Microbial Ecology

An Indigenous Drought-Tolerant Strain of *Glomus intraradices* Associated with a Native Bacterium Improves Water Transport and Root Development in *Retama sphaerocarpa*

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Received: 25 January 2006 / Accepted: 25 January 2006 / Online publication: 31 October 2006

Abstract

The effects of interactions between *Bacillus thuringiensis*, a drought-adapted bacterium, and two isolates of *Glomus intraradices*, an arbuscular mycorrhizal (AM) fungus, on *Retama sphaerocarpa*, a drought-adapted legume, were investigated. The fungal isolates were an indigenous drought-tolerant and a nonindigenous drought-sensitive isolate. Shoot length and root growth, symbiotic parameters, water transport (in terms of percent relative plant water uptake), and volumetric soil moisture and soil enzymatic activities in response to microbial inoculations were evaluated. *Retama* plants colonized by *G. intraradices* plus *Bacillus* possessed similar shoot length after 30 days from sowing compared with noninoculated *Retama* plants after 150 days. Inoculation with drought-adapted bacterium increased root growth by 201%, but maximum root development was obtained by co-inoculation of *B. thuringiensis* and the indigenous *G. intraradices*. Nodules were formed only in plants colonized by autochthonous AM fungi. Relative water uptake was higher in inoculated than in noninoculated *Retama* plants, and these inoculants depleted soil water content concomitantly. *G. intraradices*-colonized *Retama* reached similar shoot length irrespective of the fungal origin, but there were strong differences in relative water uptake by plants colonized by each one of the fungi. Indigenous *G. intraradices*-colonized roots (evaluated as functional alkaline phosphatase staining) showed the highest intensity and arbuscule richness when associated with *B. thuringiensis*. The interactive microbial effects on *Retama* plants were more relevant when indigenous microorganisms were involved. Co-inoculation of autochthonous microorganisms reduced by 42% the water required to produce 1 mg of shoot biomass. This is the first evidence of the effectiveness of rhizosphere bacterium, singly or associated with AM fungus, in increasing plant water uptake, which represents a positive microbial effect on plants grown under drought environments.

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Introduction

Drought stress is considered one of the most important ecological factors limiting plant establishment and survival [20]. Microbial communities are able to develop a range of activities that are very important in maintaining biological balance and sustainability in soil particularly under stress conditions [6, 18]. In stressed areas, plants are more dependent on microbial activity, and the microorganisms are able to enhance their metabolic activity to combat stress [29].

Apart from the natural protection system that plants possess against stresses, plants interact with a variety of soil microorganisms that can alleviate the stress symptoms [47]. The plant root and surrounding soil form an interface where plant root and soil constituents interact with saprophytic and symbiotic microorganisms [4, 5]. Ecophysiological studies demonstrated that arbuscular mycorrhizal (AM) symbiosis is important in protection against drought stress.

It is accepted that the role of AM symbiosis in contributing to plant establishment, growth, and drought tolerance when growing under water-stress conditions is the result of the sum of nutritional, physiological, and cellular effects [2, 36, 37].

Recent studies by our group investigated the effectiveness of bacterial inoculation (*Bacillus* sp.) on the development and physiology of AM symbiosis [47]. In AM-colonized plants grown under axenic conditions, inoculation with *Bacillus* sp. enhanced fungal development and metabolism. Under stress conditions, the bacterium also had an important stimulatory effect on the

 DOI: 10.1007/s00248-006-9078-0 • Volume 52, 670–678 (2006)

development of *Glomus intraradices*. The plant–gas exchange and other plant physiological values were modulated by the bacterium. Changes in photosynthetic rate affect the translocation of soluble sugars to the root, which can enhance the metabolic activity of the fungus in the root. The highest amount of active AM mycelium developed by this fungus was obtained after co-inoculation with this bacterium. Nevertheless, this investigation was done under particular environmental conditions, since plants were grown in a soilless medium and nutrients were sequentially supplied in an available form during the plant growth period [47].

Recent studies have reported that different AM fungi are highly variable in their tolerance to drought stress [23], but there is no information on improving plant water uptake.

Therefore, the objectives of this study were (1) to compare the effect of autochthonous microorganisms, such as *Bacillus thuringiensis*, and a *G. intraradices* strain isolated from a dry Mediterranean area, with a nonautochthonous *G. intraradices* strain from a collection (isolate BEG 123) not adapted to drought; (II) to determine the effect of the interaction between *B. thuringiensis* and each one of the AM isolates on plant tolerance to drought stress. The comparative effects were measured in terms of plant growth, relative plant water uptake and volumetric soil moisture, symbiotic parameters, and soil enzymatic activities.

