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Effect of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) on Salt Stress Tolerance of *Casuarina obesa* (Miq.)

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Salinity is one of the main abiotic stresses limiting plant growth and development. However, the use of salt-tolerant plants combined with beneficial soil microorganisms could improve the effectiveness of biological methods for saline soil recovery. The aim of this study is to identify the *Casuarina obesa*/ Arbuscular Mycorrhizal fungi (AMF)/Plant Growth Promoting Rhizobacteria (PGPR) association that could be used in salt-land rehabilitation programs. Thus, the plants were grown under greenhouse on sandy soil, inoculated either with PGPR (*Pantoea agglomerans* and *Bacillus* sp.), or with AMF (*Rhizophagus fasciculatus* and *Rhizophagus aggregatum*) or co inoculated with PGPR and AMF and watered with a saline solution (0, 150, and 300 mM). After 4 months of cultivation, the plants were harvested and the results obtained showed that inoculation improves the survival rate, height and biomass of the plants compared to the control plants. The results also showed that inoculation increases the total amount of chlorophyll and the accumulation of plant proline at all levels of salt concentration. However, *P. agglomerans* and *Bacillus* sp. strains alone or in combination with *R. fasciculatus* increased plant growth. This study showed that these strains of PGPR, whether or not associated with AMF, could be biological tools to improve *C. obesa* performance under saline stress conditions.

Keywords: *Casuarina obesa*, beneficial microorganisms, land rehabilitation, salt stress, plant tolerance

INTRODUCTION

Soil salinization is one of the major environmental stress for plant growth (Barnawal et al., 2014). About 10% of the total arable land as being affected by salinity and sodicity (Shahid et al., 2018). This phenomenon is constantly increasing and constitutes an obstacle to agricultural production (Munns and Tester, 2008). In Senegal, about 55% of arable land is affected by salinization (LADA, 2009), which considerably affects the potential for national agricultural production. Land salinization is most often the result of natural factors such as rainfall deficiency, seawater intrusion, capillary rise due to evaporation and rock solubilization, but also of anthropogenic factors such as poor agricultural practices (Legros, 2009). This salinization leads to a degradation of the biological, chemical and physical properties of soils, resulting in decreased of soil fertility and crop yields (Ndour, 2006). With increasing population growth, access to land is becoming more difficult. Therefore, there is an urgent to develop effective strategies to rehabilitate salt land despite the many economic and climatic constraints around the world. Thus, several strategies have been adopted by rural populations and development actors. These include: (i) mechanical control through the installation of anti-salt structures (dams, or bunds), (ii) chemical control through the application of chemical amendments such as gypsum, sulfur or sulphuric acid to neutralize alkalinity, and (iii) biological control through the introduction of salt-tolerant plant species or organic matter to rehabilitate saline soils (Fall et al., 2018). The use of salt-tolerant plants is an appropriate approach to salt land management (Singh, 2000). Indeed, the presence of trees favors the development of micro flora and micro fauna essential for biodegradation processes, which represent the basis for the soil fertility. However, the success of a revegetation strategy in these salt areas will require the use of local or introduced plant species that are best adapted to environmental conditions. To this end, trees of the *Casuarinaceae* family are widely used in these programs because of their ability to grow on very poor soils (Patil et al., 2005; Diagne et al., 2014). The growth of Casuarina species on these degraded soils may be related to their ability to associate with nitrogen-fixing bacteria (*Frankia*) or PGPR and AMF that improve the plant's nitrogen and phosphate nutrition. Indeed, these symbiotic associations constitute means of adapting plants to unfavorable environmental conditions such as saline stress (Beltrano et al., 2013). They also play an important role in plant growth under various conditions by modifying their root systems and increasing the mobilization and absorption of essential elements such as N and P (Hashem et al., 2016). The aim of this study were to select the most salt-tolerant *C. obesa*/PGPR/AMF combinations for the rehabilitation of land degraded by salinization.

MATERIALS AND METHODS

Plant Material

The plant species used in this experiment were derived from seeds of *C. obesa* (lot number: 17994) provided by CSIRO/ATSC (Australian Tree Seed Centre; https://www.csiro.au/en/Resarch/Collections/ATSC Purchasing-seed). They were collected in the localities of Mullewa (latitude 28°15' North, longitude 115)38' East, altitude 250) in Australia. About 2,800 viable seeds were counted in a 10 g lot. These seeds were not pre-treated and were stored at 4°C in the laboratory.

