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Original Research Article

Effectiveness of dual inoculation with native arbuscularmycorrhizal fungi and *Pseudomonas fluorescens* on growth of *Tamarix* shrubs in saline arid soils

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Abstract

The present work aims at comparing the effectiveness of native arbuscularmycorrhizal fungi (AMF) inoculum, combined or not with a native endophytic bacteria *Pseudomonas fluorescens,* originating from saline Algerian arid and semi-arid soils, with a commecial AMF inoculum on *Tamarix articulata* growth in non-saline (0.6 ds.m^{-1}), moderate saline (2.3 ds.m^{-1}) and saline soils (7.5 ds.m^{-1}). Root mycorrhizal rates were improved by native inoculums more than with the commercial inoculum in saline and moderately saline soils. They were about 4 fold higher with the co-inoculum and by 2 fold with native AMF inoculum. Improvements of shoot biomasses were about 1.4 fold higher with native inoculums as compared to commercial inoculum in moderate saline soil. The positive effect on plant growth of the co-inoculation was associated with shoot phosphorus and nitrogen content enhancements. These findings confirm the capacity of native arbuscular inoculum and of endophytic bacteria to promote the installation of *T. articulata* plantations in saline soils.

Keywords: Arbuscularmycorrhizal fungi, *Tamarix* growth, Mycorrhizal inoculum, Soil salinity, *Pseudomonas fluorescens*.

INTRODUCTION

Tamarix plants have a worldwide distribution including arid and semi-arid Algerian areas (Makhlouf et al., 2012). These trees were introduced from Europe to United States in the 1800's (Beauchamp et al., 2005) and to North Africa in the first middle of 1900's (Makhlouf et al., 2012), as windbreakers and for erosion control (Khabtane, 2012). *Tamarix* are also deep rooted plants highly tolerant to drought and salinity (Makhlouf et al., 2012). During the last decade, several extensive restoration projects expanded using *Tamarix* species alone or associated with other species such as cypress (*Cypresusspp*) (Khabtane, 2012) and poplar trees (*Populusspp)* (Beauchamp et al., 2005). A neglected aspect of those restoration efforts is the role played by mycorrhizal symbiosis and root-inhabiting soil microorganisms in the revegetation of disturbed areas (Smith and Read, 2008).

Some previous studies indicated that *Tamarix* are mycotrophic plants naturally associated with the arbuscularmycorrhizal fungi (AMF) (Beauchamp et al., 2005; Chaudhry et al., 2013; Bencherif et al., 2015; 2016). Symbiotic fungi involved in plant community interactions and in the ecosystem productivity and plant diversity (Smith and Read, 2008). AMF and symbiotic

bacteria closely interact with roots, improving plant growth and health (Smith and Read 2008; Ramasamy et al. 2011; Bona et al., 2015), enhancing biocontrol efficiency, abiotic stress management and phytoremediation (Ramasamy et al., 2011). AMF improve plant mineral nutrition, hormone biosynthesis and consequently plant metabolism (Lingua et al. 2012; Baslam et al., 2013; Bona et al., 2015). The impact of AMF on host plants depend on the mycorrhizal potential of the fungal inoculant, on the plant host mycorrhizal dependency as well as on the soil and environmental conditions in which the association occurs (Hoeksma et al., 2010; Sikes et al., 2014). *Pseudomonas* bacteria are known to stimulate plant growth both through biosynthesis of plant hormones, reduction of stress-ethylene produced by plants, nitrogen fixation and phosphate solubilization (Bonfante and Anca, 2009). *Pseudomonas* bacteria may also promote plant growth by improving mycorrhizal symbiosis establishment and functioning by supporting the spore germination and mycelia extension of AMF (Frey-Klett et al., 2007; Ramasamy et al., 2011).

In stressed soils, such as saline soils of arid and semi-arid areas, it has been found that shrub trees like *Tamarix* species were more appropriate for the revegetation than forest trees (Makhlouf et al., 2012). It has also been shown that AMF assist shrub nutrition and growth (Saxena et al., 2017) and insure protection under saline stresses (Evelin et al. 2009; Estrada et al. 2013). AMF inoculation associated with symbiotic bacteria can improve plant growth (Xun et al., 2015) and positively influence the physiology of plants (Ramasamy et al., 2011). This emphasis the importance of testing the effectiveness of different AMF strains to develop inoculum suitable for *Tamarix* seedling production with the aim to revegetate the stressed soil areas.

Several investigations using *Tamarix ramossisima* have already been carried out under greenhouse conditions using inoculated plants (Beauchamp et al., 2005; Meinhardt and Gehring, 2012; Tanigushi et al., 2015), but limited investigations concerned *Tamarix articulate* species frequently encountered under steppic Algerian environments.

The first investigation on the determination of microorganisms associated to *T. articulata*rhizosphere revealed an interesting AMF biodiversity under adverse saline environmental conditions. Moreover, it was shown that even if mycorrhizal colonization rate of *T. articulata* decreased when salinity level increased, it was not completely inhibited and remained possible (Bencherif et al., 2015). In the present study, *T. articulata,* pot-culture greenhouse experiments were undertaken in order to (i) evaluate the benefit of AMF inoculation on *T. articulata* growth and mineral nutrition under soil salinity conditions and (ii) to compare the efficiency of commercial and native AMF inoculum while tested alone or in combination with the endophytic bacteria *Pseudomonas fluorescens*.

