

## Full Length Research Paper

# Effect of arbuscular mycorrhizal fungal inoculation on growth, and nutrient uptake of the two grass species, *Leptochloa fusca* (L.) Stapf and *Sporobolus robustus* Kunth, under greenhouse conditions

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The aim of our work was to assess the effect of inoculation with three arbuscular mycorrhizal fungi (AMF) (*Rhizoglyphus aggregatum* (N.C. Schenck and G.S. Sm.) Sieverd., G.A. Silva and Oeh., *Funneliformis mosseae* (T.H. Nicolson and Gerd.) C. Walker and A. Schüssler. and *Rhizoglyphus intraradices* (N.C. Schenck and G.S. Sm.) Sieverd., G.A. Silva and Oehl.), and a mixed inoculum of these AMF on root colonization, biomass production, mycorrhizal dependency (MD) and shoot mineral contents of two salt tolerant grasses *Leptochloa fusca* L. Stapf and *Sporobolus robustus* Kunth. After four months of growth in a sterilized soil and greenhouse conditions, grasses inoculated with AMF showed significantly higher total biomass production than non-inoculated seedlings. MD and shoot mineral contents (especially P) varied with AMF host plants. Maximum values of MD (13%) were observed in *L. fusca* and *S. robustus* seedlings when inoculated with *R. intraradices* and *F. mosseae*, respectively. Only P contents were higher in the *S. robustus*/mixed-AMF combinations than the other treatments. These results demonstrate the potential benefits in our experimental conditions of AMF inoculation for improving growth and P acquisition particularly in the *L. fusca*/*F. mosseae* and *S. robustus*/mixed-AMF combinations.

**Key words:** Grass species, symbiosis, mycorrhizal dependency, mineral nutrition.

## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are beneficial and ubiquitous fungi in natural and agricultural ecosystems (Smith and Read, 2008). These fungi establish symbiosis

with the majority of terrestrial plants, supplying mineral nutrients to the plants in exchange for photosynthetically fixed carbon (Smith and Read, 2008). The extraradical

phase of the fungi acts as an extension of the root system for the uptake of mineral nutrients, especially immobile nutrients such as P, Cu and Zn. There is considerable evidence that AMF can improve plant growth and nutrition in soils subject to a range of saline soils (Tian et al., 2004; Ruíz-Lozano et al., 2011; Dodd and Ruíz-Lozano, 2012; Mbadi et al., 2015). Their importance was recognized in a broad range of basic and practical studies (White et al., 2008). Several previous studies highlighted potential of AMF for grasses growth improvement. Johnson (1998) found that AMF inoculum increased the growth of a native grass (*Panicum virgatum*) at low phosphorus levels in taconite mine tailings. Smith et al. (1998) found that AMF inoculation effectively increased AMF colonization in a roadside prairie restoration site and that plots so inoculated had greater cover by native prairie species than uninoculated plots. Cavender and Knee (2006) found that prairie inoculum increased colonization of potted *Andropogon gerardii* relative to controls. However, plant growth responses to AMF vary widely along a continuum from positive to negative despite the ubiquity of the AM symbiosis (Maherali, 2014) and there was a great variation in degree of mycorrhizal dependency (MD) in different grass species (Wilson and Hartnett, 1998). The structure and function of the root system of grasses are expected to influence their response to inoculation with AMF (Anjum et al., 2006). Grasses with a coarse root architecture and low root hair density (warm season C4 grasses), derive the greater growth benefits from AMF than grasses with highly fibrous root system and root hair density (eg. cool season C3 grasses). This is because fine root architecture is considered as an alternative to mycorrhizae in P-limited soils. Nevertheless, in some cases, the absence of a significant correlation between density and length of root hairs and MD does not support this hypothesis (Guissou et al., 1998). The effects of AMF inoculation on grass species may also vary with AMF composition indicating that not all AMF are functionally equivalent (Rengel, 2002).