Materials and Methods

Experimental Design. The experiment consisted of nonmycorrhizal controls with or without inoculation with an autochthonous strain of *B. thuringiensis* and two AM fungi, which were assayed singly or in co-inoculation with *B. thuringiensis*. The two fungal strains were *G. intraradices* autochthonous from Mediterranean soil and *G. intraradices* from a collection (BEG 123). All treatments were replicated five times (a total of 30 pots) and placed in a random complete block design.

Soil Characteristics. A calcareous loamy soil from a Mediterranean zone (Spain) was sieved (2 mm), diluted with quartz–sand (<1 mm) (1:1 soil/sand, v/v), and sterilized by steaming (100°C for 1 h along 3 days). The soil had a pH of 7.2 (water) and contained 1.6% organic matter. Nutrient concentrations were 2.1 mg kg^{-1} nitrogen, 1.7 mg kg^{-1} phosphorus ($NaHCO_3$-extractable phosphorus), and 0.8 mg kg^{-1} potassium. The soil texture was made up of 57.8% sand, 19% clay, and 23.2% silt.

Microbial Selection and Soil Inoculation. Soil samples for microbial inocula production were taken from the described Mediterranean soil. The predominant bacterium and autochthonous AM fungus were isolated from this soil and were cultivated for inocula production.

The bacterial isolation was carried out following the conventional procedure: Briefly, 1 g of homogenized rhizosphere soil was suspended in 100 mL of sterile water (dilution, 10^2) and 1 mL of this suspension was serially diluted to reach dilutions of 10^4 to 10^7. These were plated in agar nutrient broth medium (8 g L^{-1}) and cultivated for 48 h at 28°C.

Once selected, the most abundant bacterial type was independently grown in 250-mL flasks containing 50 mL of nutrient broth medium (8 g L^{-1}) in shake culture. The predominant indigenous mycorrhizal inoculum was isolated by wet sieving and decanting [45]. It was morphologically identified as a *G. intraradices* isolate. The mycorrhizal inoculum was bulked in an open-pot culture of red clover and consisted of soil, spores, mycelia, and infected root fragments having a colonization of 70%. Ten grams of mycorrhizal inoculum was added to corresponding pots at transplanting time just below the root of clover seedlings.

A second strain of *G. intraradices* (isolate BEG 123) from our collection was used as a reference inoculum. It was also bulked in an open-pot culture of clover. The AM inoculum consisted of 10 g of soil, spores, mycelia, and infected root fragments with 80% colonization. It was added to the appropriate pots at transplanting time just below the clover seedlings.

The bacterial strain was later identified as *B. thuringiensis* [47]. It was the most abundant cultivable bacterial type in such a soil. Correspondingly, pot seedlings were inoculated with 1 mL of bacterial culture (10^8 cfu mL^{-1}) grown in nutrient broth medium for 24–48 h at 28°C. The bacterial culture was centrifuged at 4000 rpm for 5 min and the sediment was resuspended in sterilized tap water. The bacterial suspension contained 10^8 cfu mL^{-1}. The bacterium was inoculated at transplanting time over the root of the *Retama* seedlings and 15 days later.

A suspension of *Rhizobium* sp. (nonidentified autochthonous strain) was added to each pot (1 mL, 10^8 cfu per pot). It was prepared following standard procedure [3].

Pots containing 500 g of sterilized soil/sand mixture were inoculated either with the *G. intraradices* selected from the original Mediterranean soil or with the reference *G. intraradices* (BEG 123 strain). Nonmycorrhizal pots received the same amount of autoclaved inoculum together with a 2-mL aliquot of a filtrate (<20 mm) of the AM inoculum to provide a general microbial population free of AM propagules.

Plant Growth Conditions. The test plant selected for the present study was *Retama sphaerocarpa*, a legume plant commonly used for revegetation purposes in Mediterranean areas characterized by infertile soils (low nitrogen, phosphorus, other nutrients, and organic matter) [10].

R. sphaerocarpa plants were grown for 5 months in 500-mL pots in a greenhouse under controlled cli-

matic conditions (18–24°C, with an 18:6-h light/dark period and 50% relative humidity). A photoperiod of 16 h at a photosynthetic photon flux density (PPFD) of 350 μmol m^{-2} s^{-1} as measured with a light meter (model LI-188B; Licor Inc., Lincoln, NE, USA) was maintained during the experiment by supplementary light to compensate for natural illumination.