MATERIAL AND METHODS

Native AMF trapping and inoculum production and *Pseudomonas fluorescens* propagation

Native rhizosphere of *Tamarix plants* was harvested from naturally saline sites located in arid and semi-arid Algerian areas (Table 1). One volume of soil was mixed with one volume of sterilized peat soil in pot culture (300 ml) to trap native AMF on alfalfa (*Medicago sativa*) roots according to Fortin et al. (2008). Seeds of alfalfa were previously sterilized in ethanol 90% for 5 min and rinsed with sterile distilled water. Pots culture were placed under growth chamber conditions (16/8 h, 20/ 25°C, 70% relative humidity) and watered twice a week. After 10 months, aerial parts were cut and roots were mixed with soil, and new seeds planted for 12 months. The inoculum produced after 22 months consisted of a mixture of different native AMF spores, mycorrhizal alfalfa roots, hyphae and the inert substrate. After the trapping period, spores were extracted from the soils using the wet-sieving method described by Gerdemann and Nicolson (1963). Spores were identified on the basis of their morphological characteristics (Blaskowski 2012; *http://www.zor.zut.edu.pl/Glomeromycota*). Alfalfa roots colonization rate was evaluated according to McGonigle et al. (1990). Each 6 months, spores were extracted and verified, the same AMF species were observed.

Pseudomonas fluorescens endophytic bacteria were originally isolated from the *Tamarix* roots and identified according to the methods described by Fages and Mulard (1988). The concentration of the bacterial suspension used for inoculation was 3.3 10^8 cell ml^{-1} and 30 ml of the suspension was added to 50g of native AMF inoculum in order to produce the co-inoculum. This mixture was added to five pot culture of *T. articulata* seedlings (Fages and Mulard, 1988; Adam, 2008).

Mycorrhizal inoculation treatments of *T. articulate* seedlings

The experimentation involved three different AMF inoculation treatments and three soil salinity levels. Three soils harvested from arid and semi-arid areas were used in this experiment. They are classified according to their salinity levels as non-saline (0.6±0.14 ds m^{-1}) (NS), moderately saline (2.3±0.4 ds m^{-1}) (MS) and saline soils (7.5±3.01ds m^{-1}) (S). Soil chemical characteristics are described in Table 1. Cuttings of *T. articulata* were stratified at 4°C for 15 days and planted into pots filled with substrate consisting of autoclaved sand and natural sampled soil (1:1). Three types of inoculum were used: (1) Commercial inoculum (Symbivit®; Inoculum plus, France) consisting of a mix of 6 Glomeromycota: *Rhizophagusintraradices* BEG140, *Funneliformis-mosseae* BEG95, *Glomusetunicatum* BEG92, *Funnelifor-*

Table 1. Studied soil characteristics

Soil characteristics	Non-saline soil	Moderate saline soil	Saline soil
Salinity level (ds m^{-1})	0.6± 0.14	2.33± 0.4	7.52±3.01
pH	7.96±0.15	7.77±0.18	7.52±1.9
Organic matter (%)	0.59± 0.11	1.66± 0.21	1.16± 0.06
Total nitrogen (%)	0.21± 0.13	0.22± 0.01	0.24± 0.02
Phosphorus (mg g^{-1})	0.21± 0.14	0.41± 0.18	0.11± 0.04
Clay (%)	15.33± 0.01	15.14± 0.08	8.63± 0.15
Silt (%)	16.99± 0.12	26.92± 0.20	40.25± 0.23
Sand (%)	67.67± 0.13	56.71± 0.15	51.16± 0.17
Texture	Sandy	Sandy-silty-clayey	Sandy-silty

misclaroideum BEG96, *Glomusmicroaggregatum* BEG56, *Funneliformisgeosporum* BEG199. This mixture of Glomeromycota is deposed on natural clay carriers and natural ingredients supporting mycorrhiza (hamates, ground minerals, extract from sea organisms), naturally degradable granules of a water-retaining gel, with an average of 50 spores/g, (2) Native inoculum made of trap cultured inoculum containing a mixture of propagules composed of40 spores/g and 0.68 g/g of AMF trapped colonized root fragments. (3) Co-inoculum with native AMF plus native *P. fluorescens* containing an average of 40 spores/g supplemented with 30 ml of a bacterial solution ($3.3x10^8$ cell ml^{-1}) diluted in $MgSO_4$ (0.01M). The inoculation methodology described by Adam (2008) modified was used to inoculate *Tamarix* seedlings. Whatever the inoculum type, each pot (capacity of 500 ml) was supplemented with 50 g of inoculum according to Inoculum plus use instructions. Non mycorrhizal plants were planted in natural arid and semi arid soil to evaluates the effectivness of inoculation treatment on natural aerea., they were supplemented with the equivalent amount of autoclaved native inoculum, in order to provide plants with the same organic substances as inoculated plants. The inoculum were placed in the layer beneath the cutting in the pot cultures to facilitate fungal colonization of plant roots. Seedlings were incubated in controlled conditions at 22°C/25°C night/day, 10-14 hours photoperiod and 70% humidity. Five replicates were carried out for each treatment.