Two halophytic forage grasses, *Leptochloa fusca* (L.) Stapf and *Sporobolus robustus* Kunth are widely distributed in the Sine Saloum Delta, Senegal. They are perennial, rhizomatous and C4 chloridoid grasses. They can be easily propagated and established from seed, stem cutting, root stumps or rhizomes, especially in salt-affected and waterlogged areas where other forage species may not grow successfully (Abdullah et al., 1990; Ahmad, 2010). These grasses provide a good quality forage and highly palatable for sheeps and goats. However, little is known about the domestication of these

halophytic forage grasses with AMF and the management practices for their cultivation and adaptation must be developed and tested. The determination of plant growth responses under controlled conditions is a valuable first step in evaluating the importance of the symbiosis for grasses in nature and understanding ecosystem function.

To our knowledge, although some *Sporobolus* and *Leptochloa* species have already been studied, our work is the first to investigate the effect of AMF (*Rhizoglossum aggregatum*, *Funnelliformis mosseae* and *Rhizoglossum intraradices*) on the growth and mineral nutrition of *L. fusca* and *S. robustus* seedlings in greenhouse conditions.

## MATERIALS AND METHODS

### Soil preparation

The substrate used in the experiment was a non saline sandy soil from Sangalkam, Senegal (Duponnois et al., 2002). The soil is characterized by a low content available P (4.8 mg P kg<sup>-1</sup> soil). The soil was crushed, passed through a 2 mm sieve and autoclaved for 1 h at 120°C to eliminate native microorganisms. Some chemical and physical characteristics of the soil were pH (H<sub>2</sub>O) 5.3; electrical conductivity, 0.1 mS/cm at 25°C; clay, 3.6%; fine, silt 0.0%; coarse silt ,0.8%; fine sand, 55.5%; coarse sand, 39.4%; total carbon, 0.17%; total nitrogen, 0.02%; C/N 8.5; total P, 39 mg P kg<sup>-1</sup> soil.

### Fungal inocula and inoculation

Arbuscular mycorrhizal fungi used were *F. mosseae* (T.H. Nicolson and Gerd.) C. Walker and A. Schüssler (Redecker et al., 2013), *R. aggregatum* (N.C. Schenck and G.S. Sm.) Sieverd., G.A. Silva and Oehl., and *R. intraradices* (N.C. Schenck and G.S. Sm.) Sieverd., G.A. Silva and Oehl (Sieverding et al., 2014). The AM fungi used in the present study are salt tolerant. Previous results obtained by Diatta et al. (2014) showed a positive effect of inoculation with the same strain (*R. intraradices*) on growth of date palm seedlings under conditions of salt stress. Soumaré et al. (2008) found that the NaCl level of 200 mM in the rooting medium stimulated shoot and root dry weight in *Acacia nilotica* plants inoculated with *R. intraradices*, *F. mosseae*, *R. aggregatum* and *Rhizoglossum fasciculatum*. Similar results have been previously reported for *A. auriculiformis* plants inoculated with *R. intraradices* (Diouf et al., 2005). These AM fungi were obtained from pot cultures maintained in the collection at the *Laboratoire Commun de Microbiologie*, Senegal. The AMF were propagated on maize (*Zea mays* L.) for 12 weeks on sandy soil in greenhouse conditions. The maize plants were then uprooted. The roots were gently washed and cut into 1 cm pieces. The crude inoculum (20 g) of AMF consisted of sand, spores, fragments of hyphae and root fragments. It contained an average of 40 spores per gram of soil and roots fragments with at least 80% of colonized roots length. Furthermore, these AM fungi have approximately the same infective propagules according to

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**Abbreviations:** AMF, Arbuscular mycorrhizal fungi; MD, mycorrhizal dependency.

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**Table 1.** Effect of inoculation with arbuscular mycorrhizal fungi (AMF) and mixed-AMF on stem numbers, growth variables and AM colonization of *L. fusca* and *S. robustus* seedlings.