Four weeks before transplanting, seedlings were grown under conditions of high humidity. Once transplanted, water was supplied daily during the following 6 weeks to maintain constant soil water close to field capacity (17% volumetric soil moisture). After this time, and for a period of 15 weeks, these plants were allowed to dry until the soil water content was 80% of field capacity (13% volumetric soil moisture).

Measurements. Plants were harvested 5 months after transplanting. The dry biomass of roots, nutrient-related water parameters, and symbiotic development (mycorrhizal infection and nodulation) were determined. Soil moisture was measured with an ML2x ThetaProbe (AT Delta-T Devices Ltd., Cambridge, UK), which measures volumetric soil moisture content by responding to changes in the apparent dielectric constant of moist soil [1, 35, 48]. However, during the 24-h period between each rewatering, the soil water content progressively decreased to a minimum value of 70% of field capacity. Water uptake per shoot unit was calculated as the ratio between volumetric soil moisture and plant shoot yielded. Shoot concentrations (milligrams per gram) of nitrogen (micro-Kjeldahl) and potassium [21] were measured.

Roots were carefully washed and then divided into three batches: One batch was stained by the classical non-vital trypan blue (TB) staining [31] and the others were used for histochemical vital staining with succinate dehydrogenase (SDH) or alkaline phosphatase (ALP) to measure total (TB), living (SDH), or active (ALP) AM fungal development. SDH and ALP activities were determined according to previously described procedures [38, 41]. Root fragments were then stained overnight at room temperature and cleared for 15–20 min in 1% active chlorine solution in sodium hypochlorite.

Mycorrhizal development was also evaluated [42] and expressed as intensity of AM colonization "M," which gives an estimation of the amount of root cortex that became mycorrhizal and is referred to the whole root system. "A" refers to the arbuscule abundance and gives an estimation of the arbuscule richness in the whole root system.

In rhizospheric soil, acid phosphatase activity was determined by using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as a substrate [30]. The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm [40].

Dehydrogenase activity was also determined [12], and iodo-nitrotetrazolium formazan (INTF) was measured spectrophotometrically at 490 nm.

The indole acetic acid (IAA) content in rhizospheric soil was determined by a previously developed colorimetric method [14, 26]. Then the absorbance of the red solution was measured with a spectrophotometer (Turner Model 350) adjusted to a wavelength of 535 nm [49].

Molecular Identification of the Bacterial Strain. Total DNA from bacterium was obtained as described [13, 46].

Results

Inoculation with *B. thuringiensis* increased shoot length by 34%, as compared to the control plants, and increased plant colonization by the reference and the autochthonous strains by 38% and 42%, respectively (Fig. 1). The bacterial effect was more evident regarding root improvement for the autochthonous strains (Table 1).

In nonmycorrhizal control plants, the bacterial strain increased root development by 101%. This effect of the bacterium in enhancing root development was similar to the effect of AM colonization in single-inoculated plants. Nevertheless, *Retama* plants achieved further root development after co-inoculation with indigenous *G. intraradices* and *B. thuringiensis*, which enhanced root biomass by 140% over noninoculated plants (Table 1).

All *Retama* plants were inoculated with the appropriate culture of *Rhizobium* sp., but nonmycorrhizal plants and plants colonized by reference *G. intraradices* did not form nodules under these experimental conditions (Table 1). In fact, nodulation was evident only in plants colonized by the indigenous *G. intraradices*. The co-inoculation of this fungus with the bacterium did not affect the formation of this symbiotic structure (Table 1).

Inocula clearly reduced water uptake by shoot unit (Table 2). This is a relevant microbial effect for plants growing in dry soils. The lowest amount of water required for producing 1 mg of shoot tissue was observed in plants colonized by autochthonous microorganisms (IA + B). This treatment reduced by 42% the water acquired in noninoculated control plants (Table 2).

Time course measurements showed the effectiveness of dual inoculations in shortening the juvenile growth period of *Retama*. In fact, after 30 days of transplanting, the shoot lengths of dually inoculated *Retama* were similar to the shoot length of control plants after 150 days of transplanting (Fig. 1).

With regard to the comparative effect of both *G. intraradices* isolates, the autochthonous isolate increased the relative plant water uptake more than the reference AM, although the increase in shoot lengths was similar for both fungi.