Determination of root and shoot dry weight and mineral nutrition

After 24 weeks of culture, shoots and roots were harvested and then dried in a hot air oven at 70°C for 72 hours until constant weight.

Dried shoots (1g) were digested in a mixture of boiling sulfuric acid and hydrogen peroxide. Nitrogen content was determined using Kjeldhal method (Muñoz-Huerta et al. 2013). Phosphorus was extracted by HCl acid and evaluated by spectrophotometric method using molybdenum sulfate (Drogba, 2011).

Isolation, quantification and identification of AMF spores and Determination of mycorrhizal rate

AMF spore extraction was performed after 24 weeks plant cultures using a wet sieving method (Gerdemann and Nicolson 1963). Spore morphotypes were described and species tentatively identified using original descriptions of species, identification keys (Blaszkowski, 2012) and specialized websites *http://www.zor.zut.edu.pl/ Glomeromycota/index.html* and *http:// invam.caf.wvu.edu.*

All events related to AMF root colonization, namely internal hyphae, arbuscules and vesicles were visualized after 10 (burgeoning stage) and 24 weeks (ramification stage) of culture by microscopic observation on Trypan blue-stained roots according to Phillips and Hayman (1970) method modified by Dalpé and Seguin (2013). Root colonization rate (containing arbuscules, vesicles and hyphae) was estimated using the grid line intersect method (McGonigle et al., 1990). Two thousand and two hundred fifty root fragments of approximately 1 cm/fragment from 60 seedlings were observed under an optical microscope (x100 magnification).

Statistical analysis

Data presented are mean values based on five replicates per treatment. All results were subjected to a two way analysis of variance (ANOVA) using Graph pad Prism 5.0 for windows for all main effects (AMF inoculation treatments, soil salinity) and their interactions. Comparisons among means within each dependent variable were made using Tukey HSD test ($p<0.05$). Pearson's correlation (r) was used to determine the relationship between various dependent variables within total mycorrhizal rate and soil salinity level.

RESULTS

AMF trap culture evaluation under different soil salinity levels

The number of spores isolated, from *Medicago sativa*

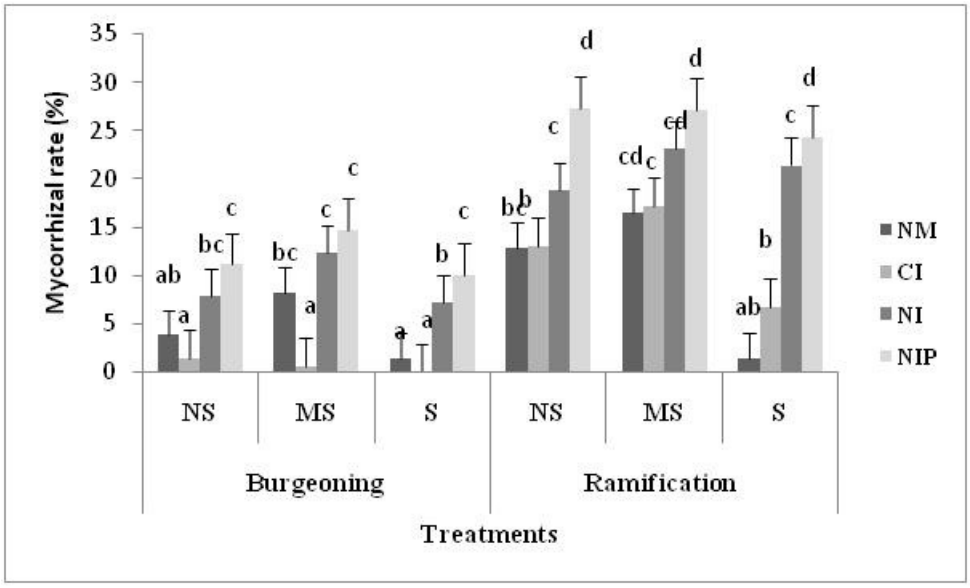

Figure. 1 Root colonization rates of *T. articulata* at 10 (burgeoning) and 24 weeks (ramification) for each treatment (NM: Non Mycorrhizal. CI: commercial inoculum. NI: native inoculum. NIP: native inoculum plus *Pseudomonas fluorescens*) in each soil (NS: non-saline soil, MS: moderate saline soil, S: saline soil). Data are means based on five replicates ±standard error (S.E) and different letters indicates significant difference among treatments ($p<0.05$, TukeyHSD test).