Grasses	AMF	Stem numbers	Height (cm)	RDW (g/plant)	SDW (g/plant)	TDW (g/plant)	Root/shoot ratios	Mycorrhization (%)
<i>L. fusca</i>	Control	15.60±3.65 <sup>b</sup>	74.90±7.24 <sup>a</sup>	04.81±0.84 <sup>ab</sup>	09.60±0.85 <sup>b</sup>	14.42±1.38 <sup>b</sup>	0.50±0.08 <sup>a</sup>	-
	<i>F. mosseae</i>	20.50±4.16 <sup>a</sup>	70.60±9.29 <sup>a</sup>	04.19±0.55 <sup>b</sup>	10.17±0.66 <sup>b</sup>	14.36±1.29 <sup>b</sup>	0.41±0.08 <sup>a</sup>	23.06±7.74 <sup>a</sup>
	<i>R. aggregatum</i>	18.80±3.01 <sup>ab</sup>	75.90±5.06 <sup>a</sup>	04.58±0.89 <sup>b</sup>	09.71±1.01 <sup>b</sup>	14.30±1.47 <sup>b</sup>	0.47±0.09 <sup>a</sup>	11.61±6.30 <sup>b</sup>
	<i>R. intraradices</i>	20.80±5.13 <sup>a</sup>	72.80±5.86 <sup>a</sup>	05.59±0.71 <sup>a</sup>	11.29±0.67 <sup>a</sup>	16.88±1.89 <sup>a</sup>	0.50±0.15 <sup>a</sup>	19.73±5.89 <sup>a</sup>
	Mixed-AMF	18.10±3.28 <sup>ab</sup>	77.20±8.63 <sup>a</sup>	04.20±0.69 <sup>b</sup>	10.27±0.78 <sup>b</sup>	14.48±1.58 <sup>b</sup>	0.41±0.06 <sup>a</sup>	10.97±4.42 <sup>b</sup>
<i>S. robustus</i>	Control	16.00±2.90 <sup>a</sup>	105.30±6.23 <sup>a</sup>	04.80±0.87 <sup>b</sup>	14.23±1.61 <sup>b</sup>	19.04±2.74 <sup>b</sup>	0.33±0.04 <sup>b</sup>	-
	<i>F. mosseae</i>	16.80±2.61 <sup>a</sup>	109.00±6.35 <sup>a</sup>	06.17±0.64 <sup>a</sup>	15.83±1.20 <sup>ab</sup>	22.01±1.15 <sup>a</sup>	0.39±0.05 <sup>a</sup>	18.51±6.10 <sup>ab</sup>
	<i>R. aggregatum</i>	16.80±1.87 <sup>a</sup>	104.90±6.06 <sup>a</sup>	04.97±1.01 <sup>b</sup>	14.94±1.47 <sup>ab</sup>	19.92±2.22 <sup>ab</sup>	0.33±0.05 <sup>b</sup>	17.60±5.06 <sup>b</sup>
	<i>R. intraradices</i>	16.10±3.10 <sup>a</sup>	109.80±7.17 <sup>a</sup>	04.89±0.71 <sup>b</sup>	15.93±0.93 <sup>a</sup>	20.82±1.43 <sup>ab</sup>	0.30±0.03 <sup>b</sup>	23.73±4.88 <sup>a</sup>
	Mixed-AMF	16.80±2.20 <sup>a</sup>	109.10±9.75 <sup>a</sup>	05.89±1.07 <sup>a</sup>	14.28±1.03 <sup>b</sup>	20.17±1.89 <sup>ab</sup>	0.41±0.05 <sup>a</sup>	16.92±5.92 <sup>b</sup>

RDW, root dry weight; TDW, total dry weight; SDW, shoot dry weight. For each plant species, values within a column followed by different letters are significantly different according to Student-Newman-Keuls test ( $P < 0.05$ ).

Guissou et al. (1998). The inoculum was placed in a hole during transplanting. The mixed-AMF contained approximately the same infective propagules of each fungal species. Non inoculated controls also received 20 g of autoclaved sand-root mixture.