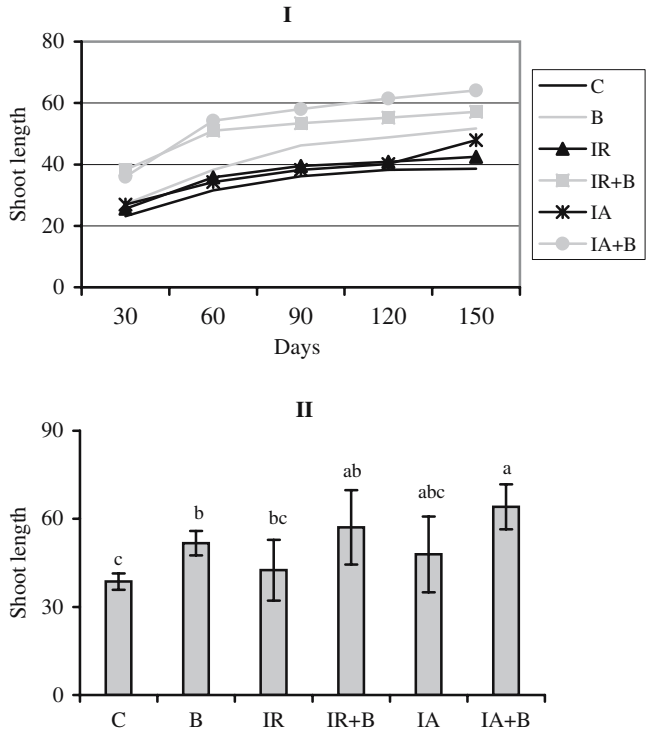

Figure 1. Effect of *Bacillus* sp. (B) or *G. intraradices* [autochthonous (IA) or from collection (IR)] with or without B on *Retama* shoot length (centimeters) in time course 30, 60, 90, 120, and 150 days after sowing [I] and at yield time [II] compared to noninoculated control (C) plants. Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

Nitrogen and potassium plant acquisition increased by 61% (N) and by 48% (K) when the most effective microbial treatments (combination of indigenous microorganisms) were applied (Fig. 2).

The relative plant water uptake was determined as an index of pot water loss (Fig. 2). Setting the water uptake of control plants as 100%, we found important differences when comparing the nontreated with inoculated plants. Differences in such values ranged from 101.8% (single *B. thuringiensis*-inoculated plants) to 113.8% (autochthonous *G. intraradices*-colonized plants).

Mycorrhizal plants increased the daily water loss, and *B. thuringiensis* only changed this physiological process when it was applied together with the reference fungus. The volumetric soil moisture in nonmycorrhizal soil (and *B. thuringiensis* treatments) decreased in single or dually AM-inoculated soil (Fig. 2).

Mycorrhizal colonization was tested after TB, SDH, or ALP staining to estimate total (TB), living (SDH), and functional (ALP) fungal colonization (Fig. 3).

Colonization intensity (M) and arbuscule richness (A) was similar in all TB-stained roots irrespective of the applied treatments. The highest arbuscular vitality (SDH staining) was observed in plants colonized by autochthonous *G. intraradices* co-inoculated with *B. thuringiensis*.

Table 1. Effect of *Bacillus* sp. (B) or *G. intraradices* [autochthonous (IA) or from collection (IR)] with or without B on *Retama* root dry weight (milligrams) and nodule number compared to noninoculated control (C) plants

Microbial treatments	*Root dry weight (mg)*	*Nodule no.*
C	140 c	–
B	282 b	–
IR	284 b	–
IR + B	270 b	2 b
IA	318 a	26 a
IA + B	336 a	28 a

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

Similarly, greater hyphal (M) and arbuscular (A) activities (ALP staining) were observed in plants colonized by autochthonous *G. intraradices* than in those colonized by reference *G. intraradices*, but in both mycorrhizal treatments, *B. thuringiensis* maximized the functionality of both fungal developments (M and A) (Fig. 3). Nevertheless, *B. thuringiensis* was only effective in increasing arbuscule richness and activity when associated with the autochthonous endophyte (Fig. 3).

Regarding AM-colonizing processes, indigenous *G. intraradices*-colonized roots showed higher ALP functional activity than reference *G. intraradices* particularly when associated with *B. thuringiensis*. No AM colonization was formed in noninoculated plants.