Table 2. Correlation between soil salinity and total mycorrhizal rate at ramification stage with different measured parameters

Parameters	CI		NI		NIP	
	AMF	S	AMF	S	AMF	S
Mycorrhizal rate atburgeoning	-	-0.96**	-	0.43*	-	0.69*
Mycorrhizal rate at ramification	-	-0.63**	-	0.48**	-	0.6**
Shoot biomass	0.32ns	-0.61*	0.56**	0.58*	0.69**	0.54**
Rootbiomass	0.56*	-0.74**	0.67*	0.67*	0.97*	0.56**
Phosphorus content	0.34ns	-0.64*	0.65*	0.58*	0.53**	0.86*
Nitrogen content	0.23ns	-0.25ns	0.72*	0.32ns	0.5*	0.65*
Total spore number	0.17ns	0.38ns	0.55*	0.7*	0.71**	0.54*

Correlation was established according to Pearson coefficient test; * $p<0.05$.** $p<0.01$, ns: not-significant. CI: commercial inoculum, NI: native inoculum, NIP: co-inoculum (native inoculum plus *Pseudomonas fluorescens*), AMF : total mycorrhizal rate, S : soil salinity level.

trap cultures, was about 46.32 and 16 spores.g^{-1} of soil in moderate, saline and non-saline soils respectively (S1). *Medicago sativa* root colonization rates followed the same pattern (S1). Moderate saline soils showed the highest root colonization rate (65%) and the lowest rate was found in the non-saline soil (35%). The root colonization rate in the most saline soils was about 53%. Native AMF species were identified as *Septoglomusconstrictum*, *Funneliformisgeosporum*, *F. mosseae*, *F. coronatum*, *F. caledonium*, *Rhizoglomusfasciculatum*, *Rhizoglomus. diaphanum* and *Gigasporagigantea* (S1). *S. constrictum* dominated in non-saline and moderate saline soils, while *F. geosporum* was abundant in saline soil.

Impact of mycorrhizal inoculation on *T. articulata* root colonization rate under different soil salinity levels

Tamarixarticulata root colonization rates after 10 and 24 week-culture durations, corresponding respectively to burgeoning and ramification stages are presented in figure 1. After 10 weeks of plant culture (burgeoning stage), mycorrhizal rate were 1.7 and 1.5 folds higher when the soil was inoculated with native AMF and co-inoculated with AMF plus *P. fluorescens* respectively as compared to non mycorrhiral soil. In contrast, in the presence of commercial AMF inoculum, whereas the roots were not colonized in saline soil, low root colonization rates were observed in non-saline and

Table 3. Result of two way ANOVA test for independent variables, soil salinity, inoculation treatment and interaction among them in *T. articulata* inoculated with commercial inoculum, native inoculum, co-inoculum under different levels of soil salinity

Parameter	Soil salinity	Inoculation treatments	Soil salinity X Inoculation treatments
Mycorrhizal rate atburgeoning	3.60*	26.09***	0.71ns
Mycorrhizal rate at ramification	1.02ns	6.04**	0.5ns
Total spore number	0.75ns	7.5*	0.12ns
Shoot biomass	26.09***	4.16*	0.71ns
Rootbiomass	1.16**	6.60*	1.17*
Phosphorus content	17.43***	0.89***	0.8ns
Nitrogen content	1.2ns	0.5ns	0.4ns

Data was established by two-way ANOVA analysis. Means were obtained from three replicates for each condition. * p<0.05, **p<0.01, *** p<0.001, ns: not significant.

moderate saline soils (Figure 1). After 24 weeks (ramification stage), the native inoculum and co-inoculum increased significantly total arbuscularmycorrhizal rates comparatively with non mycorrhizal plants in non-saline and saline soil. They were about 2 and 17 folds higher with the co-inoculation in non-saline and saline soil respectively and about 1 and 15 folds higher with native AMF inoculum in non-saline and saline soil respectively. With commercial inoculum, mycorrhizal rate was 6.6 folds higher at ramification stage than at burgeoning stage for saline soil and about 33.4 folds higher in moderate saline soil. While positive correlations were recorded between mycorrhizal rate and salinity level both at burgeoning and ramification stages in the presence of native inoculum and co-inoculum respectively at p<,0.05 and 0.01, negative correlation was recorded with commercial inoculum at p<0.01 (Table 2). At burgeoning stage, significant effect of soil salinity at p<0.05 and of inoculation treatment at p<0.001 were recorded for total mycorrhizal rate. However, at ramification stage, only inoculation treatment has a significant effect at p<0.01 on root colonization (Table 3).

Impact of mycorrhizal inoculation on AMF spore abundance and biodiversity in *T. articulata*rhizosphere under different soil salinity levels

AMF spores isolated from *T. articulate* rhizosphere cultured in pot during 24 weeks under different soil salinity levels were identified and quantified (S2). They belong to *Funneliformis*, *Septoglomus*, *Rhizoglomus* and *Gigaspora* genera. Eight species were identified as *F. geosporum*, *F. mosseae*, *F. caledonium*, *F. coronatum*, *S. constrictum*, *Gi. gigantea*, *R. diaphanum*, *R. fasciculatum* and one non-identified glomoid spore species. While, *S. constrictum* was the most abundant species in non mycorrhizal soils regardless soil salinity level, *F. geosporum* and *F. mosseae* were the most abundant species in co-inoculated non-saline, moderate and saline soils. With AMF native and commercial inoculums, *F. geopsorum* was the most abundant species in the three studied soil. *Gi.gigantea* was not recorded in saline soils. Positive correlation was recorded between total mycorrhizal rate at ramification stage (24 weeks) and total spore number for native inoculum at p<0.05 and for co-inoculum at p<0.01. Soil salinity level affected total spore number under native inoculum and co-inoculum at p<0.05 (Table 2). Inoculation treatment showed significant effect on total spore number at p<0.05 (Table 3).