Bacterial and Fungal Material

The strains *Pantoea agglomerans* lma2 (*P. agglomerans*) and *Bacillus* sp. were provided by the Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, Ferhat Abbas Setif-Algeria University. These strains have been isolated in the Bou-saâda region of Algeria (Hasfa, 2014). They were isolated from the rhizosphere of wheat under arid soil affected by salinity. *P. agglomerans* (accession number GQ478022) is a Gram-negative bacterium and has been identified by sequencing RNA16S. These strains were initially cultured in tubes containing 5 ml of liquid Luria-Bertani (LB) Broth medium and placed under agitation in a bacterial culture chamber at 30°C for 48 h. After a second culture was made in tubes containing 500 ml of Broth LB medium and placed in a culture chamber for 24 h under the same conditions. The liquid bacterial cultures thus produced were used for inoculation of the plants.

The arbuscular mycorrhizal fungi used in this study were: *Rhizophagus fasciculatus* (Rf) (Thaxt.) C. Walker and A. Schüßler DAOM227130 isolated in Quebec (Schüßler and Walker, 2010) and *Rhizophagus aggregatum* (Ra) (N.C. Schenck and G.S. Sm.) C. Walker DAOM2277128 isolated in Burkina Faso. These AMF provide from the collection of "Laboratoire Commun de Microbiologie" (LCM). AMF inoculum was produced during 6 months under greenhouse using a mycotrophic plant (*Zea mays* L.) grown in the presence of AMF in pots containing 1.5 kg of sandy soil previously sterilized at 120°C for 2 h. The soil used for the study was collected at Sangalkam in Senegal (14° 46'52" N, 17° 13'40" O) with the following physico-chemical characteristics: pH (H_2O) 6.5; clay 3.6%; fine silt 7.4%; fine sand 36.6%; coarse sand 21.55%; total carbon 0.54%; total nitrogen 0.06%; C/N 8.5; total phosphorus 39 mg kg-1 and soluble phosphorus 4.8 mg kg-1 (Diouf et al., 2005). The inoculum consisted of a mixture of spores and root fragments. The spore count was estimated using the Gerdemann and Nicolson (1963) method. The spore density of *R. fasciculatus* and *R. aggregatum* per 100 g of soil was 1,210 and 1,635 spores, respectively.

Plant Growth, Experimental Design, and Application of Salt Stress

C. obesa plants were cultivated in nursery (30°C) on sterile soil and watering with tap water at a frequency of 2 times/day. After 3 months of cultivation the seedlings were carefully dug out and transferred into pots of dimensions (25 ×12 ×50 cm) filled with a sandy soil sieved to 2 mm and autoclaved at 121°C for 120 min. Each pot was received a seedling and kept in a nethouse at 30°C at ISRA/CNRA (Institute Senegalese of Agriculture Research/National Center for Agronomical Research, 14°71 North - 16°48 West Bambey, Senegal). The soil used in our experiment was taken at CNRA (14°71 North - 16°48 West Bambey, Senegal). The physicochemical characteristics of this soil were determined at Laboratory of soil, plant and water,

ISRA/CNRA, Bambey, Senegal and are as follows: pH (H_2O) 7.78; pH (KCl) 7.46; EC 218.4 μS/cm; assimilable phosphorus 8.915 ppm; total phosphorus 3.896 ppm; total nitrogen 0.057%; organic carbon 0.631%; organic matter 1.09% and C/N 11.15. Fungal inoculum was applied at the time of plant transplantation. Treatments with AMF received 10 g of inoculum *R. aggregatum* strain or 10.12 g of the *R. fasciculatus* strain placed at about 3–5 cm depth. Bacterial inoculum was applied 1 week after transplanting close to the seedling root system. Treatments with bacteria received 5 ml of bacterial inoculum from *P. agglomerans* or the *Bacillus* sp. strain with a final absorbance of 0.2 measured at $\lambda = 595$ nm for each strain.