No general trends were observed in soil enzymatic activities (phosphatase, β-glucosidase, and dehydrogenase) (Fig. 4). The highest dehydrogenase activity was observed in the rhizosphere of (AM + B)-inoculated plants with the indigenous microorganisms. The inoculation with *B. thuringiensis* increased this enzymatic activity in control and *G. intraradices*-colonized soil. In fact, all the treatments applied increased dehydrogenase activity in rhizosphere soil (Fig. 4). On the contrary, β-glucosidase as well as phosphatase activities were mainly increased by *G. intraradices* from a collection, whereas autochthonous *G.*

Table 2. Effect of *Bacillus* sp. (B) or *G. intraradices* [autochthonous (IA) or from collection (IR)] with or without B on water uptake by root units compared to noninoculated control (C) plants

Microbial treatments	*Water uptake (mg/shoot)*	*% Decrease*	*% Water uptake reduction (mg/shoot)*
C	0.122 b	100.0 b	0 b
B	0.061 a	60.3 a	39.7 a
IR	0.068 a	67.4 a	32.6 a
IR + B	0.066 a	64.8 a	35.2 a
IA	0.063 a	61.5 a	38.5 a
IA + B	0.059 a	58.0 a	42.0 a

Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

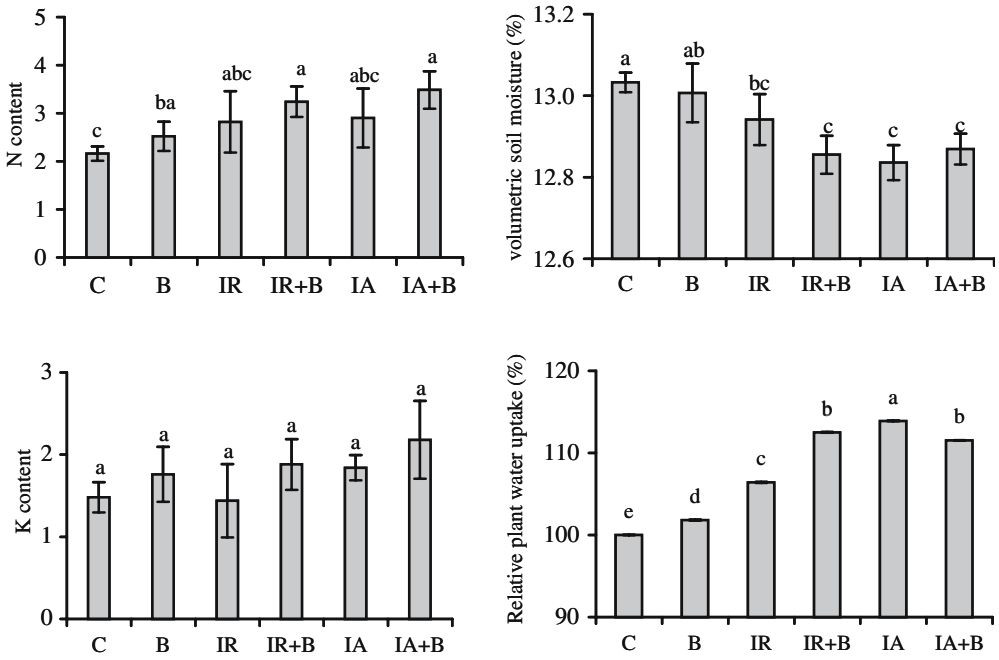

Figure 2. Effect of *Bacillus* sp. (B) or *G. intraradices* [autochthonous (IA) or from collection (IR)] with or without B compared to noninoculated control (C) on N content (milligrams), K content (milligrams), and percentages of volumetric soil moisture and relative plant water uptake in *Retama* plants. Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