Impact of mycorrhizal inoculation on *T. articulata* growth under different soil salinity levels and on mineral nutrition

Impact of inoculation by the three different mycorrhizal inoculum types (commercial inoculum, AMF native inoculum and co-inoculum with AMF plus *P. fluorescens*) on *T. articulata* growth is presented in Figure 2 A, B. Whereas, no effects of inoculation on root and shoot biomasses were observed with commercial AMF inoculum in saline soils, a significant increase was recorded in non-saline and in moderate saline soils as compared with non mycorrhizal seedlings in the same soils at p< 0.05. In contrast, positive effects on shoot biomasses were observed with the native AMF inoculum and co-inoculum in the three studied soils. Shoot biomasses were about 1.25, 1.49 and 1.09 fold higher with native co-inoculum comparatively to commercial AMF inoculum respectively in saline, moderate saline and non saline soils. Interestingly commercial AMF inoculum and native AMF inoculum improve equitably roots biomasses in moderate and saline soils. As wel as native co-inoculum incresed roots biomasses about 1.55 fold higher than both commercial and native AMF inoculum. While negative correlation was recorded under commercial AMF inoculum between soil salinity level and shoot and root biomasses (p<0.05; p<0.01), a positive correlation was recorded under native AMF inoculum

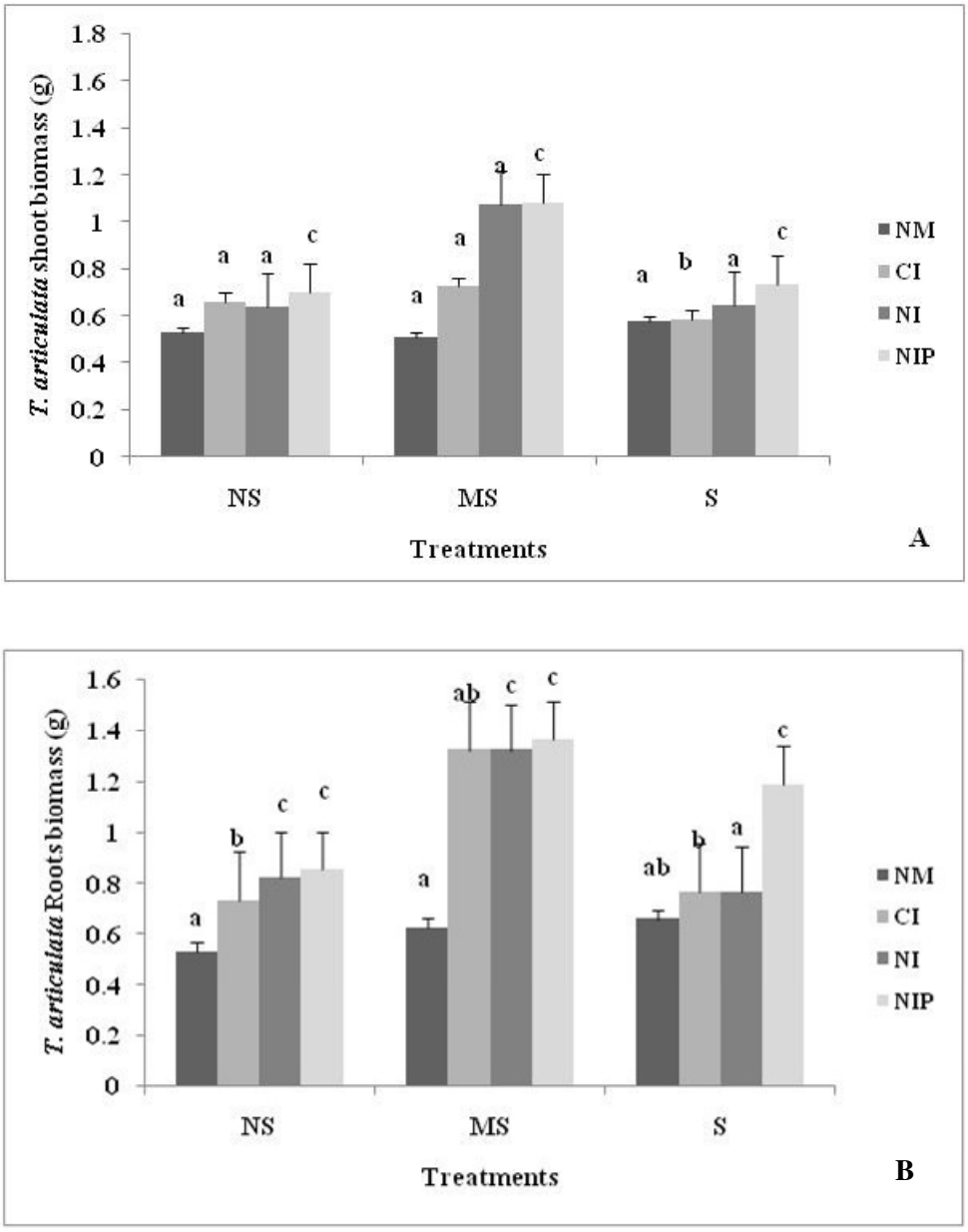

Figure 2. *T. articulata* shoot (**A**) and root (**B**) biomasses after 24 weeks of culture in a greenhouse. Data are means based on five replicates ±standard error (S.E) and different letters indicates significant difference among treatments ($p<0.05$, Tukey HSD test). NM : Non Mycorrhizal. CI: commercial inoculum. NI: native inoculum. NIP: native inoculum plus *Pseudomonas*. NS: non-saline, MS: moderate saline soil, S: saline soils.