Salt stress was applied 2 months after inoculation to allow the establishment of mycorrhizal symbiosis. For each treatment, salt stress was gradually applied twice a week. Then, a weekly increase in NaCl concentration was performed to avoid osmotic shock. After 3 weeks of acclimatization, the control plants were watered with 0 mM and the stressed plants with 150 and 300 mM of NaCl. The choice of these concentrations was made on the basis of previous studies on Casuarina by Djighaly et al. (2018).

After 4 months the plants were harvested. Parameters such as height growth, total biomass, chlorophyll and proline contents and mycorrhizal frequency and intensity rate were evaluated.

The following treatments were applied: C: control plants not inoculated; P. agg: inoculated with *Pantoea agglomerans*; B25: inoculated with *Bacillus* sp.; Rf: inoculated with *Rhizophagus fasciculatus*; Ra: inoculated with *Rhizophagus aggregatum*; B25 + Rf: co-inoculated with *Bacillus* sp. and *Rhizophagus fasciculatus*; B25 + Ra : co-inoculated with *Bacillus* sp. and *Rhizophagus aggregatum*; P. agg + Ra: co-inoculated with *Pantoea agglomerans* and *Rhizophagus aggregatum*; P. agg + Rf: co-inoculated with *Pantoea agglomerans* and *Rhizophagus fasciculatus*. For each treatment seven repetitions were applied randomized device separated into blocks for each treatment to avoid contamination.

Measurement of Height Growth, Above-Ground, Root, and Total Biomass

The height (cm) growth of the plants was measured each month using a graduated ruler. The survival rate was determined using the formula:

$$\textbf{Survival rate}\ (\%) = \frac{\textbf{number of survival plants}}{\textbf{number total of plants}} * \textbf{100} \qquad (1)$$

After 4 months of greenhouse cultivation, the plants were harvested and the fresh weight of the aerial (BA) and root (BR) biomass produced for each treatment was weighed. The aerial and root parts were dried in the oven at 65°C for 1 week. Once the samples were completely dry, their dry weight was determined using an electronic precision balance TE1245 (Sartorius AG, Germany).

Determination of Chlorophyll and Proline Content

The chlorophyll content (a) and (b) of the aerial parts of the plants was determined using Arnon (1949) method, modified and described by Tahri et al. (1998). The total chlorophyll content (a+b) was calculated according to Arnon (1949) formula:

CHt (mg/l) = [8, 02 x DO (663 nm) + 20, 2 × DO (645 nm)] × V / M where V = volume of acetone (10 ml); M = foliar mass (100 mg); DO = optical density in nm.

Proline levels in the leaves were determined using the method described by Monneveux and Nemmar (1986). A quantity of 100 mg of fresh leaves were used and the samples were measured with a spectrophotometer at a wavelength of 520 nm. Proline contents were calculated using the equation derived from the calibration curve constructed from a range of known and increasing proline concentrations from 0 to 800 μmoles.

Mycorrhization

To determine the frequency and intensity of mycorrhization, the roots of the plants were cleaned and stained using the Phillips and Hayman (1970) method. For each plant, 100 fragments of 1 cm were used for the observations under the microscope. The frequency of mycorrhization was determined by the formula: F% = (number of mycorrhized fragments/total number of observed fragments) × 100. Mycorrhization intensity was analyzed using the method of Trouvelot et al. (1986) using a range of colonization intensity noted from zero (0) to five (5). It was estimated by the formula: I% = (95n5 + 70n4 + 30n3 + 5n2 + n1)/total number of observed fragments where n5 = number of noted fragments 5; n4 = number of noted fragments 4; n3 = number of noted fragments 3; n2 = number of noted fragments 2; n1 = number of noted fragments 1.

Statistical Analysis

The data were subjected to a two-factor analysis of variance (ANOVA) (inoculation and salinity level). The averages of the variables measured at the 5% probability threshold ($p \leq 0.05$) were compared using the Student Newman-Keuls test. The tests and statistical analysis of the results were performed with GenStat Edition 17 software.

RESULTS

Analysis of the variance reveals a significant effect of factor Inoculation and salt concentration (NaCl) on growth parameters such as plant height, shoot and root biomass (**Supplementary Table 1**). The interaction "Inoculation × [NaCl]" had significant effects on chlorophyll and proline content.