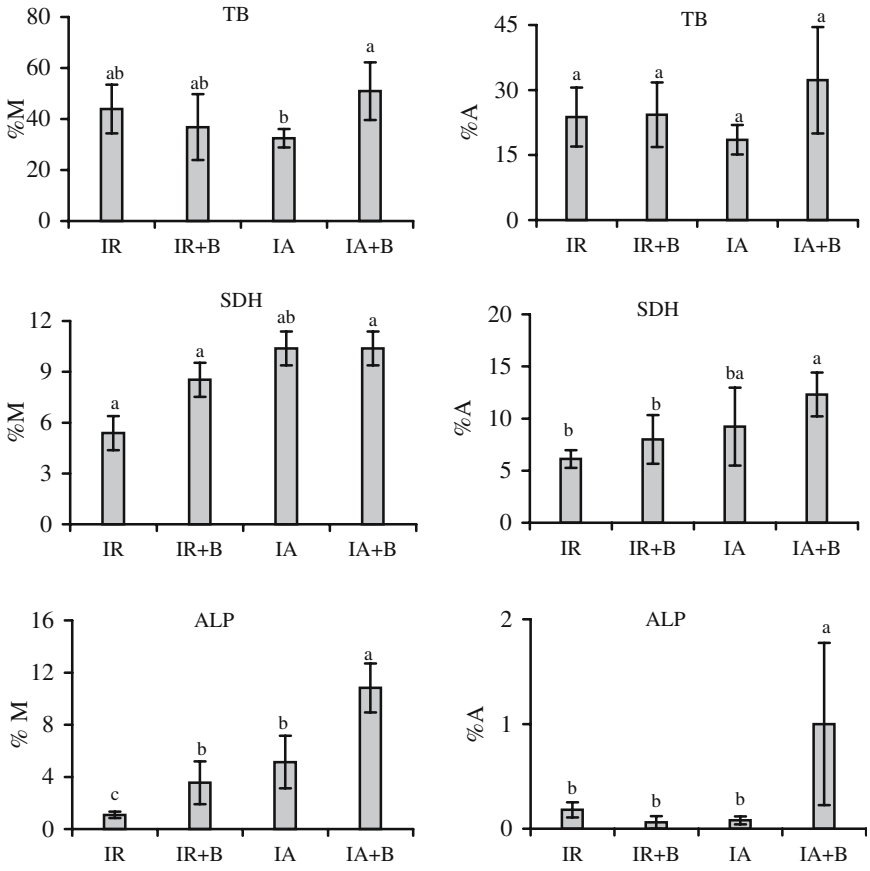

Figure 3. Effect of *Bacillus* sp. on *Retama* AM colonization by *G. intraradices* [autochthonous (IA) or from collection (IR)] as measured by trypan blue (TB), succinate dehydrogenase (SDH), or alkaline phosphatase (ALP) staining. % M is the colonization intensity and % A is arbuscule abundance, both (M and A) relative to the whole root system. Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

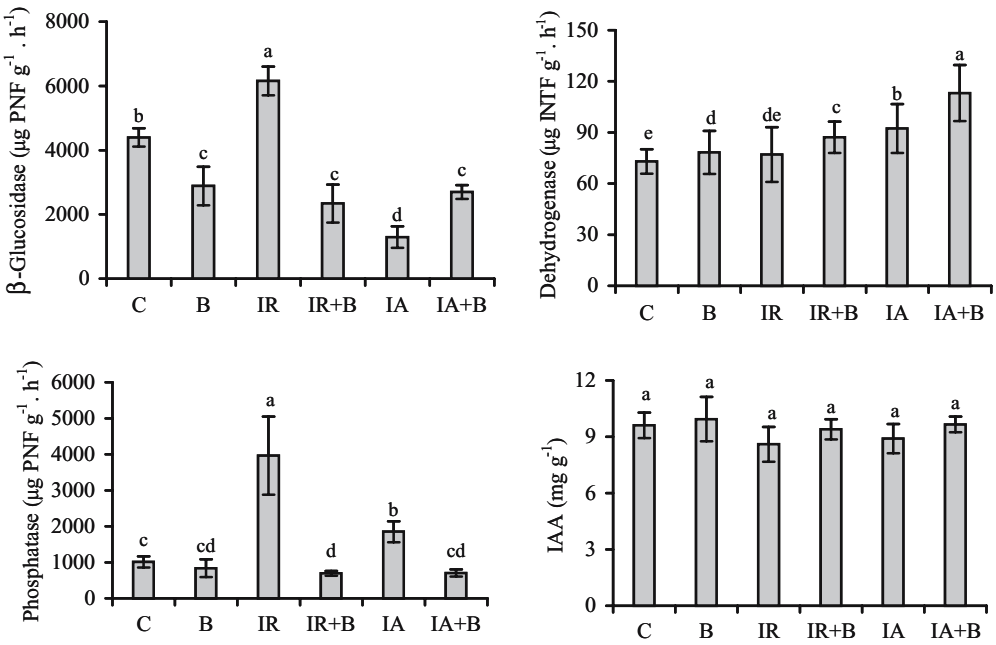

Figure 4. Effect of *Bacillus* sp. (B) or *G. intraradices* [autochthonous (IA) or from collection (IR)] with or without B on β-glucosidase, phosphatase, dehydrogenase activity, and indole acetic acid (IAA) content in rhizospheres of *Retama* compared to noninoculated control (C) plants. Means followed by the same letter are not significantly different ($P < 0.05$) as determined by Duncan's multiple range test ($n = 5$).

intraradices was less effective than the reference, but *B. thuringiensis* decreased both activities. IAA content in the rhizosphere did not change by the treatments applied (Fig. 4).