($p<0.05$) and co-inoculum ($p<0.01$) with shoot biomasses. For root biomasses positive correlation was recorded with total mycorrhizal rate under the three inoculation treatments at $p<0.01$ (Table 2). Soil salinity level showed a significant negative effect on shoot and root biomasses ($p<0.001$; $p<0.01$). In addition, inoculation treatment recorded positive effect at $p<0.05$ (Table 3). At the same way, significant positive effect of interaction between soil salinity level and inoculation treatment was recorded at $p<0.05$ on root biomasses (Table 3).

In order to evaluate the effect of different AMF inoculation treatments on mineral uptake, *T. articulata* shoot contents of two minerals essential for plant growth, phosphorus and nitrogen, were assessed under three soil salinity levels .

Phosphorus shoot content is showed in figure 3A. Soil salinity significantly reduced *T. articulate* shoot phosphorus content in non mycorrhizal soils. However, addition of native inoculum and co-inoculum enhanced phosphorus content in the three studied soils. In the

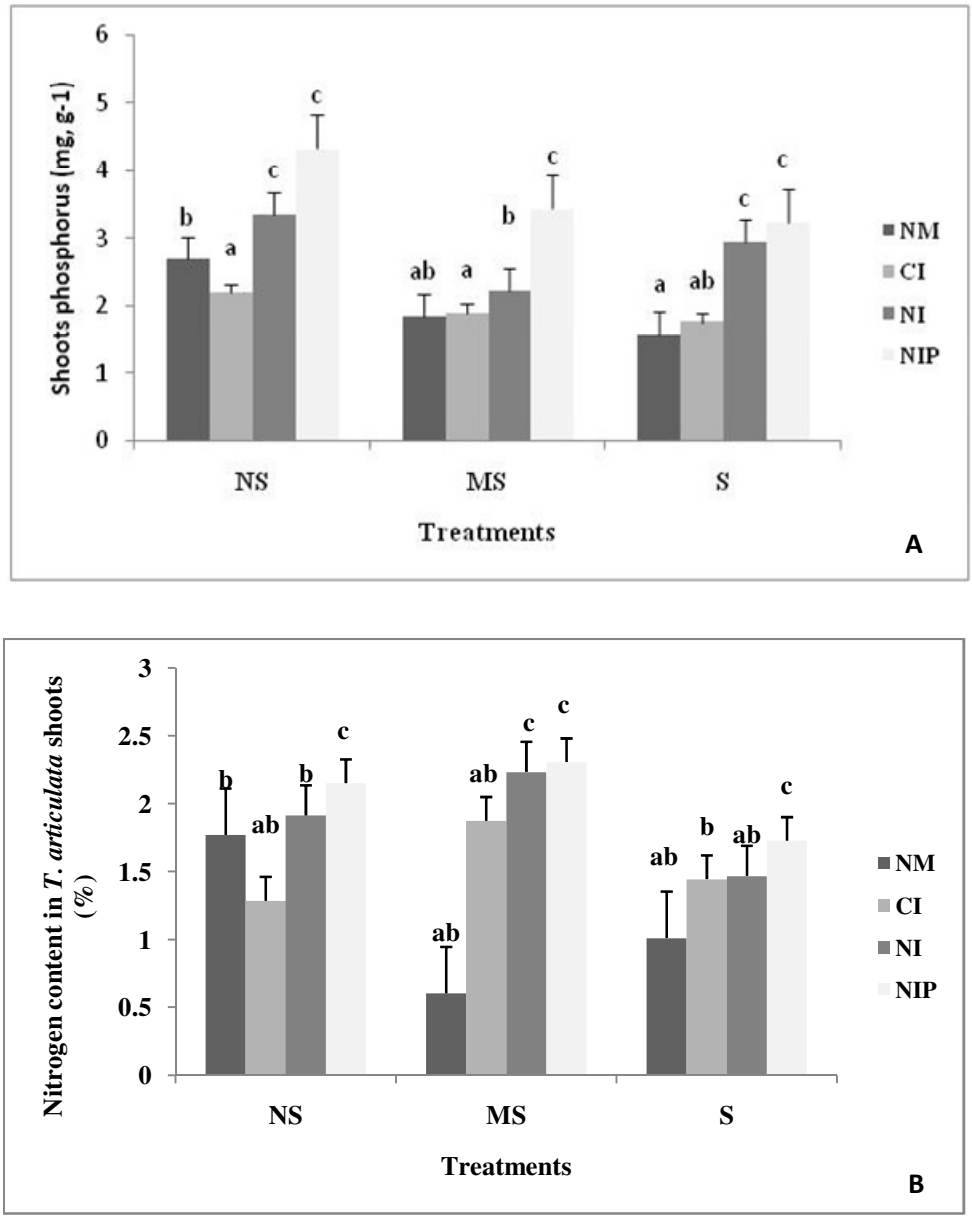

Figure 3. Phosphorus (**A**) and Nitrogen (**B**) shoot contents after 24 weeks of *T. articulata* growth under the four-inoculum treatments in each soil. Data are means based on five replicates ±standard error (S.E) and different letters indicates significant difference among treatments ($p<0.05$, TukeyHSD test). NM: Non Mycorrhizal. CI: commercial inoculum. NI: native inoculum. NIP: native inoculum plus *Pseudomonas*. NS: non-saline soil, MS: moderate saline soil, S: saline soil.