Effect of Inoculation With PGPR and/or AMF on the Survival Rate of *C. obesa* Plants Under Salt Stress Conditions

The results showed that all plants survived 1 month after salt stress application (**Table 1**). At harvest (2 months after salt application), plant mortality was noted only at 300 mM NaCl. The highest survival rate was observed in plants inoculated with *P. agglomerans* (100%) at 300 mM. However, this rate decreased in plants inoculated with B25 + Ra and Ra (80.95%), followed by control plants (C) and those inoculated with Rf + B25 and Rf

TABLE 1 | Survival rate of *C. obesa* plants after 1 and 2 months of salt application.

	Survival rate of *C. obesa* plants (%)					
Treatments	**After one (1) month of salt**			**After (2) months of salt**		
NaCl	**0 mM**	**150 mM**	**300 mM**	**0 mM**	**150 mM**	**300 mM**
C	100 a	100 a	100 a	100 a	100 a	85,71 ab
P.agg	100 a	100 a	100 a	100 a	100 a	100 a
B25	100 a	100 a	100 a	100 a	100 a	90,47 a
Rf	100 a	100 a	100 a	100 a	100 a	85,71 ab
Ra	100 a	100 a	100 a	100 a	100 a	80,95 b
B25 + Rf	100 a	100 a	100 a	100 a	100 a	85,71 ab
B25 + Ra	100 a	100 a	100 a	100 a	100 a	80,95 b
P.agg + Rf	100 a	100 a	100 a	100 a	100 a	90,47 a
P.agg + Ra	100 a	100 a	100 a	100 a	100 a	90,47 a

C, control plants not inoculated; P. agg, inoculated with Pantoea agglomerans; B25, inoculated with Bacillus sp.; Rf, inoculated with Rhizophagus fasciculatus; Ra, inoculated with Rhizophagus aggregatum; B25 + Rf, co-inoculated with Bacillus sp. and Rhizophagus fasciculatus; B25 + Ra, co-inoculated with Bacillus sp. and Rhizophagus aggregatum; P. agg + Ra, co-inoculated with Pantoea agglomerans and Rhizophagus aggregatum; P. agg + Rf, co-inoculated with Pantoea agglomerans and Rhizophagus fasciculatus. Each value represents the mean of plants used for each treatment (n = 7); lower case letters (a,b) indicate significant differences to a two-factor analysis (inoculation and salinity level) according Student Newman-Keuls test ($p < 0.05$).

with (85.71%) and in plants inoculated with P.agg + Rf, P.agg + Ra and B25 (90.47%) at 300 mM (**Table 1**).

Effect of Inoculation With PGPR and/or AMF on Height, Total Dry Biomass of *C. obesa* Plants Under Saline Stress Conditions

Results obtained after 4 months of growing in greenhouse showed that the increasing of salt concentration lead to a decrease of plant height from 35.39% to 150 mM and 64.67% to 300 mM compared to controls at 0 mM (**Table 2**). In the presence of 150 mM, inoculation with P. agg + Rf improved the height of *C. obesa* plants by 13.27% compared to control plants (**Table 3**). At 300 mM, inoculation with PGPR and/or AMF have no significative effect on *C. obesa* height.

The Aerial biomass of *C. obesa* was improved by the inoculation with P. agg + Rf compared to control plants at all concentration of NaCl. At 300 mM, only inoculation with P.agg + Rf improved the root biomass of *C. obesa* plants.

The total biomass of *C. obesa* was improved by the inoculation with P. agg, P. agg + Rf and B25 + Rf compared to control plants in the absence of salt. At 150 and 300 mM, a significant increase was observed in plants co-inoculated with P. agg + Rf of 34.24 and 76.62%, respectively, compared to control plants (**Table 2**).

Effect of Inoculation With PGPR and/or AMF on the Total Chlorophyll and Proline Contents of *C. obesa* Under Salt Stress Conditions

The total chlorophyll contents of *C. obesa* plants was evaluated after 4 months of salt stress and the results obtained show a significant increase in chlorophyll contents in plants inoculated with *P. agglomerans* compared to control plants at all concentration. The inoculation with P. agg increase chlorophyll content by 25.56, 28.85, and 51.55% at 0, 150, and 300 mM, respectively, compared to controls plants (**Table 3**). At 150 mM, inoculation with P. agg + Rf had increased the total chlorophyll content of *C. obesa* compared to the control plants.