*L. fusca* and *S. robustus* seeds were collected from Fatick (16°46' W and 14°17' N) in March 2010. The seeds of grasses were surface sterilized with 5% (p/v) sodium hypochlorite (NaClO) solution for 2 min. They were subsequently rinsed three times with sterilized distilled water and germinated on a sterilized sandy soil. Two weeks after germination the seedlings were individually transplanted in plastic bags (24 cm high and 12 cm in diameter) filled to 4/5 of the volume of soil. The experiment was randomized in complete block with two factors and ten treatment combinations. It was set-up as a 2 × 5 factorial design consisting of two plant species and five AMF treatment including nonmycorrhizal control, which were arranged in a completely randomized design with 10 replicates per treatment combination.

#### Quantitative evaluation and mycorrhizal colonization

The experiment was conducted over four months in greenhouse conditions at Dakar, Bel-Air (14°44'N, 17°30'W) under natural sunlight (35°C day, 27°C night and 14 h photoperiod). The relative humidity was about 75%. Plants were watered every day with tap water until soil saturation. No additional nutrients were supplied during the experiment. After four months of growth, plant parameters such as height of the culm and stems number were determined. After harvesting, the shoots and roots were separated and dried at 65°C for 10 days. The shoots and roots dry weight and total plant dry weight, for each of the ten plants replicates per treatment, were determined. The mycorrhizal dependency (MD) of each plant species was calculated using the formula:  $MD (\%) = 100 \times (TDW_M - TDW_{NM}) / TDW_M$ , where  $TDW_M$  and  $TDW_{NM}$  are total dry weight of mycorrhizal and non mycorrhizal plants, respectively (Plenchette et al., 1983). Dried shoots were chemically analyzed. Total P content was determined by automatic calorimetry according to the method of Dabin (1965). The total C and N contents were quantified using the combustion system CHN Thermo Finnigan EA 1112 Series Flash Elemental Analyzer. Total K and Mg contents were determined by the atomic absorption spectrometry. Physical and chemical analysis of soils was performed in the Laboratoire des

Moyens Analytiques (LAMA), certified ISO 9001-2000, Dakar, US Imago, IRD, www.lama.ird.sn., Senegal. Subsamples of the total root mass were cleared at 90°C for 30 min in 10% KOH, rinsed with tap water and stained with 0.05% Trypan blue (Phillips and Hayman, 1970). Mycorrhizal root colonization was evaluated by using the method of Trouvelot et al. (1986).

#### Data analysis

Data were subject to one-way analysis of variance (ANOVA), and mean values were compared using Newman-Keul's test. Data of AM colonization were arcsine-transformed before analysis when needed to fulfill the assumptions of the ANOVA. We carried out this analysis separately for each species. All ANOVAs mentioned above were done with XLSTAT (v2010.5.04) software. Pearson correlations between dependent variables were performed by the same software.

## RESULTS

### Effect of AMF on root colonization and growth of grasses

All inoculated grass seedlings were colonized by AMF. The extent of AM colonization by the three AMF and mixed-AMF varied depending on grass species (Table 1). AM colonization of *L. fusca* by *R. intraradices* and *F. mosseae* was more marked than those inoculated by *R. aggregatum* and mixed-AMF. For *S. robustus* seedlings, AM colonization was significantly higher with *R. intraradices* than the other AM treatment. No contamination by AMF was observed on the non-inoculated grass seedlings (Table 1). Mycorrhizal root colonization of *L. fusca* and *S. robustus* was not correlated with any growth parameters (unpresented Tables). ANOVA of plants growth parameters with all the AMF treatments showed that the response to AMF

**Table 2.** Mycorrhizal dependency (MD) of *L. fusca* and *S. robustus* seedlings inoculated with arbuscular mycorrhizal fungi (AMF) and mixed-AMF.

AMF	<i>L. fusca</i>	<i>S. robustus</i>
<i>F. mosseae</i>	0.00±8.50 <sup>b</sup>	13.12±6.43 <sup>a</sup>
<i>R. aggregatum</i>	0.00±11.49 <sup>b</sup>	03.27±11.46 <sup>a</sup>
<i>R. intraradices</i>	13.69±8.92 <sup>a</sup>	07.12±13.12 <sup>a</sup>
Mixed-AMF	0.72±9.89 <sup>b</sup>	04.40±12.33 <sup>a</sup>

Values in a column followed by different letters are significantly different according to Student-Newman-Keuls test ( $P < 0.05$ ).