Discussion

Retama sphaerocarpa is a key component for preventing the processes of erosion and desertification in semiarid and arid areas [10, 34]. The root system of this plant has access to deep water sources and thus is able to support the typical droughts of arid and semiarid areas. In this study, native *G. intraradices* was more effective in improving root growth and relative water uptake in *Retama* plants than the reference fungus. Our results showed the range of plant responsiveness to AM colonization, which varied according to the origin of the AM fungus and associated microorganisms. Knowledge about the characteristics of indigenous AM isolates involved in stress adaptation and the improvement of plant drought tolerance (in terms of plant water uptake and growth) is of great interest regarding the possible use of microbial inoculation for revegetation practices under drought conditions.

The autochthonous *G. intraradices* was also more effective for active AM colonization and nodule development than the reference *G. intraradices*.

According to these and other reported results [22], it is important to know the particular effectiveness of microbial isolates and the intrinsic capacity to maintain plant stress tolerance and adaptation under a range of stress conditions. This fact could indicate a level of ecological adaptation of the microbial groups to the environment. The elimination of microbial populations leads to problems with plant establishment, survival, and development [5, 32–34].

The rhizobial association in this legume plant is a source of nitrogen input in the soil, and thus it can be considered as very important in revegetation strategies. Despite the limited water and nutrients and the poor quality of soils in semiarid zones, reforestation strategies involving this plant are scarce. Water is one of the main factors controlling the effectiveness of AM colonization [1]. In nodulating plants, the highest growth, as a result of symbiotic associations, must be taken into account in future revegetation of degraded soils.

Therefore, the application of the most effective microbial groups is recommended for a better plant establishment. One important aspect for successfully applying microbial inoculants is the appropriate selection of effective isolates. Our results showed that the successful survival and development of *Retama* plants in degraded dry soils are highly dependent upon the activity of autochthonous microbial populations. The improvement of *Retama* establishment is achieved by microbial inoculants, particularly those adapted to local conditions. Such microbial management can improve the biochemical soil properties in degraded areas. Root and associated microorganisms improved aggregate formation. Such improvement of the structural stability of the rhizosphere is very important in the recovery of soil in semiarid areas. The aggregate stabilization has a biological origin [9].

The presence of PGPR microorganisms (bacteria and/or AM fungi) antagonize the negative effect of detrimental factors caused by lack of nutrients, presence of organic matter, or drought [17]. Nevertheless, different isolates of the same *Glomus* sp. were found to show variability in their tolerance and adaptation to detrimental factors [28, 47].

Inoculated plants responded better to native symbionts adapted to the environmental conditions and showed the highest effectiveness in terms of root development and symbiotic parameters. Isolation of indigenous and presumably drought-stress-adapted microorganisms increased the survival and stimulated plant growth to a higher extent than nonindigenous, exotic, isolates, as was determined in terms of relative plant water uptake. The results highlighted that fungal isolates within one species can vary in their symbiotic effectiveness [27], which is a function of the compatibility of the fungus and other soil microorganisms with plants in the mycorrhizosphere environment.

Comparable shoot growth of AM-colonized plants (by native or nonadapted fungus) did not result in a comparable volumetric soil moisture or relative plant water uptake. These results suggest that native drought-adapted fungus can particularly affect physiological capacities in colonized plants. The adaptation to drought of AM plants may be caused by changes in transpiration and stomatal control [36, 37]. Such effects could be due to high root and/or hyphal development.

According to Koide [19], mycorrhizal roots can absorb a higher volume of water per unit of root length than nonmycorrhizal roots, apart from a direct hyphal water uptake. Mycorrhizal colonization might indirectly increase water uptake by improving root conductance to water flow and directly via extraradical mycelia that might transport water to mycorrhizal roots [8, 36]. According to the results, plants colonized by each one of the two AM fungi depleted soil water to a higher extent than nonmycorrhizal plants. The highest volumetric soil moisture was tested in soil colonized by autochthonous *G. intraradices* and resulted in an increase (by 113.9%) of relative plant water uptake. *B. thuringiensis* did not change the percentage of volumetric soil moisture.

Changes in the affinity of nutrient uptake and threshold concentration by AM colonization are also based on physiological and structural root characteristics.