The results obtained show an increase in proline synthesis as a function of salt concentration. In the absence of salt, the proline content is not significantly different between inoculated and non-inoculated plants. In the presence of 150 mM, there is an increase in proline levels in plants inoculated with P. agg and B25 compared to control plants. At 300 mM, a significant difference was noted in plants inoculated with P. agg and B25 compared to plants (**Table 3**).

Effect of Salt Stress on the Mycorrhization of *C. obesa* Plants Under Salt Stress Conditions

The highest frequency of mycorrhization was observed in co-inoculated plants inoculated with P.agg + Rf at all concentration. Concerning the intensity of mycorrhization, the higher intensity was observed with plants co-inoculated P. agg + Rf compared to controls plants at 0 and 150 mM. At 300 mM, no difference was noted between treatments compared to controls plants (**Table 4**).

DISCUSSION

The effect of inoculation with AMF and PGPR strains was studied on *C. obesa* plants subjected to different concentrations of NaCl (0, 150, and 300 mM) for 4 months under greenhouse. The results obtained show that inoculation with *P. agglomerans* strain improves the survival rate of plants compared to control plants. After 2 months of salt stress application, the survival rate of plants inoculated with *P. agglomerans* strain was 100%. This result could be explained by the intrinsic ability of this strain to tolerate salt. The works of Hasfa (2014) was showed that *P. agglomerans* lma 2 tolerated high salt concentrations and that its growth increased to NaCl concentrations between 100 and 400 mM. Inoculation with *Bacillus* sp. and *P. agglomerans* strains combined with *R. fasciculatus* or *R. aggregatum* also increased the survival rate of *C. obesa* (90.47%) compared to control plants*R. aggregatum* strain compared to controls did not improve plant survival.

The efficacy of co-inoculation with P.agg + Rf and P.agg + Ra could be related to the ability of PGPR to solubilize phosphates and the effective absorption of solubilized P from soil through AMF hyphae (Richardson et al., 2009; Saia et al., 2020). This association allows better colonization of the plant by AMF through these PGPR, which are well known as Mycorrhiza Helper Bacteria (Garbaye, 1994). This improved colonization results in better soil exploration by the mycorrhized roots of the plant and better absorption of mineral elements by the hyphae of fungi (Smith and Read, 2008). This statement is in agreement with our results which showed that the frequency of mycorrhization was significantly improved by co-inoculation P.agg + Rf compared to plants inoculated with AMF strains

TABLE 2 | Effect of inoculation with PGPR and/or AMF on height, shoot biomass, root biomass and total dry biomass of *C. obesa* plants under salt stress conditions.

	Control	P.agg	B25	Ra	Rf	P.agg + Rf	P.agg + Ra	B25 + Rf	B25 + Ra
HEIGHT (cm)									
0 mM	45.52 abc	52.50 a	52.21 ab	48.32 abc	44.41 abc	49.08 abc	43.78 c	45.11 abc	43.92 bc
150 mM	29.41 de	28.34 de	31.12 cd	26.22 de	29.81 de	33.91 c	25.54 de	27.98 de	23.21 e
300 mM	16.08 f	28.88 de	20.85 e	11.24 f	20.80 e	23.88 e	21.21 e	18.47 e	13.60 f
AERIAL BIOMASS (g)									
0 mM	1.26 bcd	1.65 ab	1.58 abc	1.22 cd	1.37 abcd	1.72 a	1.15 d	1.46 abcd	1.54 abcd
150 mM	0.38 fgh	0.40 fgh	0.44 ef	0.23 gh	0.42 efg	0.61 e	0.20 h	0.34 fgh	0.30 fgh
300 mM	0.12 i	0.42 efg	0.34 fgh	0.11 i	0.12 i	0.57 e	0.25 h	0.27 h	0.09 j
ROOT BIOMASS (g)									
0 mM	0.32 b	0.47 ab	0.44 b	0.32 b	0.39 b	0.48 ab	0.30 b	0.65 a	0.33 b
150 mM	0.12 cde	0.13 cd	0.11 cde	0.08 e	0.11 cde	0.14 c	0.08 e	0.11 cde	0.09 de
300 mM	0.05 e	0.12 cde	0.08 e	0.02 e	0.05 e	0.20 c	0.09 de	0.06 e	0.03 e
TOTAL DRY BIOMASS (g)									
0 mM	1.58 bc	2.12 a	2.03 ab	1.55 bc	1.76 abc	2.21 a	1.45 c	2.11 a	1.87 abc
150 mM	0.50 de	0.53 de	0.55 de	0.31 f	0.54 de	0.76 d	0.28 f	0.45 de	0.39 ef
300 mM	0.18 f	0.54 de	0.42 de	0.14 g	0.19 f	0.77 d	0.34 ef	0.33 f	0.13 g