**Table 3.** Shoot P (‰), K and Mg (ppm), C and N (%) concentrations of *L. fusca* and *S. robustus* seedlings inoculated with arbuscular mycorrhizal fungi (AMF) and mixed-AMF.

Grasses	AMF	N	P	K	Mg	C
<i>L. fusca</i>	Control	0.77±0.02 <sup>a</sup>	0.96±0.05 <sup>ab</sup>	7.52±0.26 <sup>a</sup>	5.81±0.16 <sup>a</sup>	43.05±0.32 <sup>a</sup>
	<i>F. mosseae</i>	0.83±0.06 <sup>a</sup>	1.17±0.11 <sup>a</sup>	7.50±0.13 <sup>a</sup>	5.88±0.65 <sup>a</sup>	42.46±0.52 <sup>a</sup>
	<i>R. aggregatum</i>	0.64±0.02 <sup>a</sup>	0.99±0.12 <sup>ab</sup>	6.40±0.55 <sup>ab</sup>	4.97±0.34 <sup>a</sup>	42.99±0.23 <sup>a</sup>
	<i>R. intraradices</i>	0.82±0.09 <sup>a</sup>	0.86±0.04 <sup>b</sup>	5.50±0.89 <sup>b</sup>	5.60±1.03 <sup>a</sup>	43.11±0.04 <sup>a</sup>
	Mixed-AMF	0.64±0.04 <sup>a</sup>	1.00±0.10 <sup>ab</sup>	6.63±0.60 <sup>ab</sup>	4.37±0.35 <sup>a</sup>	43.25±0.35 <sup>a</sup>
<i>S. robustus</i>	Control	0.39±0.29 <sup>a</sup>	0.88±0.19 <sup>b</sup>	4.96±0.56 <sup>a</sup>	2.77±0.41 <sup>a</sup>	43.74±0.14 <sup>a</sup>
	<i>F. mosseae</i>	0.61±0.02 <sup>a</sup>	1.04±0.02 <sup>ab</sup>	5.78±0.69 <sup>a</sup>	2.43±0.21 <sup>a</sup>	43.90±0.37 <sup>a</sup>
	<i>R. aggregatum</i>	0.61±0.05 <sup>a</sup>	1.03±0.00 <sup>ab</sup>	5.29±0.46 <sup>a</sup>	2.49±0.20 <sup>a</sup>	43.38±0.18 <sup>a</sup>
	<i>R. intraradices</i>	0.61±0.06 <sup>a</sup>	0.90±0.04 <sup>b</sup>	5.16±0.72 <sup>a</sup>	2.48±0.21 <sup>a</sup>	43.56±0.21 <sup>a</sup>
	Mixed-AMF	0.51±0.39 <sup>a</sup>	1.23±0.04 <sup>a</sup>	5.57±0.26 <sup>a</sup>	3.03±0.50 <sup>a</sup>	43.46±0.27 <sup>a</sup>

For each plant species, values within a column followed by different letters are significantly different according to Student-Newman-Keuls test ( $P < 0.05$ ).

inoculation is different among the plant species (Table 1). No significant effect of inoculation was observed on height of *L. fusca* and *S. robustus*. Inoculation with *F. mosseae* and *R. intraradices* increased significantly stem number of *L. fusca* seedlings compared to control but had no significant effect in *S. robustus*. The root dry weight of *S. robustus* seedling was significantly increased by inoculation with *F. mosseae* and mixed-AMF, compared to non-inoculated seedlings. In contrast, the root dry weight of *L. fusca* seedlings inoculated with AMF did not differ from the control treatment. The shoot dry weight of the two grasses was significantly increased by inoculation with *R. intraradices*. The total dry weight of *L. fusca* and *S. robustus* colonized by *R. intraradices* and *F. mosseae*, respectively, were higher compared to control. Inoculation with *F. mosseae* and mixed-AMF increased significantly the root/shoot ratios of *S. robustus* seedlings compared to control but had no significant effect in *L. fusca*.