The greatest shoot biomass production was reached in autochthonous *G. intraradices* plus *B. thuringiensis*-inoculated plants and this treatment did not show the maximum soil water consumption. The highest water use efficiency, not determined here, may probably be involved in this effect. In addition, it is known [16, 25] that extraradical mycelia increase soil aggregation, which is related to the hydraulic continuity in the soil. *B. thuringiensis* effectiveness in increasing root elongation in *Retama* may be relevant to water uptake [15]. In addition, it may produce exocellular polysaccharides able to stabilize aggregates [7].

Here, we demonstrated the ability of native-adapted microbial interactions to maintain and to enhance the proportion of AM intraradical active mycelium (ALP staining) throughout the experiment. The soil used here caused limited symbiotic developments (AM colonization and nodulation), but the management of native microorganisms (AM fungus and the bacterium) resulted in a very efficient inoculum for increasing nodulation, mycorrhization, and, consequently, plant establishment. A close relationship seems to exist between the quality of AM colonization and the plant growth responses as was observed in this study.

Nonautochthonous *G. intraradices* was also able to colonize plant roots and to promote plant growth under dry conditions. It is assumed that any association between microbial populations not inhabiting the same rhizospheres, as reference *G. intraradices* and *B. thuringiensis* are, may be functionally incompatible.

These results confirm the importance of some characteristics related to the effectiveness of autochthonous microorganisms in dry soils, which resulted in greater nutrient acquisition and relative plant water uptake. In addition, they showed that the ability of autochthonous *G. intraradices* for increasing plant development is highly related to its effect to increase root biomass and its interaction with the autochthonous *B. thuringiensis*.

Differences between both *G. intraradices* in the development of mycelium (M) and arbuscules (A) were only observed in terms of activity (ALP staining), particularly intensity of infection (M), which was highest with the autochthonous AM strain. The highest development of abuscule (A), in terms of activity and quantity, by autochthonous fungus inoculated with *B. thuringiensis*, must account for the greatest nutrient transfer between symbionts [44]. As soil inoculum potential depends on the number of active propagules, the stimulating effect of *B. thuringiensis* can be used for increasing such a potential as shown here.

The most important differences between the isolates of *G. intraradices* used were observed in terms of the percent relative plant water uptake that was higher than the control by 106.4% in the case of the reference *G. intraradices*-colonized plants and by 113.9% in autochthonous *G. intraradices*-colonized plants.

The positive benefit from *B. thuringiensis* inoculation was particularly shown on root growth. Such effectiveness was stronger in single inoculation than in co-inoculation with either AM isolate. The bacterial effect may be attributed to several mechanisms other than the secretion of plant growth hormones, as indicated by IAA content determined in the rhizosphere. In fact, phytoactive sub-

stances can cause morphological and physiological changes in the root system [11].

Biological changes in the rhizosphere soil promoted by the microbial inoculants, applied in this study were evaluated as soil enzymatic activity (index of the soil/plant system functioning) [39].

Soil microbial activities are required to assess soil quality. Soil enzymatic activity is a sensitive indicator of changes produced in soil by environmental conditions such as drought. Nevertheless, there are few studies that use these biological values to indicate soil quality in systems treated with microbial inoculants. These biological parameters related to soil microbial activity are used as indicators of changes produced by management practices such as microbial inoculation [30]. These activities are sufficiently sensitive to give an indication about ecosystem functioning [24]. In fact, oxidoreductases, such as dehydrogenase, are responsible for oxidative processes in soil. Regarding these results, except IR inoculation, all the microbial treatments applied increased this parameter. This enzymatic activity depends on the metabolic state of soil biota and microbial activity [12]. The inoculation with IA + B was the most effective treatment to increase dehydrogenase activity. On the contrary, phosphatase activity was particularly increased by IR treatment. It was reported [30] that this hydrolase activity, capable of hydrolyzing organic phosphate esters, decreased with the inoculation of *Pseudomonas fluorescens*.

Results indicate the positive effect and interaction of adapted microorganisms (AM fungus and *B. thuringiensis*) on plant growth under drought conditions. Such effectiveness in increasing the potentiality of inocula is concomitant with highest plant growth, nitrogen and potassium nutrition, and values related to relative plant water uptake and volumetric a soil moisture. This study reinforces the benefit of manipulating autochthonous microorganisms.

Retama, as with some other legume plants, can be used for revegetation of dry soils with low availability of nutrients [43]. The mechanisms of stress tolerance may involve an increase in root development, promotion of mineral nutrition, and water uptake [37]. Thus, AM fungi and rhizosphere bacteria are considered as an alternative plant strategy for coping with environmental limitations. As it was shown here, the combination of microbial groups may be used to increase plant growth-stimulating effects and survival of *Retama* plants used in revegetation programs.

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