Each value represents the mean of plants used for each treatment (n = 7); lower case letters (a–j) indicate significant differences to a two-factor analysis (inoculation and salinity level) according Student Newman-Keuls test ($p < 0.05$).

TABLE 3 | Total chlorophyll and proline content of *C. obesa* plants inoculated with PGPR and/or AMF subject to 0, 150, and 300 mM NaCl.

	Chlorophyll content (mg.g-1FM)			Proline content (μmol/g MF)		
	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM
Control	2.33 b	2.12 b	0.96 f	57.14 e	51.82 e	25.03 fg
P.agg	3.09 a	2.98 a	1.98 bc	49.54 e	103.24 bc	90.19 c
B25	1.44 d	1.88 c	1.25 e	63.14 de	156.21 a	87.82 c
Ra	1.68 cd	1.82 c	0.58 g	53.97 e	136.52 ab	65.64 de
Rf	1.51 d	1.60 cd	0.98 f	47.18 e	61.4 de	77.71 d
P.agg + Rf	1.89 c	2.19 b	1.49 d	37.23 f	36.06 f	17.00 g
P.agg + Ra	1.92 bc	1.96 bc	1.25 e	31.85 f	70.21 d	33.45 f
B25 + Rf	1.93 bc	2.07 b	1.19 e	34.12 f	67.44 de	50.9 e
B25 + Ra	1.90 bc	1.57 d	0.92 f	52.13 e	46.61 e	38.92 f

Each value represents the mean of plants used for each treatment (n = 7); lower case letters (a–g) indicate significant differences to a two-factor analysis (inoculation and salinity level) according Student Newman-Keuls test ($p < 0.05$).

alone in the presence of salt. Several studies have shown that symbiotic associations between beneficial soil bacteria (PGPR or Rhizobia) and the PGPR can improve root colonization of plants by AMF in many species such as: *Acacia auriculiformis* and *Acacia mangium* (Diouf et al., 2005), *Acacia senegal* (Ndoye et al., 2012) and *Litchi chinensis* (Visen et al., 2017). These microorganisms are regulators of stress by adjusting nutritional and hormonal balance and inducing systemic stress tolerance (Ruiz-Lozano et al., 2012). This regulation can induce an increase in K+ content accompanied by an effective decrease in Na+ in plant tissues. Additional experiments aimed to evaluate the variations of K+ and Na+ and Cl- concentrations in response to the inoculation with P.agg + Rf and P.agg + Ra will help to determine whether a similar mechanism is involved. However, the result obtained with other combinations could be explained by a less effective symbiosis between the two strains. Artursson et al. (2006) showed that the success of co-inoculation depends not only on the symbiotic efficacy of microorganisms but also on the compatibility between different symbiosis partners. Moreira et al. (2019) showed a synergistic effect between AMF and PGPR in increasing maize growth in saline conditions. This effect was related to an increase in K+ content accompanied by an effective decrease in Na+ in plant tissues.

The positive effect co-inoculation P. agg + Rf on growth parameters could be explained by the fact that *P. agglomerans*, is a halophilic, nitrogen-fixing and phosphate-solubilizing strain, capable of producing of indole acetic acid (AIA) up to 600 mM NaCl (Hasfa, 2014) and AMF (*R. fasciculatus*) in improving salt tolerance stress among Casuarina species (Djighaly et al., 2018). In the presence of salt, these microorganisms participate

TABLE 4 | Effects of the co-inoculation of PGPR and AMF on the frequency and intensity of mycorrhization of *C. obesa* plants under salt stress.