presence of the native co-inoculum, phosphorus content was about 1 fold higher in non-saline and moderate saline soils and about 2 folds higher in saline soils comparatively to non mycorhizal soils. Addition of native AMF inoculum, enhanced phosphorus shoot content about 1 fold higher in non-saline and saline soils as compared with non mycorrhizal soils. While negative correlation was recorded between soil salinity level and shoot phosphorus contentin the presence of commercial inoculum ($p<0.05$), a positive one was recorded with native AMF inoculum and co-inoculum ($p<0.05$) (Table 2). Shoot phosphorus content was affected negatively at $p<0.001$ by salinity level and positively at $p<0.001$ by inoculation treatment (Table 3).

Increases in nitrogen shoot content were obtained with co-inoculum as compared with non mycorrhizal soils whatever the salinity soil levels (Figure 3B). They were about 1 fold higher in non-saline and saline soils and

about 3.8 folds higher in moderate saline soils. In addition, native co-inoculum and native AMF inoculum increased shoot nitrogen content more than commercial inoculum regardless soil salinity level. In saline soils shoot nitrogen was about 1.22 fold higher with co-inoculum than with commercial AMF inoculum. No significant correlation was recorded with soil salinity level and nitrogen shoot content in the presence of native inoculum and co-inoculum, while positive correlation was showed with co-inoculum at $p<0.05$.(Table 2).

DISCUSSION

The data on *T. articulata* root colonization rates through plant maturation clearly showed that the commercial inoculum took more time to establish compared to the native arbuscular inoculums, particularly in saline soils. This could be interpreted as a better adaptation of the native AMF to saline soil conditions, through spore germination capacity, hyphal spread and/or mycorrhizae formation (Juniper and Abbott, 2006; Garg and Pandey, 2015; 2016). In saline soils, co-inoculation allowed higher root colonization rate both at burgeoning and ramification stages as compared to commercial inoculum and non-inoculated soil. At burgeoning stage, absence of root colonization level was recorded despite presence of autochthonous AMF in studied soils, which must be attributed to the negative effect of commercial AMF inoculum on soil natural microbiota. These findings are in accordance with those of Garg and Pandey (2016) who observed higher root colonization rate in two genotypes of *Cajanuscajan* with native inoculum when compared to commercial one in salt conditions. Likewise, Bona et al. (2015) observed high AMF root colonization rate in strawberry culture inoculated with a mixture of AMF and *Pseudomonas ssp*. The difference in mycorrhizal rate between both native inoculum and co-inoculum as compared with commercial inoculum must be due to the characteristics of AMF species (Evelin et al.; 2009) and to their specific adaptation to environmental conditions (Symanczik et al., 2015). Moreover, compatibility between host plant and inoculum may be influenced also by root exudates. Similar result was reported by Estrada et al. (2013) who showed an increased colonization ability of *Asteriscusmaritimus* inoculated with *R. irregularis* as native isolate under saline conditions when compared to *R. irregularis* isolate used in commercial inoculum.

On the other hand, AMF spore isolation from the *T. articulata* pot after 24 weeks of culture indicated that *F. geosporum* and *F. mosseae* were the most abundant species in inoculated soils while *S. constrictum* dominated in nonmycorrhizal soils. Some species of *Rhizoglomus* and *Gigaspora* genera were also detected. Such fungal diversity occurs frequently in natural ecosystems where many AMF can colonize the same root system (Sharma et al., 2009). *F. geosporum* predominance may indicate a better adaptation of this species to saline soils. In fact, *F. geosporum* has already been described in *T. ramosissima* in salt marsh by Taniguchi et al. (2015) and in field saline soils in the rhizosphere of *T. articulata* by Chaudhry et al. (2013) and by Bencherif et al. (2015; 2016). No significant differences on AMF spore number were detected between the different inoculation treatments. However, a tendency to get higher spore content in the saline soils, in particular, under the co-inoculum was noticed. Such a result suggested that the addition of *P. fluorescens* to the native AMF inoculum can potentially stimulate AMF sporulation in saline soil. This is in agreement with the study of Panneerselvam et al. (2012), which reported that combined inoculation of *G. mosseae* with *Pseudomonas putida*, improved significantly AMF colonization and spore proliferation in addition to enhancement of the growth promotion in guava seedlings.