Results show that the two grass species differed in their MD following the inoculated AMF (Table 2). *L. fusca*

and *S. robustus* had similar MD values when inoculated with *R. intraradices* and *F. mosseae*, respectively (Table 2). *L. fusca* in symbiosis with *F. mosseae*, *R. aggregatum* and mixed-AMF showed the lowest MD. *S. robustus* in symbiosis with *R. intraradices*, *R. aggregatum* and mixed-AMF also showed the lowest MD.

### Shoot mineral content of grass species

N, K, Mg and C concentrations did not differ significantly in shoots of the two grasses colonized by the different AMF and mixed-AMF compared to those of the non-inoculated grasses (Table 3). P concentration did not differ significantly in shoots of *L. fusca* grasses. However, P concentrations in shoots were significantly improved in the *S. robustus*/mixed-AMF combinations when compared to non-inoculated grasses and other plant-AMF combinations. P concentrations probably contributed to total biomass production of *S. robustus* more than the other nutrients. There was a positive correlation between

mycorrhizal root colonization and shoot N concentration of *L. fusca* ( $r = 0.981$ ,  $p < 0.05$ ). For *S. robustus*, there was a significant positive correlation ( $r = 0.994$ ,  $p < 0.01$ ) between shoot C concentration and total dry weight. Shoot K concentration had a significant positive correlation with root dry weight ( $r = 0.980$ ,  $p < 0.05$ ).

## DISCUSSION

The present study shows the importance of AMF for the growth of *L. fusca* and *S. robustus*. Our findings agree with some studies which showed that AMF increased growth of different plant species (Ramakrishnan and Bhuvanewari, 2014; Jan et al., 2014; Khakpour and Khara, 2012; Graham and Abbott, 2000). The root/shoot ratios of *S. robustus* changed significantly in response to *F. mosseae* and Mixed-AMF inoculation. This could probably be explained by the fact that these AMF strains positively influence RDW, whereas they gave large decreases in SDW production of *S. robustus*.

However, root colonization was mainly influenced by host plant factor (Klironomos, 2003) which was responsible for the differences between the averages. The root colonization percentages observed in *L. fusca* and *S. robustus* could be due to compatibility between the AMF present in the inoculum and the plants. The lack of significant increase on growth and mycorrhizal root colonization by mixed-AMF might be due to a competition between AMF strains as reported by Declerck et al. (2002).

In our study, the reduction in AM root colonization in response to inoculation with multiple AM fungi was observed. Previous works have demonstrated significant genetic variability within plants and/or fungal species for symbiotic capacity in mycorrhizal interactions. Tagu et al. (2005) showed that the ability of poplar to form ECMS is under its genetic control.

Other studies with contrasting results have found that the plant genotype can play a dominant role in controlling the associated soil microbial communities (Mari et al., 2003; Korkama et al., 2007). Short-term experiment have either shown variations in mycorrhizal colonization, in microbial and in mycorrhizal communities (Barbour et al., 2009; Lojewski et al., 2009), or few differences in arbuscular fungal and bacterial communities (Madritch and Hunter, 2002).

MD refers to the degree of plant responsiveness to mycorrhizal root colonization by producing maximum growth or yield at a given level of soil fertility (Plenchette et al., 1983). Measurements of MD could be highly variable but could also be very similar, depending on the plant species response to the non-inoculated treatment. Cruz et al. (1999) categorized the MD in three groups: high dependency ( $MD > 40\%$ ), moderate dependency ( $10 < MD < 40\%$ ) and not dependent ( $MD \leq 10\%$ ). Considering this classification of MD made by Cruz et al.

(1999), *L. fusca* and *S. robustus* were classified as moderately dependent plants to *R. intraradices* and *F. mosseae*, respectively. However, grasses were not dependent on all other AMF tested because the non-inoculated plants were also able to grow. Our findings indicate that *L. fusca* and *S. robustus* plants response to AMF inoculation which depends on fungi-plant combination. Several previous studies have demonstrated that MD varies also with plant species (Zangaro et al., 2007) as well as with the mycorrhizal species (Nogueira and Cardoso, 2007; Othira et al., 2012).