	Frequency of mycorrhization (%)			Intensity of mycorrhization (%)		
	0 mM	**150 mM**	**300 mM**	**0 mM**	**150 mM**	**300 mM**
Ra	26.25 b	23.75 b	16.00 b	1.37 c	3.14 b	1.57 c
Rf	34.50 a	31.25 b	15.00 b	6.34 b	3.26 b	0.82 c
P.agg + Rf	37.00 a	45.50 a	27.25 a	9.14 a	12.41 a	2.88 bc
P.agg + Ra	22.00 b	43.25 a	16.75 b	0.54 c	8.12 ab	0.45 c
B25 + Rf	15.00 c	35.00 ab	22.00 ab	1.12 c	4.91 b	0.90 c
B25 + Ra	23.00 b	29.55 b	25.75 a	1.74 c	2.35 bc	2.94 bc

Each value represents the mean of plants used for each treatment (n = 7); lower case letters (a–c) indicate significant differences to a two-factor analysis (inoculation and salinity level) according Student Newman-Keuls test ($p < 0.05$).

in the selective absorption of ions such as phosphorus, nitrogen and magnesium and in the reduction of Na+ ion absorption (Giri and Mukerji, 2004; Chen et al., 2014; Paul and Sinha, 2017). According to Ruiz-Lozano et al. (2012), improving plant tolerance to salinity by adding endomycorrhizae leads to increased photosynthetic activity and better water absorption efficiency.

The higher frequencies and intensities in co-inoculated P.agg + Rf plants would explain the salt tolerance of this strain. The work of Djighaly et al. (2018) showed significant metabolic activity of *R. fasciculatus* at 300 mM NaCl. But also to the additional effect of PGPR which improves the phosphate nutrition of the plant (Egamberdieva et al., 2019).

The high total chlorophyll content observed in plants inoculated with *P. agglomerans* in the presence of salt could explain the better growth rates observed in these plants. However, we noted that *P. agglomerans* strain associated with *R. fasciculatus* was more effective than *R. aggregatum* strain associated with the same strains. This result could be explained by a less effective symbiosis between the AMF (*R. aggregatum*) strain co-inoculated with the PGPR (*P. agglomerans*). A significant accumulation of proline was obtained with inoculated plants *P. agg* and B25 in the presence of salt (150 and 300 mM) compared to control plants. Proline accumulation is a mechanism of resistance to saline stress by adjusting intracellular osmotic pressure. These results are in agreement with works of Hasfa (2014) who showed that inoculation with *Bacillus* sp. and *Pantoea agglomerans* strain improve salt stress tolerance of wheat. However, our results showed that *P. agglomerans* + *R. fasciculatum* treatment had no significantly improve proline concentration in presence of NaCl. This result does not explain the good behavior of *C. obesa* plants co-inoculated with *P. agglomerans* + *R. fasciculatus*. However, it could also be supposed that this combination (*P. agglomerans* + *R. fasciculatus*) could establish other salt tolerance mechanisms such as the production of antioxidant enzymes, stress hormones and the overexpression of genes involved in salt stress tolerance. The works of Gond et al. (2015) showed that *P. agglomerans* improved the growth capacity of tropical maize compared to uninoculated controls. These results were explained by the up-regulation of the aquaporin gene family, especially plasma membrane integral protein (ZmPIP) genes in *P. agglomerans*-treated plants.

CONCLUSION

The results of the experiment show that inoculation with PGPR and/or AMF can improve resistance to salinity of *C. obesa* plants by increasing their growth parameters. In general, chlorophyll and proline content were also improved in plants inoculated under salt stress. This positive effect of inoculation was more pronounced with *P. agglomerans* and with *P. agglomerans* + *R. fasciculatus*. Thus, inoculation with salt tolerant PGPR and/or AMF could be a solution for the rehabilitation of land affected by the salt in Senegal. It would be interesting to carry out further research in field conditions to confirm the performance of these strains and then isolate indigenous strains of PGPR and *Frankia* from saline soils and determine their impact on salt tolerance in Casuarina inoculated with AMF.

As Casuarina species are associated with Frankia strains that play a critical role on their performance. It would be interesting the study the interaction of Casuarina species, salt tolerant AMF and PGPR strains.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MNd, PID, MNg, GNd and ND did the experimental work and analysis thereof and wrote the manuscript. ND, DNg, SS, and HC-S contributed in designing, supervision, and interpretation of the results. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2020.601004/full#supplementary-material

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