An interesting result found in the current study is the ability of the native microorganisms to enhance Tamarix growth in saline stressful condition. Indeed, a positive effect of native inoculum and co-inoculum was observed on *T. articulata* shoot and root biomasses as compared to commercial inoculum, in particular, in moderate saline soils. This result corroborates with recent studies of Ortiz et al. (2015) and Bona et al. (2015) who showed that native inoculum, based on AMF and *Pseudomonas sp,* increased *Trifoliumrepens* and strawberry growth. *Pseudomonas* being a good auxin and cytokinin producer may solubilize phosphate and stimulate AMF root colonization and promote root growth (Frey-Klett et al., 2007). The synergistic interaction between AMF and bacteria as *Rhizobia, Azospirilum* and *Pseudomonas* can stimulate plant growth (Vyas and Vyas, 2014). In contrast, non-significant difference was recorded between commercial, co-inoculum and native AMF inoculum in the non-saline soils on *T. articulate* growth except for root biomass with commercial inoculum. This finding underlined the beneficial effect of AMF for plant survival and growth in harsh environments (Garg and Pandey, 2015; Saxena et al., 2017). The main mechanism explaining *T. articulata* growth enhancement under inoculation conditions seems to be improvement in nutrient uptake. Positive correlation was recorded between mycorrhizal rate and *T. articulata* growth, phosphorus and nitrogen contents in the presence of native AMF inoculum and co-inoculum, which indicates that native AMF still existed in soils and have beneficial effects on plant growth or physiological process. This result is in agreement with those of Liu et al. (2016) in extremely high salinity up than 4 $ds.m^{-1}$. In contrast, the commercial inoculum showed only one positive correlation between AMF mycorrhizal rate and root biomass. Indeed, co-inoculation enhanced phosphorus and nitrogen shoot contents under non-saline, moderate and saline soils. Co- inoculation of *T. articulata* by AMF

and *Pseudomonas* increased phosphorus shoot content approximately by two folds compared to the commercial mycorrhizal inoculum. These findings are in accordance with previous data which demonstrated that AMF improved plant phosphorus uptake (Hamel, 2004) and that this effect is enhanced by the co-inoculation of AMF with *Pseudomonas spp* (Artursson et al., 2006). In the same way, Vyas and Vyas (2014) recorded that plant phosphorus content in *Capsicum mathania* was observed upon dual inoculation (*Glomusdeserticola* plus *Azospirillum*) rather than single inoculation. Likewise, dual inoculation with *Pseudomonas striata* followed by *Glomus* fasciculatum enhanced phosphorus value in leaves of *Triticumaestivum* (Sadhana, 2014). Co-inoculation can activate the production of phosphatases by the mycorrhizal fungus and increase the production, by mycorrhizal hyphae, of organic acids that mineralize phosphorus (Hamel, 2004; Sadhana, 2014). In addition, our findings showed that inoculation treatment enhanced nitrogen shoot content. This can be attributed to increased nitrogen availability by mycorrhizal inoculum (Hamel 2004; Garg and Pandey, 2016). In saline soils, plants increasingly accumulate Na^+, which directly damages membrane systems and disturb N-reductase activity (Evelin et al., 2009; Saxena, 2017). To counteract this detrimental effect, the AMF inoculation tends to enhance K^+ and decreased Na^+ by preselecting nutrients and preventing Na^+ ion accumulation (Garg and Penday, 2016). Likewise, AMF have been reported to directly take up both organic and inorganic nitrogen from the soil solution and transfer it to their host plants (Ferrol and Pérez-Tienda, 2009).

On the other hand, it is noteworthy that soil salinity significantly reduces phosphorus shoot content in non-inoculated plants. This result could be explained by the fact that phosphate ions precipitate in the presence of Ca^{2+}, Mg^{2+} and Zn^{2+}ions in saline soils and became unavailable to plants (Evelin et al., 2009). The detrimental salinity effect can be attenuated by AMF inoculation (Garg and Pandey, 2016; Liu et al., 2016). In addition, the positive correlation recorded with native AMF incoculum and co-inoculum as compared with commercial inoculum must be explained by low mycorrhizal dependency of *T. articulata* to commercial inoculum.

CONCLUSION

The application of native AMF inoculum and AMF/*P. fluorescens* co-inoculum significantly enhanced growth and affected mineral uptake of *T. articulata* on saline soil. Native mycorrhizal inoculum single or combined with native *P. fluorescens* conferred higher salt tolerance to *T. articulata* than mycorrhizal commercial inoculum. Although the mechanisms are not well understood, this study supports the hypothesis that *P. fluorescens* and AMF induce changes in plant physiology and create favorable microenvironments. This can be regarded as an efficient biotechnology to improve plant development under stressed conditions of arid and semi-arid areas such as soil salinity. However, the interaction of non-native isolates with the local microflora needs to be explored. Whereas, it is imperative to understand the nature of the interaction between native beneficial endophytic bacteria and native AMF in order to explore and manage the co-inoculation for obtaining sustainable plant development. It will also be interesting in the future, to produce co-inoculum originated from arid and semi-arid areas for Tamarix species and other shrubs to increase plant establishment and growth, phosphorus and nitrogen uptake. On the other hand, it must be useful to enlarge this experimentation to several sites and diversified studies on abiotic stresses to confirm the universality of the co-inoculation efficiency.

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