus *G. intraradices*), there was a reduction in the AM colonization but no changes in *Rhizobium* colonization were found. Therefore, it is possible that the nodules of R3 may be be more efficient when co-inoculated with *G. intraradices*. Niranjan et al. (2007) also found higher efficiency of nodule nitrogen fixation in a legume tree when co-inoculated with *G. Fasciculatum*, even with less AM colonization than single AM inoculated plants.

All of these data highlight the importance of soil water regime in the growth responses of bean plants to different rhizobial strains. Therefore, the well described positive effect of the dual inoculation between AM fungi and rhizobial bacteria (Ibijbijen et al. 1996; Tajini et al. 2011, 2012) may not be valid under drought conditions. This fact could be essential since most of the land area is becoming arid or semi-arid (García-Tejero et al. 2012).

The negative interaction found in terms of AM root colonization by almost all of the tested rhizobial strains could be caused by competition for C resources (Denison et al. 2003). However, a direct negative effect of some of the rhizobial strains in AM development could have taken place. Thus, Catford et al. (2003) using a split-root system found that formation of nodules in one side of the roots inhibited the AM colonization in the other side of the roots. This effect was also corroborated by adding Nod factors. To assay the direct effect of rhizobial bacteria on AM colonization, we used a non-legume plant (maize) single- or double-inoculated with *G. mosseae* and with each of the six tested rhizobial strains.

Although it has been observed that some rhizobial strains have PGPR properties in non-legume species (Galleguillos et al. 2000; Antoun et al. 1998), in the present study such properties were not found. However, soil application of R4 strain further increased shoot maize growth in *G. Mosseae*-inoculated plants. This growth stimulation by R4 strain was not related to AM root colonization, stomatal conductance or to N, P or K shoot concentration. However, these results showed that these maize plants (R4 plus *G. mosseae*) had higher mineral use efficiency: they were able to grow more with the same concentration of minerals. This higher efficiency could be related to higher activity of C metabolism enzymes or to higher photosynthetic rate. In a compiled research, Kaschuk et al. (2009) described that rhizobial inoculation increased photosynthetic rate by a carbon sink mechanism which could not operate in maize plants. However, Weston et al. (2012) found that a PGPR bacteria modified shoot primary metabolism and may also modify the photosynthetic rate. Curiously, R4 strain decreased yield in bean, but increased shoot growth in maize. The different growth responses of plants to a given applied bacteria have been seen elsewhere (Franzini et al. 2010; Rincon et al. 2008). This positive effect of R4 strain on *G. mosseae* maize plants could be of interest when maize plantations are intercropping with legume plants (Siame et al. 1998). Under such conditions, maize plants could benefit from the presence of rhizobial bacteria around their roots. However, this beneficial effect will depend on the rhizobial bacterium involved and on the soil water regime. Thus, to achieve better maize water use efficiency under intercropping system, Sileshi et al. (2011) found that certain amount of nutrient application is necessary if water availability is restricted.

The R6 strain decreased total dry weight of maize plants by diminishing root dry weight in *G. Mosseae*-inoculated plants. Hence, R6 plus *G. mosseae* roots were more efficient in supporting shoot growth, even with less stomatal conductance and similar AM colonization. It was previously reported that a particular PGPR induced the capacity of the roots to take up water (Marulanda et al. 2010). This could be happening also with the R6 strain since nutrient

absorption is linked to water uptake (Rouphael et al. 2012). However, N shoot concentration was reduced by R6 soil application to *G. mosseae*-inoculated plants. Therefore, R6 strain inhibited the capacity of *G. mosseae* to enhance root N uptake and/or its own capacity to absorb N compounds (George et al. 1992; Cappellazzo et al. 2008).

Four out of the six rhizobial strains reduced *G. mosseae* colonization in maize plants and three of them (R1, R4 and R5) had also the same effect in *P. vulgaris* plants. However, R3 and R6 strains, which diminished AM colonization in *P. vulgaris*, had no effect in maize plants. These data support the idea that the inhibition of AM colonization by some rhizobial strains (R1, R4 and R5) under moderate drought stress is not related to competition for C resources, but to a direct inhibition of AM fungi growth (Catford et al. 2003). Both symbionts alter plant hormonal pattern (León-Morcillo et al. 2012; Ryu et al. 2012) and cause certain defence signals (Campos-Soriano et al. 2012; Xin et al. 2012). These changes caused by certain rhizobial strains may limit AM colonization. However, R3 and R6 strains should compete for C resources with the AM fungus when photosynthesis is restricted due to drought conditions (Flexas et al. 2004). In fact, when both symbionts were co-inoculated, a reduction of the nodule number and weight was also observed. Therefore, some rhizobial strains inhibit directly the AM colonization, and others compete with AM fungi for C resources under drought stress conditions. This difference between plants grown under optimal and limited water conditions could also be caused by hormonal changes triggered by the drought treatment (Davies and Wilkinson 2012).

We conclude that under moderate drought conditions, the interactions between AM fungi and rhizobial bacteria may not produce the positive growth responses that were previously described in plants (Azcón and Barea 2010) and may even result in deleterious effects. Also, the inhibition of AM root colonization by certain rhizobial strains under mild drought conditions may not be caused by C resource competition, but by direct inhibition of fungal growth. Future studies should be designed to shed more light on this process.

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