AMF have been shown to positively influence the composition mineral nutrients especially P in plant tissues (Smith and Read, 2008). In this study, inoculated grasses with the mixed-AMF had significantly higher total P accumulation than non-mycorrhizal *S. robustus* grasses. Similar results were obtained by Liu et al. (2001) who reported that high shoot P content and growth of wheat plants inoculated with *G. mosseae* than the non-mycorrhizal. It is known that AM symbiosis plays a vital role in improving the P nutrition of the host plants (Rohyadi et al., 2004; Smith and Read, 2008). It has been estimated that external hyphae of AM fungi deliver up to 80% of plants P requirements (Marschner and Dell, 1994). This is probably due to the extended network of AM fungi hyphae that allow them to explore more soil volume than non-mycorrhizal plants (Ruiz-Lozano and Azcon, 2000). Indeed, mycorrhizal hyphae extend beyond the depletion zones around roots and acquire nutrients that are several centimeters away from the root surface (Ezawa et al., 2002). Surprisingly, P concentration did not differ significantly in shoots of *L. fusca* grasses. It is important to note that the nutritional benefits of mycorrhizas are not confined to P alone, but to increased uptake of other nutrients, such as Mg, K and N have been demonstrated in a variety of plants (Clark et al., 1999). This was not the case in this study because all AMF tested did not significantly increase Mg, K and N concentrations in shoot of *L. fusca* grasses and *S. robustus*. The lack of positive response of shoot nutrient concentration to AMF treatment is more closely related to that grasses species have an extensive enough root system such that, nutrient demand was accomplished without the mycorrhizal symbiosis.

We demonstrated that plant species vary in the degree they respond differently to AM inoculation. We found significant positive correlation between shoot N concentration and the mycorrhizal root colonization in *L. fusca* grasses, while this relationship did not exist in *S. robustus* grasses. This indicates that the amount of N taken up should be related to the extent of root colonization by AMF. There was no significant correlation between root dry weight and mycorrhizal root colonization. This means that mycorrhizal root colonization levels were not directly associated with better plant growth. Our findings corroborate those of Hetrick et al. (1992) who

suggested that mycorrhizal root colonization is not necessarily related to plant growth. Diagne and Ingleby (2003) reported that in semi arid areas, a high mycorrhizal root colonization rate does not necessarily result in better plant growth. A significant positive correlation was showed between shoot K and P concentration ( $r = 0.991$ ,  $p < 0.01$ ) in *L. fusca* grasses, meaning that increased K concentrations can be a consequence of increased P availability on plant growth. A significant positive correlation showed between the shoot K concentration and root dry weight occurred in *S. robustus* grasses, while this relationship did not exist in *L. fusca* grasses. This means that AMF increase plant biomass through an increased uptake of K for the plant. Shoot C concentration had positively correlated with total dry weight and MD in *S. robustus* grasses. Based on the premise that plants optimize carbon expenditure to avoid limiting any one resource (N, C or P) more than another, it is logical that species of low MD would limit carbon expenditure for mycorrhizal colonization more than species highly dependent on the symbiosis. Historically, the benefit of the symbiosis for the plant has been considered to improve nutrient supply, in particular uptake of P by mycorrhizal roots as compared to nonmycorrhizal roots, especially in nutrient-poor soils (Smith and Read, 1997).

In conclusion, our results indicate that *L. fusca* and *S. robustus* are responsive to mycorrhizal inoculation. Hence, root colonization by AMF improved plant growth. The current investigation confirms that mycorrhizal symbioses can play a vital role in the improvement of the growth and P nutrition of the host plants. Thus, growth of *L. fusca* and *S. robustus* inoculated with mycorrhizal fungi was higher compared with non-mycorrhizal grasses under greenhouse conditions. From a practical point of view, the domestication of *L. fusca* and *S. robustus* with selected AMF and the management practices for their cultivation and adaptation must be developed and tested in the saline area.

### Conflict of interests

The authors did not declare any conflict of interest.

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